*Plant, Cell and Environment* (1999) **22,** 649–657

# Does elevated atmospheric $CO_2$ concentration inhibit mitochondrial respiration in green plants?

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## ABSTRACT

**There is abundant evidence that a reduction in mitochondrial respiration of plants occurs when atmospheric $CO_2$ ($C_a$) is increased. Recent reviews suggest that doubling the present $C_a$ will reduce the respiration rate [per unit dry weight (DW)] by 15 to 18%. The effect has two components: an immediate, reversible effect observed in leaves, stems, and roots of plants as well as soil microbes, and an irreversible effect which occurs as a consequence of growth in elevated $C_a$ and appears to be specific to $C_3$ species. The direct effect has been correlated with inhibition of certain respiratory enzymes, namely cytochrome-*c*-oxidase and succinate dehydrogenase, and the indirect or acclimation effect may be related to changes in tissue composition. Although no satisfactory mechanisms to explain these effects have been demonstrated, plausible mechanisms have been proposed and await experimental testing. These are carbamylation of proteins and direct inhibition of enzymes of respiration. A reduction of foliar respiration of 15% by doubling present ambient $C_a$ would represent 3 Gt of carbon per annum in the global carbon budget.**

*Key-words*: acclimation to rising $CO_2$; dark respiration; global carbon cycle; rising $CO_2$.

*Abbreviations*: ATP, adenosine triphosphate; $K_m$, Michaelis-Menton coefficient; $C_a$, concentration of $CO_2$ in the air ($\mu$mol mol$^{-1}$); NAD, oxidized nicotin adenine dinucleotide; NADH, reduced nicotin adenine dinucleotide; NADP, oxidized nicotin adenine phosphate dinucleotide; NADPH, reduced nicotine adenine phosphate dinucleotide; *R*, rate of respiration per unit DW [$\mu$mol g DW$^{-1}$], Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; $V_{c,max}$, maximum *in vivo* rate of carboxylation at Rubisco ($\mu$mol m$^{-2}$ s$^{-1}$).

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*This paper was prepared by the participants in a workshop held 8–9 January 1998 at the Smithsonian Environmental Research Center, Edgewater, Maryland, 21037 USA.*

## INTRODUCTION

There have been indications in the literature of a direct effect of elevated atmospheric $CO_2$ concentration ($C_a$) partially suppressing higher plant respiration since the late nineteenth century (Kidd 1916; Mangin 1896 quoted in Murray 1995). Over the last 15 years such an effect has been frequently reported both as rapid reversible responses to elevated $C_a$, and as longer term responses measured on plants grown continuously at elevated $C_a$ (reviewed in Amthor 1997; Drake *et al*. 1997b; Curtis & Wang 1998). Inhibition of respiration by elevated $C_a$ has also been reported for microbial respiration (Drake *et al*. 1997b). Mechanisms for this inhibition have not yet been demonstrated unequivocally, the fast acting reversible response being particularly mystifying. If there is a significant effect of the globally rising $C_a$ on higher plant or microbial respiration rates then there are likely to be substantial implications for the global carbon cycle and agricultural systems. To quantify the implications and understand them sufficiently to allow a predictive capability, requires knowledge not only of respiration and the exact character of the suppression, but also of the role of autotrophic and heterotrophic respiration in ecosystem processes.

We use the term respiration (*R*, $\mu$mol g DW$^{-1}$) to mean the consumption of $O_2$ or the efflux of $CO_2$ per unit dry matter and we concentrate on effects observed when $C_a$ is in the range of 0–1000 $\mu$mol mol$^{-1}$. Two kinds of effects of elevated $C_a$ on apparent dark respiration in intact plants or tissues have been reported (Amthor 1991; Gonzàlez-Meler, Drake & Azcon-Bieto 1996a; Drake *et al*. 1997b): a direct, immediate effect in which respiration is reversibly reduced by exposure to elevated $C_a$, and an indirect effect

in which respiration of plants grown in elevated $C_a$ differs from the respiration of plants grown in normal ambient $C_a$ when measured at a common value of $C_a$.

In this paper, we review the data available on the effect of elevated $C_a$ on respiration, discuss the potential biochemical candidates for a mechanism whereby changes in ambient $C_a$ could bring about changes in the rate of respiration, and consider the potential effect of elevated $C_a$ on changes in construction, maintenance and transport costs. With the aid of a model for the global carbon cycle, we evaluate the significance for the global carbon budget of reduced dark respiration in foliage. We also suggest research goals for determining the mechanistic basis for the impact of rising $C_a$ on dark respiration of plants.

## EVIDENCE FOR THE EFFECT OF ELEVATED $C_a$ ON RESPIRATION

An increasing number of studies have reported direct and/or acclimation responses of higher plant respiration to $C_a$ enrichment (Table 1). These responses have been observed in leaves, whole-plants, roots and in the stems of woody plants (Gonzàlez-Meler *et al*. 1996a; Ryan *et al*. 1996; Wullschleger, Norby & Hanson 1995).

Poorter *et al*. (1992), who produced the first quantitative analysis of data on the tissue-specific response of respiration to elevated $C_a$, reported both increases and decreases in leaf, root and whole-plant respiration as a result of exposure to elevated $C_a$. Estimated changes in respiration due to elevated $C_a$ averaged across 41 $C_3$ and six $C_4$ species ranged from a 16% *stimulation* of respiration when expressed on a leaf-area basis to a 14% *inhibition* when expressed on a dry-mass basis. By contrast, Curtis (1996) conducted a statistical meta-analysis of published data on leaf respiration for trees grown at elevated $C_a$ and reported a significant reduction in respiration regardless of whether the data were expressed on a mass or area basis. This analysis did support the basic conclusion of Poorter *et al*. (1992) that the magnitude of the effect was dependent on how the data were expressed, with the apparent elevated $C_a$-induced effects on area-based rates of respiration being only about half those expressed on a dry-mass basis. In an expanded meta-analysis, Curtis & Wang (1998) reported that leaf respiration expressed on a dry-mass basis was reduced by 18% in woody plants grown at elevated $C_a$ concentrations.

**Table 1.** Estimates of the direct effect ($E$, %) of elevated $C_a$ on $R$ in leaves of plants. $E$ is expressed as the ratio of $R_e$, the rate of respiration measured in tissues grown and measured at elevated $C_a$, to $R_a$, the rate of plants grown and measured at normal ambient: $E = [(R_e/R_a - 1) \times 100]$. Elevated $C_a$ is defined in each review and the range varied from 550 to 800 $\mu$mol mol$^{-1}$. The number of species ($S$) and the number of values used in the analysis ($n$) are also indicated

| Review | $E$ (%) | $S$ | $n$ |
|---|---|---|---|
| Amthor 1997 | –15 | 23 | 140 |
| Curtis & Wang 1998 | –18 | 14 | 31* |
| Drake *et al*. 1997b | –18 | 23 | 53 |

* trees only

The analyses of Poorter *et al*. (1992), Curtis (1996), and Curtis & Wang (1998) did not attempt to separate the direct effects *per se* from the indirect effects which probably occur as a result of acclimation of the plant to elevated $C_a$. Drake *et al*. (1997b) indicated an average 18% direct inhibition for foliage while Amthor's (1997) analysis of 36 species in 45 studies reported 15% direct inhibition in shoots, leaves and roots due to a doubling of the $C_a$. Both Amthor (1997) and Drake *et al*. (1997b) reported that the indirect effect was smaller than the direct effect. The acclimation effect generally results in reduced respiration (Baker *et al*. 1992; Azcón-Bieto *et al*. 1994), although in some experiments acclimation led to increased respiration (Thomas *et al*. 1993).

Mechanisms suspected to be involved in the direct and indirect effects (discussed below) include changes in carbohydrate availability, growth rate and biomass allocation, altered chemical composition of tissues, interactions between $C_a$ and key respiratory enzymes, and dark $CO_2$ fixation (Amthor 1991). Other aspects of the responses that have been explored include the effects of $C_a$ on growth and maintenance respiration, the apparent paradox of increased growth rate and reduced respiration (Bunce 1994) the potential interaction of nutrients and temperature in the responses to $C_a$ (Wullschleger, Ziska & Bunce 1994) and the biochemical mechanisms involved in a direct effect of $C_a$ on mitochondrial respiration (Gonzàlez-Meler, Drake & Azcon-Bieto 1996a).

Is the effect of elevated $C_a$ on respiration an artifact of systematic measurement errors? Two candidates for producing systematic artifacts in gas exchange systems are dilution of the air by transpired water vapour and leaks between the chamber and the surrounding air. Although each of these could, under certain conditions, produce an apparent reduction in $CO_2$ efflux, in most available studies, insufficient data are given to conclude whether or to what extent these errors were necessarily a part of the experiment. Moreover, no one has produced a convincing set of experiments accompanying measurements of respiration to permit us to conclude that these potential sources of artifacts are capable of causing the sort of errors needed to produce the effects observed (only the direct effect is involved here since the indirect effect could only result from changes in the tissue as a result of growth in elevated $C_a$). The correction needed to account for the dilution produced by the small amount of water vapour added by transpiration in the dark, even at 30 ºC, alters the uncorrected values of respiration by less than 2%. Data obtained using the oxygen electrode (Azcon-Bieto, Gonzàlez-Meler & Drake 1994; Gonzàlez-Meler *et al*. 1996b; Reuveni & Gale 1985) and $CO_2$ infra-red gas analysers with chambers sealed and immersed in a water bath (Box and Drake; unpublished results), allowing measurement of the inhibition in the absence of leaks, show that neither of these potential errors obviate the conclusion that elevated $C_a$ reduces the rate of respiration.

Nevertheless, there remain data in the literature which do not fit the observed pattern of reduced respiration on elevated $C_a$, and these data challenge us to understand the mechanistic basis for the effects of elevated $C_a$ rather than to dismiss the phenomenon. Evidence from controlled-exposure studies and related experiments suggests that respiration may increase, decrease or remain unchanged in response to elevated $C_a$ (Gifford, Lambers & Morrison 1985). Area-based rates of respiration are sometimes higher for leaves exposed to elevated $C_a$ than are rates based on dry weight (Poorter *et al*. 1992), although Curtis and Wang (1998) found little difference between these two approaches to expressing the results. Two possible biological effects that could give very different results with elevated $C_a$ are leaf age and carbohydrate (CHO) status. When CHO is increased in leaves using light as the means for increasing CHO, respiration usually increases (Azcon-Bieto & Osmond (1983). Similarly, in young, rapidly expanding leaves, respiration was higher in plants grown in elevated $C_a$ (Hrubec, Robinson & Donaldson 1985) but this effect persisted only for the first 3 weeks of growth.

To summarize this section of the paper; there is abundant evidence that the respiration rate measured in mature foliage, stems and roots is often less in plants grown and tested in elevated than in normal ambient $C_a$. Two effects have been identified: a direct, reversible effect which occurs in all plants tested; and an indirect, irreversible effect which has been observed only in $C_3$ plants grown in elevated $C_a$. Artifacts of measurement may have altered the magnitude of the effect in the experiments reported to date but we have yet to identify a mechanism which would permit us to explain effects in terms of a physical property related to experimental protocol. We now consider the effects of elevated $C_a$ on respiratory control mechanisms.

## RESPIRATORY CONTROL MECHANISMS

### Mechanisms for the direct effect

Inhibitory effects of $CO_2$/bicarbonate levels on the *in vitro* activities of a number of respiratory enzymes in different plant tissues have been reported (Amthor 1991; Gonzàlez-Meler *et al*. 1996a). These enzymes include both adenosine triphosphate (ATP)- and PPi-dependent phosphofructokinases (glycolysis), malic enzyme (mitochondrial matrix), and cytochrome *c* oxidase and succinate dehydrogenase (mitochondrial electron transport chain). In the experiments just quoted, the $C_a$ used was much higher than the expected atmospheric $C_a$ rise. However, in the case of the two electron transport chain enzymes, recent studies (Gonzàlez-Meler *et al*. 1996b; Reuveni, Gale & Mayer 1995) have shown that inhibition by elevated $C_a$ equivalent to twice normal ambient $C_a$ occurs within minutes of the elevation of the $CO_2$ or bicarbonate.

The activity of cytochrome oxidase isolated from beef heart or obtained from soybean cotyledon and root mitochondria was inhibited about 20% by an increase in $C_a$ of 360 $\mu$mol mol$^{-1}$ (Gonzàlez-Meler *et al*. 1996b). Inhibition of cytochrome oxidase (40–50%) by much higher $C_a$ (over 1% $CO_2$ in air) has also been reported for plant cells, isolated mitochondrial and beef heart enzyme (Palet *et al*. 1991, 1992). The alternative oxidase appears to be insensitive to changes in $C_a$ (Gonzàlez-Meler *et al*. 1996b; Reuveni *et al*. 1995) even at $C_a$ up to a concentration of 5% in air (Palet *et al*. 1992). Inhibition of mitochondrial enzymes and $O_2$ uptake activity by twice normal ambient $C_a$ provides evidence of a true direct effect of elevated $C_a$ on respiration but cannot account directly for the observed magnitude of the direct effect (Gonzàlez-Meler & Siedow 1999). It is obvious that in cases where direct inhibition of respiration by elevated $C_a$ exceeds the direct inhibition of the reported mitochondrial oxygen uptake, other mechanisms are also responsible for the direct effect. Inhibition of respiration by elevated $CO_2$ as a consequence of inhibition of cytochrome oxidase is proportional to the degree of control that cytochrome oxidase exerts on respiratory metabolism. In most metabolic conditions the flux control coefficient of cytochrome oxidase is low (less than 0·10), and therefore inhibition of the enzyme will result in little inhibition, if any, of tissue $CO_2$ efflux (Gonzàlez-Meler & Siedow 1999). In mitochondria without ADP limitation of activity, the control coefficient can be as high as 0·66 (Gonzàlez-Meler & Siedow 1999) and under these conditions, one might predict that inhibition of the activity of the enzyme could result in reduction in the efflux of $CO_2$.

Several potential mechanisms exist whereby elevated $C_a$ might act on plant enzymes to modulate their activity. These include the carbamylation of proteins by $CO_2$, direct inhibitory effects of $CO_2$, bicarbonate acting either as a substrate mimic or as the end-product of a particular enzyme reaction, direct inhibition of respiratory $CO_2$ uptake by enhancement of the rate of dark $CO_2$ uptake, and effects of increased $C_a$ on intracellular pH.

Carbamylation involves the reaction of dissolved $CO_2$ with a free amino functional group, most commonly the e-amino group on the side chain of lysine residues in proteins, by the following reaction:

$$R - NH_2 + HCO_3^- = R - N(H) - C(O)O^- + H_2O$$

If the formation of the carbamate affects enzyme activity (positively or negatively), then a doubling of $C_a$ will double the amount of carbamylated protein present and affect the enzyme's activity accordingly. The site of inhibition of cytochrome *c* oxidase by $C_a$ discussed above has not been established. However, it would not be surprising to find reaction with $CO_2$ to form a carbamate responsible for reducing the activity of the enzyme. A large number of lysine residues are known to exist throughout the protein (Tsukihara *et al*. 1996).

Direct competitive effects between $CO_2$ and substrate may be another mechanism by which elevated $C_a$ can theoretically affect the activity of respiratory enzymes. This is the mechanism of inhibition of succinate dehydrogenase by bicarbonate, where a number of small anionic species are known to displace succinate at the active site (DerVartanian & Veeger 1964). It is reasonable to expect

that many enzymes whose substrates are small anions (e.g. anion transporters) could show some level of inhibition by bicarbonate. While any such bicarbonate-sensitive enzyme would presumably be affected by current levels of $C_a$, doubling of $C_a$ will enhance the inhibition beyond the level existing at present. Likewise, any enzyme, such as a decarboxylase that gives rise to $CO_2$ (or bicarbonate) as a product has the potential to be subject to end-product inhibition by that species. Increased $C_a$ would be expected to enhance the observed level of inhibition, relative to that associated with current $C_a$ levels, through simple mass action.

The enhancement of dark $CO_2$ uptake through the increased activity of PEP carboxylase in the presence of higher concentrations of its substrate, $HCO_3^-$, represents an additional mechanism by which elevated $C_a$ could inhibit the observed rate of respiratory $CO_2$ efflux. For $C_4$ and crassulacean acid metabolism plants, the magnitude of this effect would be very small because the Michaelis-Menton coefficient ($K_m$) of PEP carboxylase for bicarbonate (10–25 $\mu$M, Ting & Osmond 1973; Nott & Osmond 1982) suggests that the enzyme is acting close to maximum activity under current levels of $CO_2$ where the cytosolic bicarbonate concentration is around 200 $\mu$M. However, in some $C_3$ plants, the $K_m$ for $HCO_3^-$ has been reported to be in the range of 100–300 $\mu$M, suggesting that a doubling of $C_a$ could have a significant effect on PEP carboxylase activity. However, any long-term increase in the net uptake of $CO_2$ associated with enhanced dark $CO_2$ uptake would presumably be accompanied by a build-up of intracellular organic acids (e.g. malate) that could act to attenuate the stimulatory effect of elevated $C_a$ on PEP carboxylase activity (Amthor 1997).

Enhanced levels of dissolved $CO_2$ could theoretically act to lower intracellular pH, with consequent effects on cellular metabolism, including respiration were the magnitude of the change large enough to affect any particular enzyme's activity. However, given the presence of robust mechanisms for maintaining the constancy of intracellular pH in plants (Bown 1985), it seems unlikely that a doubling of $C_a$ would have any significant effect on cellular pH.

Other non-specific mechanisms by which $CO_2/HCO_3^-$ could operate to affect the activity of plant enzymes undoubtedly exist, but additional work is clearly needed to identify the potential role that of any of the mechanisms cited above might have on plant metabolism, including aerobic respiration, and the enzymes that might be affected by them.

To summarize what can be said about the direct effect: elevated $C_a$ has been shown to inhibit the activity of at least two respiratory enzymes (Gonzàlez-Meler *et al*. 1996b; Gonzàlez-Meler & Siedow 1999). The control coefficients for these enzymes are too low under the conditions expected to apply to mitochondria in intact leaves to explain the inhibition of whole tissue respiration by this mechanism. It is possible that many enzymes are similarly affected by elevated $C_a$ through a combination of mechanisms; the two most likely ones being carbamylation and substrate competition with $CO_2$. We now consider factors which may determine the indirect effect.

## Mechanisms for the indirect effect

As a result of effects on photosynthesis and water balance, the long-term exposure of plants to elevated $C_a$ can be expected to produce biochemical (i.e. such as increased carbohydrate concentration and reduction in protein concentration), functional (i.e. increased translocation rates) and perhaps morphological and phenological responses, all of which may result in altered rates of plant respiration.

Increased substrate supply, as occurs when light intensity increases, results in a higher respiration rate, as shown by Azcon-Bieto & Osmond (1983). But when growth is stimulated by elevated $C_a$, the respiratory activity has been shown to decrease (Azcon-Bieto *et al*. 1994). Acclimation of respiration to elevated $C_a$ in mature stems of *Scirpus olneyi* and leaves of *Lindera benzoin* grown in open top chambers in the field was correlated with a reduction in maximum activity of mitochondrial enzymes (Azcon-Bieto *et al*. 1994). No acclimation effect of elevated $C_a$ has been reported for leaves of $C_4$ plants (Azcon-Bieto *et al*. 1994) or non-photosynthetic tissues of $C_3$ plants. This difference in the indirect response of respiration in $C_3$ and $C_4$ plants to elevated $C_a$ suggests that such a reduction in respiratory capacity by elevated $C_a$ reflects a reduced need for the respiratory component of photorespiration.

In rapidly expanding leaves, an increase in the supply of substrate for respiration results from higher rates of photosynthesis (Azcon-Bieto & Osmond 1983). Mature leaves of wheat grown at elevated $C_a$ had higher rates of respiration than controls grown at low $C_a$ and this effect correlated with the concentration of carbohydrates, notably fructose and glucose. Subsequent studies by Hrubec *et al*. (1985) also documented the tendency of respiration to increase in response to elevated $C_a$-enhanced carbohydrate supplies in young, rapidly expanding soybean leaves less than 3 weeks old. During rapid leaf expansion, rates of single-leaf respiration were 50% greater for plants grown at elevated $C_a$ than they were for controls grown at ambient $C_a$ and cytox was increased at elevated $C_a$ (Hrubec *et al*. 1985; Perez-Trijo 1981). During the very early stages of development, the respiratory machinery can increase very rapidly (Robertson *et al*. 1995).

Such observations have added fuel to the belief that respiration rates will likely increase following $C_a$ enrichment due to an accumulation of carbohydrates in leaves. This is an attractive hypothesis because it has long been reported that plants grown in elevated $C_a$ have higher concentrations of carbohydrates than controls (Thomas *et al*. 1993). Amthor (1989) cited a number of experiments in which rates of respiration were not related to carbohydrate status and suggested that except in young, rapidly growing tissues, where carbohydrates are readily consumed to meet the biosynthetic demands of growth, respiration was seldom limited by substrate availability. Instead, it was suggested that respiration was regulated by the rate at which respiratory products, namely adenylates, NAD(P)H, and carbon skeletons, were used to meet the energetic needs of growth, maintenance, transport, and nutrient uptake.

While the growth of plants at elevated $C_a$ almost certainly increases leaf carbohydrate concentrations, we have little understanding how either the supply of substrate or the demand for ATP, reductants, etc. affects respiration. These processes are no doubt important to our biochemical interpretation of how respiration is controlled at the cellular scale, but we are unable to extrapolate these effects to those operating at the scale of leaves and roots, let alone to plants and ecosystems.

These additional complexities may best be dealt with by obtaining a better understanding of growth and maintenance respiration, and by separating their relative sensitivity to $C_a$ enrichment. In rapidly growing plants, the largest components of respiration are those associated with the synthesis of new plant biomass, termed growth respiration, and with the uptake of nutrients from the environment. The respiratory costs associated with growth were derived theoretically from the biochemical pathways (Penning de Vries, Brunsting & van Laar 1974), and these values have been found to agree closely with experimental data (Loomis & Lafitte 1987).

Two questions seem especially noteworthy regarding the changes observed in construction costs for plants grown at elevated $C_a$. First, are the slight reductions in construction costs sufficient to alter rates of respiration? Poorter *et al*. (1997) estimated that a 3% decrease in construction costs due to elevated $C_a$ exposure would lead to an 11% decrease (range 4% increase to a 27% decrease) in growth respiration, assuming no change in carbon concentration. Similar reductions (10–11%) in growth respiration can be estimated from the carbon concentration and construction cost data provided for both yellow-poplar and white oak saplings grown at elevated $C_a$ (Wullschleger *et al*. 1997). Such a decrease in growth respiration is well within the range of published values (Thomas *et al*. 1993; Wullschleger & Norby 1992; Wullschleger, Norby & Gunderson 1992). Second, do elevated $C_a$-induced reductions in construction costs significantly influence calculations of plant carbon budgets? Assuming all other parameters were constant, Poorter & Villar (1997) argued that a decrease in construction costs (due to elevated $C_a$) would translate into a proportional increase in relative growth rate. Noting that the effects of light and/or elevated $C_a$ on construction costs were small, Poorter & Villar (1997) suggested that the consequences of altered construction costs were also likely to be small in relation to growth. Poorter *et al*. (1997) similarly concluded that a change in chemical composition due to elevated $C_a$ was not expected to influence a plant's carbon balance to any appreciable extent. Lower protein contents in plants grown at elevated $C_a$ could reduce the respiratory costs of tissue maintenance by lowering the rates of protein turnover. Indeed, several experiments which have attempted to separate growth and maintenance respiration in plants grown at elevated $C_a$ have indicated that maintenance respiration is lower at elevated $C_a$ (Bunce & Caulfield 1991; Wullschleger & Norby 1992; Wullschleger, Norby & Gunderson 1992; Baker *et al*. 1992; Ziska & Bunce 1993; Bunce & Ziska 1996). However, none of these studies actually measured protein turnover rates and in some cases (Mousseau 1993; Ziska & Bunce 1993; Bunce 1995; Teskey 1995) the reduction in respiration at elevated $C_a$ was reversible, suggesting that it was not caused by changes in composition. Additionally, experiments which have attempted to separate growth and maintenance respiration have needed to make various assumptions, including the equivalence of green tissue respiration in light and darkness, which may well be incorrect (e.g. Atkin *et al*. 1997). Thus, the evidence that plants differ enough in their biochemical composition to substantially affect the respiratory costs of new tissue synthesis is not convincing (Chapin 1989; Poorter 1994). Therefore, the primary variation in growth respiration that might be attributable to elevated $C_a$ may arise from $CO_2$-induced differences in rates of growth and transport, and less from the nature of the materials synthesized.

The export of carbohydrates from leaves and the uptake of nutrients from soils are major energy-consuming processes. If these transport-related activities or their specific costs change with elevated $C_a$, rates of respiration needed to supply energy for these processes might also change. In a re-analysis of existing data, Amthor (1997) illustrated how failure to account for increased translocation costs at elevated $C_a$ could result in an underestimate of leaf maintenance respiration. The capacity of excised roots for $NO_3^-$ uptake was higher for plants grown at elevated $C_a$. Although the reasons for this shift in preference are not yet known, it is worth noting that the energetic cost of uptake and assimilation may be higher for $NO_3^-$ than it is for $NH_4^+$ (Bloom, Sukrapanna & Warner 1992) and it is possible that the energy expended to acquire nutrients may change in plants exposed to elevated $C_a$. Specific costs associated with nutrient acquisition also vary among species and probably depending on environmental conditions (Lambers, Atkin & Scheurwater 1996). Such variation is partly accounted for by the efflux of nitrate, which tends to increase with decreasing relative growth rate of the plant (Lambers *et al*. 1997).

Acclimation of plants to elevated $C_a$ often reduces protein while increasing carbohydrate concentrations, which ought to reduce and increase dark respiration, respectively. But analyses of these effects of elevated $C_a$ on respiration have proved inconclusive, showing that understanding of respiratory control mechanisms are too rudimentary to account for the (relatively) subtle effects of elevated $C_a$ on the respiration. We now turn our attention from the mechanistic basis for the effects of elevated $C_a$ on respiration to the impact of this effect on the rates of respiration measured for higher levels of organization.

## LINKING EFFECTS OF $CO_2$ ON SPECIFIC RESPIRATION IN CELLS AND TISSUES TO WHOLE PLANTS, ECOSYSTEMS AND THE GLOBAL CARBON CYCLE

Information (as developed above) on the physiology and biochemistry of respiration have obvious implications for plant growth, ecosystem carbon balance and global carbon cycle.

The effects of elevated $C_a$ at these scales of integration have been studied and the observed responses are certainly due to multiple effects of $CO_2$ on processes (photosynthesis, water relations, allocation, plant nutrition).

If the specific respiration rate at the organ or plant level declines, what effects can be expected at the whole ecosystem level? Respiration rate at the ecosystem level integrates the effects of elevated $C_a$ on growth rate, total plant mass and allocation, species composition, and structural aspects of the canopy. Studies that have reported the effect of elevated $C_a$ on whole ecosystem respiration are summarized in Table 2.

For the agricultural crops and native grasslands and ecosystems, ecosystem respiration (per unit ground area), was on average unaffected by elevated $C_a$ treatment although the results ranged above and below 1·0 (0·84–1·15). When respiration was normalized on biomass, there was an average of 17% reduction in the rate (excluding studies no. 3 and 7). The studies excluded are those by Bunce & Caulfield (1991), who found increases in biomass with no increase or even a decrease in respiration and a study by Schapendonk *et al*. (1997) where during the first year of growth, a *Lolium* sward exhibited an increased biomass but no increase in respiration, although both growth and respiration increased during the second year.

The response of respiration followed and was correlated with the relative effects of elevated $C_a$ on above-ground biomass. In their study in rice, Baker *et al*. (1992), study no. 7) found increased ecosystem respiration, but at the same time, decreased specific respiration. Thus, most studies of whole system respiration are consistent with the hypothesis that the response of ecosystem respiration (per unit ground area) to elevated $C_a$ is determined by the effect of elevated $C_a$ on respiration of above-ground biomass (e.g. study no. 7 Baker *et al*. 1992 and study no. 8, Drake *et al*. 1997b).

**Table 2.** Ratio of elevated to ambient treatment for biomass, respiration and normalized respiration based on literature cited in Table 1

| Study | Biomass | Respiration/ ground area | Respiration/ biomass |
|---|---|---|---|
| 1 | 4·58 | 0·87 | 0·19 |
| 2 | 1·39 | 0·99 | 0·71 |
| 3 | 1·26 | 8·46 | 6·74 |
| 4 | 1·18 | 1·02 | 0·87 |
| 5 | 1·23 | 1·28 | 1·04 |
| 6 | 1·18 | 1·28 | 1·08 |
| 7 | 1·77 | 9·06 | 5·12 |
| 8 | 1·33 | 1·22 | 0·92 |
| 9 | 1·56 | 0·74 | 0·47 |
| 10 | 1·00 | 0·98 | 0·98 |
| 11 | 1·00 | 0·95 | 0·95 |
| 12 | 1·21 | 0·50 | 0·41 |
| Ave | 1·23 | 0·99 | 0·83 |
| 90% CI | 1·12–1·34 | 0·84–1·15 | 0·67–0·97 |

CI, confidence interval; CI excluded studies 1, 3 and 7.
Studies: 1 and 2, Bunce & Caulfield 1991; 3, Nijs *et al*. 1989a; 3 and 4, Schapendonk *et al*. 1997; 5, Casella & Soussana 1997; Casella *et al*. 1996; 6, Nijs *et al*. 1989b; 7 Baker *et al*. 1992; 8, Drake *et al*. 1997b, 1997a; 9, Ham *et al*. 1995; 10, 11, Diemer 1994, 12, Navas *et al*. 1995.

Autotrophic respiration, measured as $CO_2$ emission, accounts for the release of 40 to 60% of gross ecosystem photosynthetic $CO_2$ fixation (Gifford 1994). Consequently even a relatively small diminution of plant respiration by an increase in atmospheric $C_a$ could have profound effects on global carbon budgets and crop yield. The nature of the respiratory effect of elevated $C_a$ would depend critically on whether it were an expression of increased energetic efficiency of plant respiratory carbon utilization or whether it leads to a decrease of all or some of the processes that are dependent for energy supply or carbon skeletons on respiratory activity. For example, if global gross primary production were reduced by say 20% for a doubling of $C_a$ then the 90 $\mu$mol mol$^{-1}$ increase in $CO_2$ concentration that we have seen since pre-industrial times would be causing a diminution of global plant respiratory emission of $CO_2$ relative to that in the pre-industrial atmosphere. Alternatively, if the reduction in plant respiration were matched by a reduction in all the ecosystem growth, transport and maintenance processes that are fuelled by respiration then the implications are very different indeed and the biosphere could be a diminishing resource on that account.

In order to evaluate the potential impact of the effects of elevated $C_a$ on dark respiration for the global carbon budget, a model, GTEC 2·0 (Global Terrestrial Ecosystem Carbon, Version 2·0), was run. GTEC 2·0 is a global model of terrestrial carbon storage and $CO_2$ exchange with the atmosphere. An earlier version of the model (GTEC 1·0) is described in King, Post & Wullschleger (1997) and Post, King & Wullschleger (1997). Several runs of GTEC 2·0 were made and a value of 22·6 Gt(C) for year-1 was established for global canopy (leaf) maintenance respiration. Our estimate of maintenance respiration is between the leaf-area-based estimate (27 Gt(C) year-1) and the dry-mass-based estimate (12 Gt(C) year-1) of Ruimy, Dedieu & Saugier (1996). If we assume that a doubling of $C_a$ will reduce leaf maintenance respiration by 15% (direct effect only) we calculate a 3·4 Gt(C) year-1 increase in the biospheric sink. Independent of the direct effect, if we also assume that the growth of plants ($C_3$ and $C_4$) at elevated $C_a$ will reduced foliar nitrogen concentration by 15 to 19% (Drake, Gonzàlez-Meler & Long 1997a; Cotrufo, Ineson & Scott 1998) this contributes an additional 3·4 to 4·3 Gt(C) year-1 to a potential biospheric sink.

Terrestrial carbon sinks are an important provision of the Kyoto protocol for managing greenhouse gas emissions. Carbon sequestered in forests, for example, will be taken into account in developing emissions reduction strategies; thus, factors that affect the carbon balance of plants will determine the quantity of carbon stored by forest ecosystems. The magnitude of plant respiration is one important factor governing the intrinsic capacity of forests to store carbon. If respiration is likewise reduced by rising atmospheric $C_a$, the quantity of

a carbon stored by forests will be enhanced. This amount of sink enhancement could theoretically offset an equivalent amount of carbon from $CO_2$ emissions, thereby lessening economic impacts associated with other measures.

The inhibition of the respiration observed in leaves and other organs grown in controlled environments also appears in whole ecosystem studies (Table 2). When a reduction of respiration of about 15% was tested for the impact on the global carbon budget, the result indicated that rising $C_a$ may have a significant effect on the global carbon budget.

## STRATEGIC GOALS FOR ASSESSING THE EFFECTS OF RISING $CO_2$ ON RESPIRATION

Robust and versatile prediction of large-scale responses of vegetation to $CO_2$ requires integration of cellular-level knowledge of respiration into a model which can be used for whole plant and for scaling up to ecosystems and models of the global C budget. Empirical models (for example of the McCree type) provide robust descriptions of respiration rate, but they are not versatile or fundamental enough to incorporate the acclimatory or direct effects of elevated $C_a$, and they contain implicit assumptions that are not merited (i.e. that the respiration associated with growth is supply driven and that with maintenance is demand-driven). We need a better model which is based on the biogeochemical regulation of respiration *sensu stricto* by turnover of adenylates and NAD(H) and the supply of carbon skeletons. Its construction will benefit from an understanding of where control over respiration is exercised – where the high flux control coefficients are. It should also incorporate acclimation – of changing the amount of enzymatic and electron transport machinery responsively.

The effects of elevated $C_a$ on respiration cannot be explained solely by its interaction with known targets for elevated $C_a$, namely Rubisco and stomata. One or more additional sites of $CO_2$-sensitivity must exist. Carbamylation of enzymes such a cytochrome *c* oxidase is one possibility, but does not exclude either carbamylation of a range of other proteins or a mechanism that does not involve carbamylation. A mechanistic understanding of such inhibition is an essential part of a systematic understanding of respiration in a high $C_a$ world. We need a programme of experiments at the tissue, mitochondria and enzyme level which fully explore the consequences of known effects of $CO_2$ and search systematically for other mechanisms of $CO_2$ sensitivity. These two goals require fundamental research directed at improving our knowledge of respiration, its regulation and its $CO_2$-sensitivity, independent of how $CO_2$ modulates plant respiration.

There is very convincing evidence that elevated $C_a$ reduces, rather than stimulates, mitochondrial respiration. We are unable to explain this effect as an artifact of measurement nor have we found a mechanism that could be responsible for either of the two phases of the effect. Although some data indicate that two enzymes are inhibited by $CO_2$, we are unable to extrapolate these data to the whole plant owing largely to an incomplete understanding of the control of the rate of respiration by key enzymes. Two likely candidates for a biochemical mechanism, the carbamylation of proteins and the inhibition of enzyme activity by substrate competition, await testing. Similarly, an analysis of the functional aspects of the impact of respiration from the perspective of growth, translocation or maintenance, has produced a tempting but inconclusive picture. Reduction of respiration of foliage by an amount indicated in recent reviews represents an increase in the amount of carbon sequestered in forests as atmospheric $C_a$ rises toward 500 $\mu$mol mol$^{-1}$ in the next century.

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