Chapter 13

Integrated Effects of Atmospheric CO_2 Concentration on Plant and Ecosystem Respiration

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Summary

Atmospheric CO_2 concentrations have been increasing since the industrial revolution due to fossil fuel burning and deforestation. Elevated levels of atmospheric [CO_2] are likely to enhance photosynthesis and plant growth, which, in turn should result in increased specific and whole-plant respiration rates. However, a large body of literature has shown that specific respiration rates of plant tissues can be considerably reduced when plants are exposed to or grown at high [CO_2]. Reductions in respiration by [CO_2] have been explained by either direct inhibitory effects of [CO_2] on respiratory processes or by indirect effects associated with changes in the chemical composition of tissues of plants grown at high [CO_2]. The observed reductions in plant respiration rates by elevated [CO_2] can represent a large biospheric sink for atmospheric carbon. Although doubling current ambient levels of atmospheric [CO_2] could inhibit some mitochondrial enzymes directly in the short-term, the magnitude of the direct effect of [CO_2] on tissue respiration has now been shown to be largely explained by measurement artifacts, diminishing the impact that direct effects would have on the carbon cycle. A reduction in construction and maintenance costs of tissues of plants grown at high [CO_2] can explain an indirect reduction of respiration. Such indirect effects, however, may be offset by the larger biomass of plants exposed to elevated [CO_2]. A lack of clear understanding of the physiological control of plant respiration, of the role(s) of non-phosphorylating pathways, and effects associated with plant size, makes it difficult to predict how respiration and the processes it supports respond to elevated [CO_2]. Therefore, the role of plant respiration in augmenting or controlling the sink capacity of terrestrial ecosystems is still uncertain.

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I. Introduction: Respiration and the Carbon Cycle

Respiration is essential for growth and maintenance of all plant tissues and plays an important role in the carbon balance of individual cells, whole-plants, ecosystems, and the global carbon cycle. Through the processes of respiration, solar energy conserved during photosynthesis and stored as chemical energy in organic molecules is released in a regulated manner for the production of ATP, the universal currency of biological energy transformations, and reducing power (e.g., NADH and NADPH). A quantitatively important by-product of respiration is CO_2, and therefore, plant and ecosystem respiration play a major role in the global carbon cycle.

Terrestrial ecosystems exchange about 120 Gt C per year with the atmosphere, through the processes of photosynthesis and respiration from plants and soils (Schlesinger, 1997). While a large body of literature recognizes the potential consequences on photosynthesis (Drake et al., 1997) considerably less is known about respiratory responses to elevated [CO_2]. Yet, conceptual and theoretical models predict that a small change in global plant respiration could substantially modify the sink capacity of vegetation to fix atmospheric carbon (Drake et al., 1999; Gifford, 2003). It has also been recognized that elevated [CO_2] has two type of effects on plant respiration: i) direct and ii) indirect effects (Gonzàlez-Meler et al., 1996b; Amthor, 1997; Drake et al., 1997). There is, however, little understanding of genetic and biochemical regulators of plant respiratory responses to elevated [CO_2]. This paucity of information is being slowly overcome, but it is still limiting to the development of accurate models to predict long-term effects of elevated [CO_2] on the carbon balance of the terrestrial biosphere.

Roughly, half of the annual photosynthetically fixed CO_2 is released back to the atmosphere by plant respiration (Gifford, 1994; Amthor, 1995). Because terrestrial fluxes of CO_2 between the biosphere and atmosphere far outweigh anthropogenic inputs of CO_2 to the atmosphere, a small change in terrestrial respiration can have a significant impact on the annual increment in atmospheric [CO_2]. A large body of literature has indicated that plant respiration would be reduced in plants grown at high [CO_2]. For example, it is estimated that the observed 15–20% reduction in plant tissue respiration by doubling current atmospheric [CO_2] (Amthor, 1997; Drake et al., 1997; Curtis and Wang, 1998), could increase the sink capacity of global ecosystems by 3.4 Gt of carbon per year (Drake et al., 1999), thus, offsetting an equivalent amount of carbon from anthropogenic CO_2 emissions. Therefore, not only are the gross changes in respiration important for large-scale carbon balance issues, but changes in specific rates of respiration will also have significant impact on basic plant biology such as growth, biomass allocation or nutrient uptake (Amthor, 1991; Wullschleger et al., 1994; Drake et al., 1999). New evidence, however, suggests that the theoretical increase in sink capacity of ecosystems due to reduction in respiration has been overestimated.

II. Effects of CO_2 on Respiration

Amthor (1991) described two different interactions between CO_2 and plant respiration that can be distinguished experimentally: a direct effect, in which the rate of respiration of mitochondria or tissues could be rapidly and reversibly reduced following a rapid increase in CO_2 concentration (Amthor et al., 1992; Amthor, 2000a), and an indirect effect in which high [CO_2] changes the rate of respiration in plant tissues compared to the rate seen for those grown in normal ambient [CO_2], when both are tested at a common background [CO_2] (Azcón-Bieto et al., 1994). Although little effort has been made to distinguish between the direct, reversible effect of elevated [CO_2] and the long-term indirect effect of [CO_2] on respiration, current evidence points to these effects being associated with separate phenomena (Gonzàlez-Meler et al., 1996a; Drake et al., 1999; Jahnke, 2001). Most of the studies on the effects of [CO_2] on respiration rate have focused on the magnitude (from non-significant to 60%) and direction (either stimulation, inhibition or no effect) of these two effects, with little progress in the understanding of the underlying mechanisms. Confounding measurement artifacts are an added complication for establishing the reality and magnitude of direct and indirect effects of [CO_2] on respiration (Gonzàlez-Meler and Siedow, 1999; Jahnke, 2001; Davey et al., 2003).

A. Direct effects

A rapid short-term doubling of current atmospheric CO_2 levels has been reported to inhibit respiration of mitochondria and intact plant tissues by 15–20%,

varying from no effect to more than 50% inhibition (Amthor, 1997; Drake et al., 1997; Curtis and Wang 1998). The reported magnitude of the direct effect on non-woody tissues of woody species, but not wood itself (no studies of a direct effect of $[CO_2]$ on wood have been conducted), is similar to that of herbaceous species (Gonzàlez-Meler and Siedow, 1999). Direct effects of $[CO_2]$ on respiration in trees range from about 60% inhibition in *Castanea sativa* shoots (El Kohen et al. 1991) and *Pinus radiata* fine roots (Ryan et al. 1996) to no inhibition in *Pinus ponderosa* seedlings (Griffin et al. 1996a). The magnitude of the direct effect of $[CO_2]$ on intact tissue and whole-organ respiration has now been show to be largely explained by measurement artifacts (Gonzàlez-Meler and Siedow, 1999; Jahnke, 2001), diminishing the impact that direct effects of [CO2] on respiration would have on plant growth and on the carbon cycle.

The direct effect of doubling current levels in $[CO_2]$ inhibits the oxygen uptake of isolated mitochondria and the activity of mitochondrial enzymes (Gonzàlez-Meler et al., 1996b). Dissolved inorganic carbon can also inhibit certain mitochondrial enzymes, although the $[CO_2]$ at which most of these enzymes are inhibited is higher (over 10000 μmol mol^{-1} $[CO_2]$) than the projected increase in atmospheric $[CO_2]$ (Amthor, 1991; Palet et al., 1992; Gonzàlez-Meler et al., 1996a). Increasing the $[CO_2]$ through the addition of bicarbonate in a reaction medium equivalent to a doubling of the present atmospheric $[CO_2]$, reduces the in vivo activity of cytochrome *c* oxidase and succinate dehydrogenase in mitochondria isolated from *Glycine max L.* cotyledons and roots (Gonzàlez-Meler et al., 1996b) (Fig. 1). In isolated soybean mitochondria, doubling ambient $[CO_2]$ resulted in up to 15% reduction in mitochondrial oxygen uptake (Gonzàlez-Meler et al., 1996b).

Despite the fact that cytochrome oxidase is the primary enzyme of mitochondrial respiration, only up to 50% of total respiratory control (see Kacser and Burns, 1979 for definitions) resides at the level of the mitochondrial electron transport chain (Gonzàlez-Meler and Siedow, 1999; Affourfit et al., 2001). Accordingly, Gonzàlez-Meler and Siedow (1999) argued that direct effects of $[CO_2]$ on respiration exceeding more than 10% were likely due to factors other than inhibition of mitochondrial enzymes. Amthor (1997) pointed out that elevated $[CO_2]$ could increase dark CO_2 fixation catalyzed by phosphoenolpyruvate carboxylase (PEPC), resulting in an apparent reduction of net CO_2 efflux. However, Amthor et al. (2001)

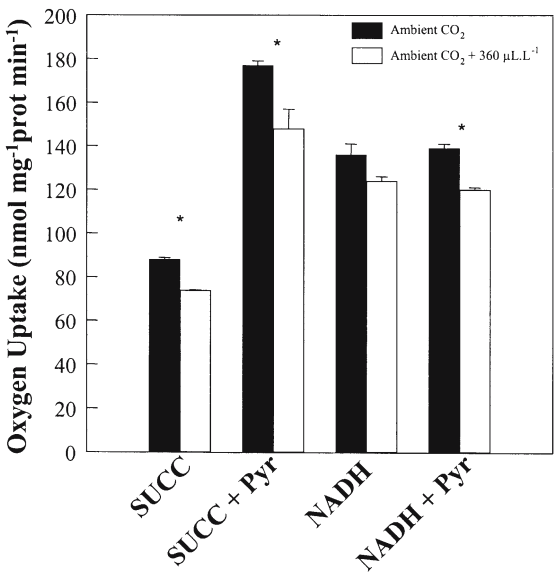

Fig. 1. The direct effect of elevated $[CO_2]$ on soybean cotyledon mitochondrial respiration. Oxidation of either succinate (SUCC), NADH and pyruvate (NADH+PYR), NADH alone (NADH) or succinate and NADH (SUCC+NADH) were measured in the presence of ADP (state 3 conditions). Mitochondria were incubated for 10 min with 0 (open bars) or 0.1 (solid bars) mM Dissolved Inorganic Carbon (DIC) at 25 °C. Values are mean ± SE of 3 to 9 replicates and * indicates a significant difference in the mean ($p<0.05$) using a Student's t test or a Rank Summary test. Modified from Gonzàlez-Meler et al. (1996b).

showed inconsistent small reduction in CO_2 efflux compared to unaltered O_2 uptake rate when CO_2 levels were increased by 300μmol mol^{-1}, suggesting a small effect of rising atmospheric levels of $[CO_2]$ on PEPC activity. Similar results were found for a variety of species, involving 600 measurements, in which CO_2 concentration increases did not alter the CO_2 and O_2 exchanges in the dark (Davey et al., 2003).

As mentioned above, recent evidence indicates that in studying direct effects of CO_2 on respiration, investigators should first be concerned about measurement artifacts. Amthor (1997) and Gonzàlez-Meler and Siedow (1999) showed that gas CO_2 exchange measurement errors could augment and explain the magnitude of the direct effect. Since then, new studies made in this new context have shown that respiration rates are little or not at all inhibited by a doubling of atmospheric $[CO_2]$ (Amthor, 2000; Amthor et al., 2001; Bunce 2001; Tjoelker et al., 2001; Bruhn et al., 2002; Hamilton et al., 2002). Indeed, Jahnke (2001) and Jahnke and Krewitt (2002) confirmed that measurement artifacts due to leakage in CO_2-exchange

systems could be as large as the previously reported direct inhibitory effects. They also found the leaks through the intercellular spaces of homobaric leaves will show a significant apparent inhibition of CO_2 efflux that is not due to an inhibition of $[CO_2]$ on respiration (Fig. 2). As such, early conclusions on the impact of direct effects of $[CO_2]$ on plant respiration on the global carbon cycle have been overstated (Gonzàlez-Meler et al., 1996a;Amthor, 1997; Drake et al., 1999).

Gas leaks through gaskets and associated with CO_2 sorption to tubing and other surfaces of the gas-exchange equipment (Jahnke, 2001) represent a complication of gas-exchange techniques than can affect both photosynthesis and respiration measurements. Corrections for these types of leaks can be applied in most cases (Pons and Welschen, 2002). However, leaks occurring through connected leaf air spaces cannot easily be corrected, and measurements in these cases can only be done when the entire leaf is exposed to the same $[CO_2]$ (Jahnke and Krewit, 2001). Applying some corrections for gas exchange leaks, Bunce (2001) reported a significant reduction in respiration after a rapid increase in $[CO_2]$, so not all reports of direct inhibition of respiration by elevated $[CO_2]$ have yet been reconciled with each other.

The observations of Jahnke (2001) and Jahnke and Krewitt (2002) are indications that direct effects of $[CO_2]$ on mitochondrial enzymes may have no consequence on the specific respiratory rate of intact tissues. Gonzàlez-Meler and Siedow (1999) provided two potential mechanisms by which inhibition of enzymes by $[CO_2]$ are not seen at the tissue level: 1) mitochondrial enzymes are 'in excess' of the levels

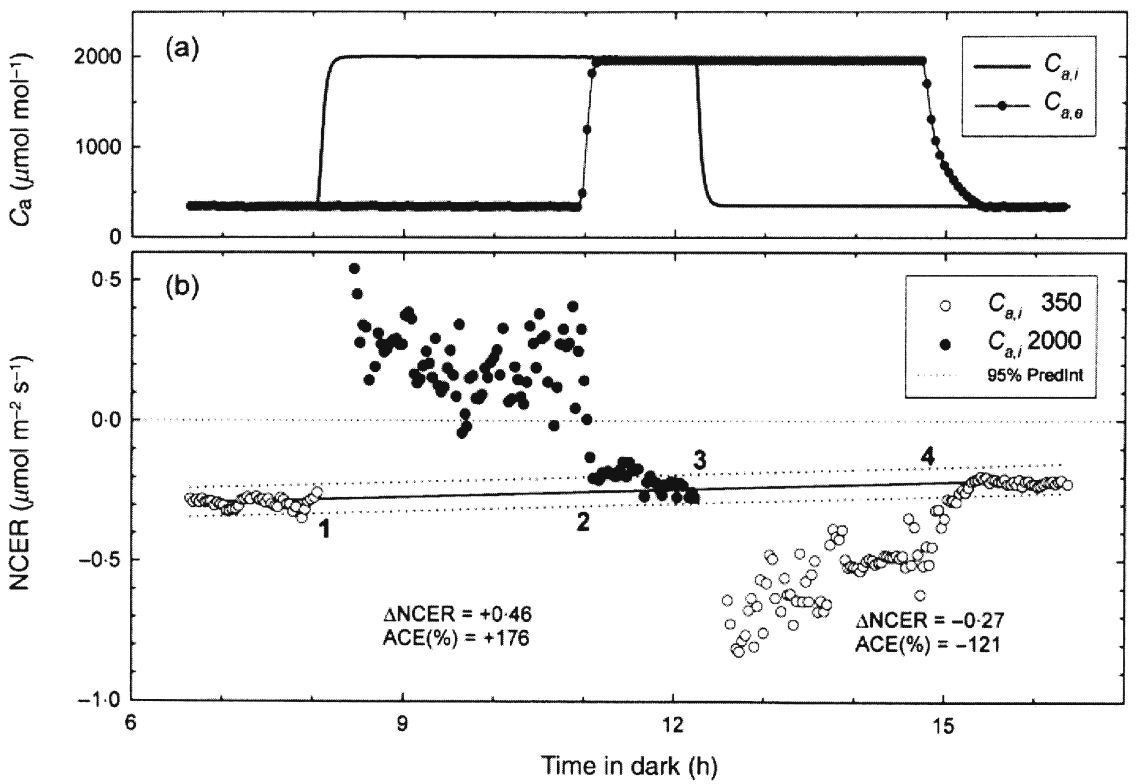

Fig. 2. Measurement artifacts are responsible for apparent direct effects of $[CO_2]$ on tissue respiration. Homobaric leaves of *Nicotiana tabacum* L equilibrate $[CO_2]$ leaf air spaces of the part of the leaf inside the measurement cuvette with that outside. When the CO_2 concentration inside the cuvette ($C_{a,i}$) increases with respect to the outside ($C_{a,e}$), CO_2 moves from the inside (high $[CO_2]$) to the outside part of the leaf (a), showing an apparent decline in the net carbon exchange rate (NCER) (b). When the whole leaf (inside and outside the measurement cuvette) is exposed to the same $[CO_2]$, no effects of changing $[CO_2]$ are seen on the leaf respiratory rate. $C_{a,i}$ was increased from 350 to 2000 μmol mol^{-1} at time mark 1 and $C_{a,e}$ at time mark 2. $C_{a,i}$ was lowered again to 350 μmol mol^{-1} at time mark 3 and $C_{a,e}$ at time mark 4. Figure 6 from Jahnke and Kewit (2002), published in Plant Cell & Environment and used with the authors' and Blackwell Publishing permission.

required to support normal tissue respiratory activity, and 2) a compensating increase in the activity of the alternative pathway upon inhibition of cytochrome oxidase by $[CO_2]$ will result in unaltered dark respiratory activity. The former mechanism implies that the control coefficient levels of mitochondrial enzymes are very low, and therefore inhibition of enzymatic activity by $[CO_2]$ will have no consequences for the overall tissue respiration rate. Interestingly, recent reports have shown that the number of mitochondria increases in leaves of plants grown at high $[CO_2]$ with no linear changes in the leaf respiratory rate (Griffin et al., 2001; see below). Hence, if the mitochondrial machinery increases in plants grown at high $[CO_2]$ with no changes in respiration rate, then the levels of cytochrome oxidase will presumably be in even more exceeding amounts than in plants grown at ambient $[CO_2]$ for any direct effect of $[CO_2]$ on mitochondrial enzyme activity to affect tissue respiration rate. In this context, it is also important to recognize that the experiments of Jahnke (2001) and Jahnke and Krewitt (2002) were done in tissues exposed to prolonged nights (up to 72 hours), so consequences of inhibition of cytochrome oxidase by $[CO_2]$ on the overall tissue respiration rate will not be expected either, because of an excess cytochrome oxidase enzyme with respect to the respiratory rate.

The latter mechanism, i.e. a compensation by the alternative path, proposed by Gonzàlez-Meler and Siedow (1999) is based upon the competitive nature of the cytochrome and alternative pathways of plant mitochondrial respiration (Chapter 1, Lambers et al.). The activity of the alternative pathway could increase upon a doubling of the $[CO_2]$, masking the direct CO_2 inhibition of the cytochrome pathway (Fig. 3). The oxygen-isotope technique (Chapter 3, Ribas-Carbo et al.) allows for the distinction of direct effects of $[CO_2]$ on the interplay between the cytochrome and the alternative oxidase of plant respiration. Figure 3 shows that mitochondrial electron transport activity is affected when CO_2 (a mild inhibitor) restricts the normal electron flow through one of the pathways (Cyt pathway – v_{cyt}). Under conditions where the alternative pathway activity is low (see Ribas-Carbo et al., 1995 for details), inhibition of the cytochrome pathway by doubling the ambient $[CO_2]$ (18%) is compensated by a similar increase in the activity of the alternative pathway (v_{alt}), resulting in no significant reduction in the overall oxygen uptake of the isolated mitochondria. These results show that increased activity of the alternative pathway upon

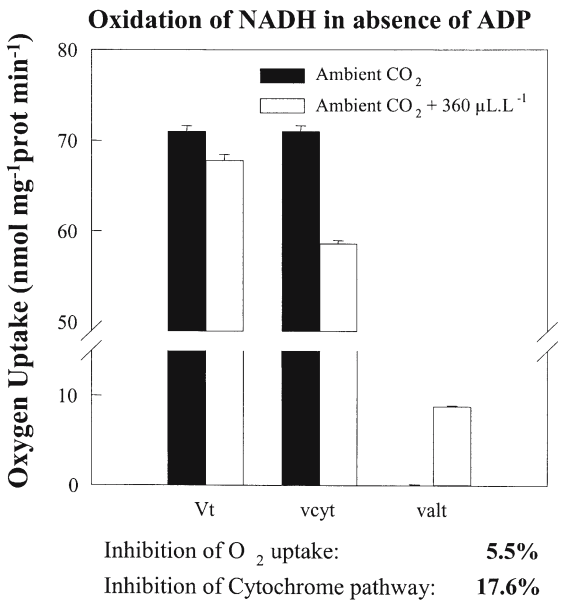

Fig. 3. Effect of doubling the ambient concentration of CO_2 on the oxygen uptake of 5-day old isolated soybean cotyledon mitochondria in state 4 conditions (i.e. ADP-limiting conditions). Experiments were done using the oxygen-isotope technique as described in Ribas-Carbo et al. (1995) and Chapter 3, Ribas-Carbo et al.) applying the treatments as for Figure 1. Results are average of four replicates; bars show standard errors (Gonzàlez-Meler, unpublished observations).

addition of CO_2 can compensate for the direct CO_2 inhibition of the cytochrome oxidase in isolated mitochondria. Although there is no indication that this mechanism is operative in intact tissues of plants exposed to rapid changes in $[CO_2]$ (for reasons exposed above; Gonzàlez-Meler and Siedow, 1999), it has been speculated that increased activity of the non-phosphorylating alternative pathway could be responsible for the altered growth characteristics of plants exposed to elevated $[CO_2]$ only during the nighttime (Reuveni and Gale, 1995, 1997; Griffin et al., 1999, Bunce, 1995, 2001, 2002).

Bunce (1995) observed that the biomass of *Glycine max* L. increased when nighttime CO_2 was elevated. Leaf area ratio increased and photosynthetic rates decreased in plants exposed to high nighttime $[CO_2]$ when compared to plants grown at normal ambient CO_2 all day. It was suggested that the direct inhibitory effect of $[CO_2]$ on leaf respiration caused the alterations in plant development. Also in *Glycine max*, Griffin et al. (1999) found that plants exposed to high nighttime $[CO_2]$ had slower leaf respiration rates and greater biomass than plants grown at normal

or elevated levels of [CO_2]. Reuveni et al. (1997) speculated that increases in biomass of *Lemna gibba* grown at high nighttime [CO_2] compared with plants grown at ambient [CO_2], were due to a reduction in the activity of the alternative pathway. Reduction in alternative pathway activity would couple respiration rates with tissue growth and maintenance, enhancing growth. Ziska and Bunce (1999) showed that elevation of nighttime [CO_2] concentration reduced biomass in two of the four C_4 species studied. In the case of *Zea maize* L. leaf, stem and root biomass were significantly reduced by high nighttime [CO_2] (Ziska and Bunce, 1999). In view of the lack of evidence for direct effects of [CO_2] on tissue or whole-plant respiration, it is unlikely that altered leaf respiration rates in plants exposed to high nighttime [CO_2] are the cause of the observed changes in plant growth characteristics. In a later study, Bunce (2002) described that carbohydrate translocation was reduced within two days of exposing plants to elevated nighttime [CO_2] when compared with plants grown at ambient conditions day and night. These results suggest that elevated [CO_2] may have other non-described direct effects of [CO_2] on the plant's physiology that may reduce energy demand for carbohydrate translocation, hence reducing the rate of leaf respiration. If so, these new types of effects cannot be catalogued as direct effects of [CO_2] on respiration (see above), but as indirect effects.

In summary, although rapid changes in [CO_2] can inhibit mitochondrial enzymes directly, previously reported direct effects of [CO_2] on tissue respiration are likely measurement artifacts. Therefore, it would appear that there are no direct effects of [CO_2] on respiration that have any impact on the amount of anthropogenic carbon that vegetation could retain. The fact that effects of [CO_2] on mitochondrial enzymes do not scale to tissue or plant level shows a lack of understanding on how respiration of tissues is regulated. Finally, the role of the alternative pathway in direct respiratory responses to elevated [CO_2], although unresolved, would have little impact, if any, on the general conclusion that direct effects of [CO_2] on plant respiration are not to be considered in plant growth or carbon cycle models.

B. Indirect and Acclimation Effects

The indirect effects represent changes of tissue respiration in response to elevated atmospheric [CO_2], and involve effects on tissue composition, especially carbohydrate accumulation in green tissues and reduced tissue [N] which is a reflection of the reduction of protein content (Drake et al., 1997). Other indirect effects are related to the effects of [CO_2] on growth, response to environmental stress, and other factors that may alter the respiratory demand for energy when compared with plants grown at ambient [CO_2] (Bunce, 1994; Amthor, 2000b). Acclimation of respiration has also been observed in photosynthetic tissues of some plants grown at elevated [CO_2] and represents the downregulation or upregulation of the respiratory machinery (i.e. amounts of cytochrome oxidase, number of mitochondria) irrespective of changes in specific respiration rates (Azcón-Bieto et al., 1994; Griffin et al., 2001). Indirect effects caused by elevated [CO_2] can be measured as reduction in CO_2 emission (or O_2 consumption) from tissues at a common background [CO_2] (Gonzàlez-Meler et al., 1996a).

As atmospheric [CO_2] rises, increased photosynthesis results in higher cellular carbohydrate concentrations (Drake et al., 1997; Curtis and Wang, 1998). Increased carbohydrate concentrations can stimulate the activity of specific dark respiration in the short-term, due to greater availability of respiratory substrates. This happens when photosynthesis is stimulated by increasing light intensity (Azcón-Bieto and Osmond, 1983) or tissues are fed exogenous sucrose (Azcón-Bieto et al., 1983). Increased carbohydrate levels should also increase the respiratory energy demand to support phloem loading and translocation of carbohydrates (Bouma et al., 1995; Amthor, 2000b). Increased carbohydrate content has also been shown to regulate the levels of cytochrome oxidase (Felitti and Gonzalez, 1998). Recent experimental evidence suggests that although carbohydrate levels are increased by elevated [CO_2], specific and whole plant respiration in many plants is reduced (Poorter et al., 1992; Amthor, 1997; Drake et al., 1997, but see below).

It has been generally accepted that leaf respiration is usually reduced as a consequence of plant growth at elevated [CO_2] (Amthor, 1997; Drake et al., 1997; Curtis and Wang, 1998; Norby et al., 1999). Early reports showed that leaf respiration is decreased at high [CO_2] under field or laboratory conditions (El Kohen et al., 1991; Idso and Kimball, 1992; Wullschleger at al., 1992a). Poorter et al. (1992) showed that leaf respiration was reduced, on average, by 14% when expressed on a leaf mass basis, but increased by 16% on a leaf area basis. More recently, Curtis and Wang

(1998) compiled respiratory data for woody plants, and observed that growth at elevated [CO_2] resulted in an 18% inhibition of overall leaf respiration (mass basis). However, many of the data compiled in these two studies compared respiratory rates of plants grown and measured at ambient conditions with plants grown and measured at elevated [CO_2]. These respiratory measurements are also affected by the measurement artifacts described above (Fig. 2). Leaks should not be important when respiratory rates are measured at a common [CO_2] for plants grown at ambient and elevated [CO_2]. An analysis of the literature focused on leaf respiratory responses (on a leaf mass basis) of plants grown at ambient and elevated [CO_2] when respiration rates were only measured at a common [CO_2] suggests that specific leaf respiration rates will be unaltered in plants grown at elevated [CO_2] (Table 1). Therefore, the generally accepted conclusion that respiration of plants grown at elevated [CO_2] will be reduced when compared with plants grown at ambient [CO_2] should be reevaluated. However, there is significant variability in the leaf respiratory response to growth at elevated [CO_2] when compared with plants grown at ambient conditions, ranging from 40% inhibition (Azcón-Bieto et al., 1994) to 50% stimulation (Williams et al., 1992). Considerations on the physiological basis by which the acclimation response of respiration to elevated [CO_2] varies, is considered next.

In boreal species, reduction of respiration of plants grown at high [CO_2] was related to changes in tissue N and carbohydrate concentration (Tjoelker et al., 1999). Tissue N concentration often decreases in plants grown at elevated [CO_2] (Drake et al., 1997; Curtis and Wang, 1998). It is expected that respiration rate would be lower in tissues having lower [N], because the respiratory cost associated with protein turnover and maintenance is a large portion of dark respiration (Bouma et al., 1994; Chapter 10, Bouma). Hence, the metabolic cost (i.e. respiratory energy demand) for construction and maintenance of tissues with high concentrations of protein (high [N]) is greater than the cost for the maintenance of the same tissue with low [N] (assuming no changes in rates of protein turnover between plants grown at ambient and elevated [CO_2]) (Amthor, 1989; Drake et al., 1999). This idea was confirmed for leaves of *Quercus alba* seedlings grown in open-top chambers in the field, where respiration was 21 to 56% lower in elevated [CO_2] than in normal ambient [CO_2] (Wullschleger and Norby, 1992). The growth respiration component of these leaves was reduced by 31% and the maintenance component by 45%. These effects were attributed to the reduced cost of maintaining tissues having lower nitrogen concentrations. Similar results were obtained in leaves and stems of plants of other species (Amthor et al., 1994; Carey et al., 1996; Dvorak and Oplustilova, 1997; Griffin et al., 1996b; Will and Ceulemans, 1997; Wullschleger et al., 1992a,b; 1997), with some exceptions (Wullschleger et al., 1995).

Plants grown at elevated [CO_2] reduce their leaf protein content by 15% on average (Drake et al., 1997). Most of this reduction is attributed to decreases in photosynthetic proteins, and little is known about changes in respiratory proteins of plants grown at elevated [CO_2]. Indirect effects of respiration to elevated [CO_2] on leaves of *Lindera benzoin* were also correlated with a reduction in maximum activity of cytochrome oxidase (Azcón-Bieto et al., 1994). This effect would represent an acclimation response of respiration to elevated [CO_2] analogous to that seen in photosynthesis (Drake et al., 1997). However, reduction of respiratory enzyme activity was not seen in rapidly growing tissues exposed to elevated [CO_2] (Hrubeck et al., 1985; Perez-Trejo, 1981). The general increase in number of mitochondria seen in leaves of adult *Pinus taeda* trees grown at high [CO_2] (Griffin et al., 2001) contrast with the reduction in maximum enzyme activity previously reported for other plants (Azcón-Bieto et al., 1994). No acclimation effect to elevated CO_2 (i.e. reduction in either respiration or

Table 1. Indirect effects of long-term CO_2 enrichment on respiration of leaves on a dry mass basis. Elevated-over-ambient (E/A) refers to the ratio of rate of leaf dark respiration of plants grown in elevated [CO_2] to the rate of plants grown in current ambient CO_2 when measured at a common CO_2 concentration. Under these conditions, effects of gas exchange leaks should be minimal in affecting the comparison of rates of respiration from plants grown at ambient and elevated CO_2.

Reference	E/A	Number of species	Observations
Amthor, 1997	0.96	21	Compiled 26 studies, crop and herbaceous and woody wild species
Davey et al., 2003	1.07	7	Original study, crop and herbaceous and woody wild species
Drake et al., 1997	0.95	17	Compiled 15 studies, crop and herbaceous wild species

cytochrome oxidase) has been observed in leaves of C_4 plants (Azcón-Bieto et al., 1994) or roots of C_3 plants (Gonzàlez-Meler, 1995). With the exception of a few studies (i.e. Azcón-Bieto et al., 1994), there are no reports on the response of membrane-associated mitochondrial enzymes to elevated [CO_2] in leaves or roots of plants. There are, however, studies showing an increased count of mitochondria in leaves of plants grown at elevated [CO_2] (Griffin et al., 2001), which may represent upregulation of mitochondrial enzymes under elevated [CO_2]. The observed increase in mitochondrial number in the study of Griffin et al., (2001), however, had no concomitant increases in leaf respiration. More research is needed in this area.

III. Growth Consequences of the Effects of [CO_2] on Respiration: A Case Study

The fact that respiration does not increase in plants grown at elevated [CO_2] (Table 1) raises a fundamental question: if respiration is important for growth and maintenance of plants, how can unaltered respiration support the increased plant productivity seen when plants are exposed to elevated [CO_2]? In some species, particularly in young, growing tissues, increased dark respiration has been shown to accompany rapid growth in the first developmental stages (see Drake et al., 1999 for references). As tissues reach maturity, respiration slows as relative growth rate (RGR) declines, because of the positive linear relationship between dark respiration per unit of mass and RGR (Hesketh et al., 1971; Amthor, 1989). Elevated [CO_2] enhances growth rates enough to show a significant increase in biomass at the end of the growing season (Kimball et al., 1993; Delucia et al., 1999; Norby et al., 2002; Karnosky et al., 2003). This relationship is also seen in CO_2 studies where faster respiration rates seem to follow a stimulation of photosynthesis by elevated [CO_2] as a result of a transient increase in RGR (see above; Amthor, 2000b; Bunce, 1994). Any factor uncoupling respiration and growth would compromise the supply of energy needed to sustain biosynthesis, and overall growth could be reduced.

The growth component of respiration (Chapter 10, Bouma) could also be reduced in plants grown at elevated [CO_2] as a result of altered tissue chemistry (Griffin et al., 1993). Based on the chemical composition of tissues, Poorter et al. (1997) found that elevated [CO_2] could reduce growth construction costs by 10–20%. Griffin et al. (1993; 1996a) observed reductions in construction costs of *Pinus taeda* seedlings grown at elevated [CO_2]. Hamilton et al. (2001) reported that elevated [CO_2] slightly reduced construction costs of leaves of mature trees (including *P. taeda*) at the top of the canopy, but not at the bottom of the canopy. Such a small reduction could be explained by reductions in tissue [N], as observed in leaves exposed to [CO_2] at the top of the canopy. Changes in construction costs did not result in a decrease in the leaf respiration rates of trees exposed to elevated [CO_2] (Hamilton et al., 2001).

The lack of long-term effects of increased [CO_2] on specific plant respiration rates could also be due to a lower involvement of the alternative pathway (Gonzàlez-Meler and Siedow, 1999; Griffin et al., 1999). Respiration through the alternative pathway bypasses two of the three sites of proton translocation; so the free energy released is lost as heat, and is unavailable for the synthesis of ATP. Respiration associated with this pathway will not support growth and maintenance processes of tissues as efficiently as respiration through the cytochrome path. The activity of the alternative pathway of respiration could decrease upon doubling [CO_2], masking increases in the activity of the cytochrome pathway. If this were the case, unaltered respiration rate in plants grown at high [CO_2] could more efficiently support growth and maintenance processes. On the contrary, excess carbohydrate levels often seen in plants grown at elevated [CO_2] could trigger the activity of the alternative pathway (see chapter 5, Noguchi), although correlations between leaf carbohydrate levels and alternative pathway activity have not been clearly demonstrated (Gonzàlez-Meler et al., 2001). It is important to determine whether inhibitory and/or stimulatory effects of [CO_2] on dark respiration have beneficial (by decreasing carbon losses) or detrimental (by reducing ATP yields per unit of N, see Gonzàlez-Meler et al., 2001) effects on overall plant biomass and allocation to different plant parts.

The oxygen-isotope technique allows for the distinction of [CO_2] effects between the cytochrome and the alternative oxidase of respiration in plants grown at ambient and elevated [CO_2]. Figure 4 illustrates the combined effects of [CO_2] on respiration on a shade-tolerant species *Cornus florida* L. and a shade-intolerant species *Liriodendron tulipifera* L. (Burns and Honkala, 1990). Although [CO_2] did not seem to affect leaf specific respiration rates, oxygen-fractionation data revealed that the growth [CO_2] environment induced important physiological

changes at the leaf level. Despite the small effect of elevated [CO_2] on respiratory CO_2 efflux, oxygen-isotope fractionation by respiration increased in plants grown at elevated [CO_2] in both shade-tolerant and shade-intolerant plants. Oxygen-isotope fractionation increased from 21.2 ‰ to 22.8 ‰ in *L. tulipifera*, and from 21.7 ‰ to 23.0 ‰ in *C. florida*. An increase in oxygen-isotope fractionation implies that the activity of the non-phosphorylating alternative pathway of respiration increases. Interestingly, ATP yields of respiration were not reduced in *C. florida* plants grown in elevated [CO_2] when compared with the plants grown at ambient [CO_2] **(Fig. 4). How**ever, *L. tulipifera* grown at high [CO_2] reduced ATP yields by 30% when compared with the plants grown at ambient [CO_2] (Fig. 4), as a consequence of a strong inhibition of the cytochrome pathway. If reduction in ATP production is maintained over time in these plants grown at elevated [CO_2], growth may be reduced. Interestingly, annual growth (measured as stem diameter increase) of *C. florida* grown at high [CO_2] increased by 10%, whereas growth of *L. tulipifera* at high [CO_2] was reduced by 35% when compared with control plants (J. Mohan, unpublished). More research is needed to establish the linkages between changes in biomass growth and the altered respiratory metabolism (Fig. 4) in response to [CO_2].

IV. Integrated Effects of Elevated [CO_2] on Respiration at the Ecosystem Level

A. Ecosystem Respiration

Terrestrial ecosystems exchange about 120 Gt C per year with the atmosphere, through the processes of photosynthesis (leading to gross primary production, GPP) and ecosystem respiration (Re) (Schlesinger, 1997). The difference between GPP and Re determines net ecosystem productivity (NEP), the net amount of carbon retained or released by a given ecosystem. An increasing body of evidence derived form direct measurements of net ecosystem exchange (NEE; CO_2 exchange between terrestrial ecosystems and the atmosphere) shows that, in general, the photosynthetic gain of carbon exceeds respiratory losses for a variety of ecosystems (Grace et al., 1995; Katul et al., 1997; 1999; Buchmann and Schultze, 1999; Luo et al., 2000). Currently, the net exchange of C between the terrestrial biosphere and the atmosphere is estimated to result in a global terrestrial sink of about 2 Gt C per year (Gifford, 1994; Schimel, 1995; Steffen et al., 1998). A significant effort has been made to identify the long-term effects of elevated [CO_2] on canopy photosynthesis and ecosystem growth. Unfortunately, the effects of [CO_2] on autotrophic and heterotrophic respiration at the ecosystem level are

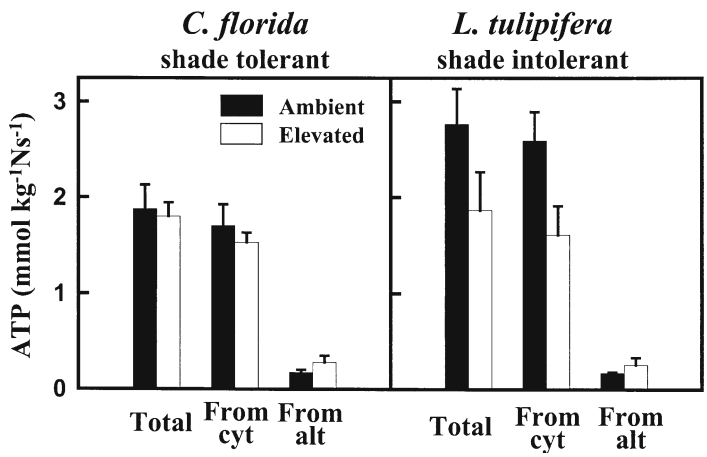

Fig. 4. Respiratory rate of ATP production per unit of tissue N in leaves of a shade-tolerant (*Cornus florida* L.) and a shade-intolerant (*Liquidambar tulipifera* L.) species grown at ambient and at ambient + 200 μmol mol^{-1} [CO_2] in the Duke FACE site. Rates of ATP production were calculated from the activities of the cytochrome and alternative pathways, following Gonzàlez-Meler at al. (2001). Total ATP production values are the addition of the ATP synthesis rates derived from the cytochrome (v_{cyt}) and the alternative (v_{alt}) activity. Rates of total oxygen uptake (in μmol kg^{-1} DM s^{-1}) for plants grown at ambient and elevated [CO_2] were 7.6±1.6 and 8.8±0.5 for *C. florida*, and 10.7±2.1 and 10.2±1.0 for *L. tulipifera,* respectively. Details on plant growing conditions and CO_2 treatment can be found in DeLucia et al. (1999) and Naumberg and Ellsworth (2000). Data are means and SE for three replicates (Gonzàlez-Meler, unpublished).

not known or well understood, despite their potential to control ecosystem carbon budgets (Ryan, 1991; Giardina and Ryan, 2000; Valentini et al., 2000).

Terrestrial plant respiration releases 40 to 60% of the total carbon fixed during photosynthesis (Gifford, 1994; Amthor, 1995) representing about half of the annual input of CO_2 to the atmosphere from terrestrial ecosystems (Schlesinger, 1997). Therefore the magnitude of terrestrial plant respiration and its responses to $[CO_2]$ are important factors governing the intrinsic capacity of ecosystems to store carbon. How plant respiration will operate in a future, high-CO_2 world requires mechanistic quantification. Plant respiration responses to high $[CO_2]$ may result from two distinct mechanisms: 1) indirect effects, and 2) changes in total plant biomass. If it is confirmed that the response of terrestrial plant respiration to an increase in atmospheric $[CO_2]$ is small (Table 1), then changes in global plant respiration should be proportional to changes in biomass. Therefore, plant respiration probably plays a small role in determining the biomass sink capacity (NPP) of global ecosystems to retain part of the anthropogenic CO_2 emissions in vegetation (Amthor, 1997; Drake et al., 1999). However, evidence suggests that in response to elevated $[CO_2]$, plant respiration at the ecosystem level does not necessarily increase with increases in total plant biomass (Drake et al., 1996; Hamilton et al., 2002).

Attempts to scale $[CO_2]$ effects on mitochondrial or tissue respiration to the ecosystem level are problematic, because, unlike photosynthesis, little is known about applicable scaling rules for plant respiration (Gifford, 2003). Attempts to build respiratory carbon budgets at the canopy level require knowledge on maintenance and growth respiration, and tissue respiratory responses to light, temperature and $[CO_2]$ (Amthor 2000b; Gifford 2003). Hamilton et al. (2002) built a model of the carbon balance of a pine-dominated forest exposed to ambient and elevated $[CO_2]$. Elevated $[CO_2]$ increased forest NPP by 27% without any increase in total plant ecosystem respiration, suggesting that the rates of specific respiration were actually decreased in tissues of plants grown at elevated $[CO_2]$. Open-top chambers allow for the measurement of canopy respiration at the ecosystem level. Table 2 shows the contribution of CO_2-induced changes in ecosystem respiration to annual NEP of a salt marsh exposed to twice ambient $[CO_2]$. In this ecosystem, elevated CO_2 consistently reduced nighttime ecosystem respiration in C_3 and C_4 community stands (Drake et al., 1996). Inhibition of ecosystem respiration by elevated $[CO_2]$ represented a substantial one fifth to one third of the total extra annual carbon gain (NEP) observed between canopies grown at ambient and elevated $[CO_2]$ (Table 2). Acclimation and other indirect effects of $[CO_2]$ on plant and soil respiration to elevated $[CO_2]$ (Azcón-Bieto et al., 1994; Drake et al., 1996) may explain this consistent reduction in ecosystem respiration.

B. Root and Soil Respiration

The largest C pool on land is in soils, with 2.5 times more C in the top meter of soil than is found in terrestrial vegetation (Schlesinger, 1997). The efflux of CO_2 from the soil occurs through the process of soil respiration, which is estimated to be around 68 to 77 Gt C/yr (Raich and Schlesinger, 1992; Raich and Potter, 1995). Soil respiration is higher than global estimates of NPP and litter production (Matthews, 1997; Field et al., 1998), because it includes respiration from autotrophs (roots) and heterotrophs. Changes in plant physiological activity strongly influence respiration from soils (Schlesinger, 1997; Högberg et al., 2001).

Soil respiration is the result of both autotrophic and heterotrophic below-ground processes, including root respiration and respiration associated with the decomposition of soil organic matter by soil microorganisms. Photosynthetic C uptake is stimulated by elevated $[CO_2]$ (DeLucia et al., 1999; Norby et al., 2002), and enhanced growth at elevated $[CO_2]$ would contribute to increased carbon inputs into the soil through more litter fall and greater root biomass production and turnover (Allen et al., 2000; Matamala and Schlesinger, 2000; King et al., 2001). However, increased C inputs into the soil do not necessarily lead to greater soil C storage (Schlesinger and Lichter, 2001) because elevated $[CO_2]$ has been shown, almost universally, to increase soil respiration rates (Zak et al., 2000; Table 3). Many studies have reported increased root growth under elevated $[CO_2]$ (Johnson et al., 1994; Vose et al., 1995; Hungate et al., 1997; Edwards and Norby, 1999; Matamala and Schlesinger, 2000; Pregitzer et al., 2000; King et al., 2001) which has been correlated with faster soil respiration rates. Greater plant C allocation below ground can result in faster soil respiration rates by 1) increasing the contribution of root respiration to total soil respiration because of greater root biomass relative to ambient $[CO_2]$, or 2) increasing the labile

Table 2. Contribution of CO_2-induced reduction in ecosystem respiration (Re) to total net ecosystem productivity (NEP) in a C_3-dominated salt marsh ecosystem exposed to elevated [CO_2] since 1988. Relative change in CO_2 stimulation was calculated form the annual net ecosystem exchange (NCE) canopy flux at plots exposed to elevated [CO_2] over that of plants growing at ambient [CO_2] (n=5). Modified from Drake et al. (1996) and Gonzàlez-Meler et al. (1995).

Year	NCE	Re	NEP	% NEP gain from change in Re
		% CO_2 stimulation		
1994	31	–57	66	33

Table 3. Percent stimulation of soil respiration in different natural ecosystems by elevated [CO_2] relative to ambient [CO_2] conditions. The table shows the dominant species or ecosystem type in the study, % stimulation of soil respiration (calculated as [(rate at elevated/rate at ambient)*100]), the level of CO_2 enrichment over the ambient CO_2 concentration and the reference.

Species	E/A*100 %	CO_2 treatment	Reference
Acer rubrum	27	+350	Edwards and Norby, 1999
Acer rubrum	15	+350 + 4 °C	Edwards and Norby, 1999
Acer saccharum	5	+350	Edwards and Norby, 1999
Acer saccharum	26	+350 + 4 °C	Edwards and Norby, 1999
Pseudotsuga menziesii	20	+200	Lin et al., 2001
Pseudotsuga menziesii	54	+200 + 4 °C	Lin et al., 2001
Pinus ponderosa	74	+350	Vose et al.,1995
Lindera benzoin	50	+340	Ball et al., 2000
Populus tremuloides	30	+200	Karnosky et al., 2003
P. tremuloides/B. papyrifera	60	+200	Karnosky et al., 2003
P. tremuloides/A. saccharum	10	+200	Karnosky et al., 2003
Pinus taeda			
Dry year	23	+200	Taneva et al., unpublished*
Wet year	12	+200	Taneva et al., unpublished*
Short-grass steppe			
Dry year	85	+360	Pendall et al., 2003
Wet year	25	+360	Pendall et al., 2003
Wetland	15	+340	Ball and Drake, 1995
California grassland	36	+360	Hungate et al., 1997

soil C pool through greater root exudation and fine root turnover, and 3) priming effect. Therefore, long-term soil C sequestration requires that a substantial proportion of the additional C assimilated by plants growing at elevated [CO_2] is allocated to roots and soil C pools that turn over slowly. Little is known about the physiological mechanisms leading to C accumulation in soils.

Although elevated [CO_2] may result in an increased transfer of C to the root and soil pool, other indirect effects associated with elevated [CO_2] or global warming may stimulate root and soil respiration (Schimel et al., 1994; Schlesinger and Andrews, 2000; but see Giardina and Ryan, 2000). For instance, Lin et al. (2001) reported that total soil respiration rates under *Pseudotsuga menziesii* grown in mesocosms were stimulated by 20% by [CO_2] enrichment; the combined effect of elevated [CO_2] and higher temperature, however, increased soil respiration rates by 54% (Table 3). In addition, the authors found that elevated [CO_2] primarily stimulated root respiration and root exudation, whereas elevated temperature had a stronger effect on decomposition of soil organic matter.

In addition to elevated [CO_2] and warming, climate change may include shifts in rainfall patterns.

Soil moisture content affects the response of soil respiration to elevated [CO_2] (Fig. 5). The percent stimulation of soil respiration rates by exposure to elevated [CO_2] in a *Pinus taeda*-dominated forest in North Carolina, USA, was greater during a dry growing season compared with soil respiration rates during a wet growing season (Fig. 5; L.Taneva et al., unpublished). Results shown in Fig. 5 indicate that during the wet year, decomposition of old C (uncoupled from recent plant activity) makes up a larger proportion of soil respiration than that under dry conditions, suggesting that respiration from older soil C pools is more sensitive to soil moisture stress than that from recent C pools, including root and rhizosphere respiration. Similarly, Pendall et al. (2003) reported that, although soil respiration rates were enhanced by elevated [CO_2] in a short-grass steppe ecosystem, the degree of stimulation largely depended on soil moisture content.

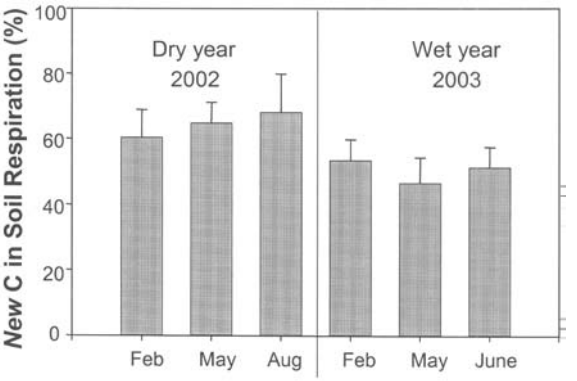

Fig. 5. Respiration of newly fixed C, as a percentage of total soil respiration (R_s), calculated with a $\delta^{13}C$ ecosystem tracer in a loblolly pine-dominated forest in North Carolina exposed to FACE since 1996. Respiration of newly assimilated C is largely due to root and rhizosphere respiration and other very active pools of soil C coupled to plant activity fixed since fumigation was turned on. Respiration of new C represents 64% and 50% of total R_S during the 2002 drought and 2003 wet year, respectively. Bars show means and SE, n=3. (L. Taneva, R. Matamala, J. S. Pippen, W. H. Schlesinger and M. A. Gonzàlez-Meler, unpublished).

V. Conclusions

Contrary to what was previously thought, respiration may not be reduced when plants are grown at elevated [CO_2]. This is because direct effects of [CO_2] on respiratory enzymes are very small. Previous direct effects of [CO_2] on respiration have been confounded with measurement artifacts due to leaks and memory effects in gas-exchange systems, and also due to leaks through leaf air spaces. Such measurement artifacts are also affecting the magnitude of the indirect and acclimation effects of [CO_2] on respiration. A re-analysis of the literature comparing respiration of leaves of plants grown at ambient [CO_2] with leaves of plants grown at elevated [CO_2] when rates are measured at the same [CO_2], indicates that leaf respiration, on average, may not be changed by increasing atmospheric [CO_2]. Increases in growth observed in plants exposed to high [CO_2], appears to be compensated for changes in tissue chemistry that reduce growth and maintenance respiration. In some species an increased activity of the alternative pathway in plants grown at high [CO_2] could counteract a positive plant growth response to elevated [CO_2]. If specific rates of respiration are not affected by growth at elevated [CO_2], respiration from the terrestrial vegetation in a high [CO_2]-world should be proportional to changes in plant mass. However, some studies show that canopy respiration does not follow the increase in biomass (NPP) observed when natural ecosystems are exposed to elevated atmospheric [CO_2]. This can be explained, in part, because root respiration at the ecosystem level seems to increase proportionally to the biomass stimulation by elevated [CO_2]. In addition, increased root exudation in ecosystems exposed to elevated [CO_2] may prompt the oxidation of stored organic carbon in soils, offsetting reductions of plant ecosystem respiration in response to high [CO_2]. The role of plant respiration in augmenting or controlling the sink capacity of terrestrial ecosystems is still uncertain.

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