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REVIEW

Physiological impacts of elevated CO_2 concentration ranging from molecular to whole plant responses

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Abstract

The dynamics of the terrestrial ecosystems depend on interactions between a number of biogeochemical cycles (*i.e.* carbon, nutrient, and hydrological cycles) that may be modified by human actions. Conversely, terrestrial ecosystems are important components of these cycles that create the sources and sinks of important greenhouse gases (*e.g.* carbon dioxide, methane, nitrous oxide). Especially, carbon is exchanged naturally among these ecosystems and the atmosphere through photosynthesis, respiration, decomposition, and combustion processes. Continuous increase of atmospheric carbon dioxide (CO_2) concentration has led to extensive research over the last two decades, during which more then 1 400 scientific papers describing impacts of elevated [CO_2] (EC) on photosynthesis have been published. However, the degree of response is very variable, depending on species, growing conditions, mineral nutrition, and duration of CO_2 enrichment. In this review, I have summarised the major physiological responses of plants, in particular of trees, to EC including molecular and primary, especially photosynthetic, physiological responses. Likewise, secondary (photosynthate translocation and plant water status) and tertiary whole plant responses including also plant to plant competition are shown.

Additional key words: acclimation to [CO_2]; carbon allocation; global change; growth; photorespiration; photosynthesis; respiration; stomatal conductance; tree physiology.

Introduction

From 1850 to 2000, approximately 270±30 Gt C has been emitted as carbon dioxide (CO_2) into the atmosphere from fossil fuel burning and cement production. About 136±55 Gt C has been emitted as a result of land-use change, predominantly as the change of forests to agriculture land. This has led to an increase of CO_2 in the atmosphere by 176±10 Gt C, *i.e.* atmospheric concentration increased from *ca.* 285 to 366 μmol(CO_2) mol^{-1} in 2002, *i.e.* by *ca.* 28 % (Scholes and Noble 2001) during the last 150 years.

The rates and trends of carbon uptake in terrestrial ecosystems are uncertain. However, during last two decades terrestrial ecosystems may have served as a small net sink for CO_2. This terrestrial sink seems to have occurred in spite of net emissions into the atmosphere from land-use change, primarily in the tropical zones. The net terrestrial carbon uptake, that approximately balances the emissions from land-use change in the tropics, results from: (*1*) land-use practices and natural re-growth in middle and high latitudes, (*2*) the indirect effects of human activities, *e.g.* atmospheric CO_2 fertilisation and nutrient deposition, and (*3*) changing climate (both

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Abbreviations: ABA – abscisic acid; ATP – adenosine triphosphate; BER – biomass enhancement ratio; C_i – intercellular CO_2 concentration; Car(s) – carotenoid(s); Chl(s) – chlorophyll(s); [CO_2] – atmospheric CO_2 concentration; D1, D2 – proteins of PS2; EC – elevated [CO_2)]; g_s – stomatal conductance; NADPH – nicotinamide adenine-dinucleotide phosphate; P_i – inorganic phosphate; P_{Nmax}, P_{Nsat} – net photosynthetic rate at saturating irradiance or saturating C_i and saturating irradiance, respectively; PAR – photosynthetically active radiation; PCO – photorespiratory carbon oxidative cycle; PCR – photosynthetic carbon reduction cycle; PS2 – photosystem 2; RuBP – ribulose-1,5-bisphosphate; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; VPD – water vapour pressure deficit; WUE – water use efficiency.

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natural and anthropogenic) (Watson *et al.* 2000).

Ecosystem models indicate that the additional terrestrial CO_2 uptake on a global scale is likely to be maintained for a number of decades in forest ecosystems, but may gradually disappear and forest ecosystems could even become a source for three reasons: (*1*) limitation of CO_2 uptake capacity by nutrients and other biophysical factors, (*2*) decrease of photosynthetic rate stimulation in response to rising [CO_2] and rise of heterotrophic respiration with increasing temperature, and (*3*) ecosystem degradation resulting from the climate change (Watson *et al.* 2000). Because of current uncertainties in our understanding with respect to acclimation of the physiological processes and climatic constraints and feed backs among these processes, studies allowing scaling-up from the leaf photosynthesis to quantify the global terrestrial C sink and assess regional C sources and sinks are highly needed (Field 2001, Scholes and Noble 2001).

CO_2 has two direct physiological effects on plants. It is (*1*) the activator of RuBPCO (EC 4.1.1.39; ribulose-1,5-bisphosphate carboxylase/oxygenase) enzymatic activity, and (*2*) the substrate of the Calvin cycle. *Ad* (*1*): The inactive RuBPCO enzyme is activated by a CO_2 molecule in a slow reversible reaction followed by a rapid reversible reaction of $RuBPCO_{inactive}$-CO_2 complex with Mg^{2+} ion (process of carbamylation). *Ad* (*2*): CO_2 is the substrate for the enzymatic reaction with the second carbon of primary acceptor RuBP (ribulose-1,5-bisphosphate). In the first step, CO_2 is bound on the active site of RuBPCO. In the second step, reaction of $RuBPCO_{active}$-CO_2 complex with RuBP leads to the formation of triose phosphate (process of carboxylation).

Effects of elevated CO_2 concentration (EC) on net photosynthetic rate (P_N) have been the subject of many studies and reported in hundreds of papers. Most of these studies show that P_N is increased by an initial exposure to EC. However, over long time periods substantial reductions in [CO_2]-enhanced photosynthesis (down-regulation) may occur. P_N may decline to the rate of the ambient control variant, but rarely below that value, *i.e.* P_N has generally not declined relative to the control, it has declined relative to the initial [CO_2]-stimulated rate (*e.g.* Lee *et al.* 1998, Luo *et al.* 1999b).

Responses at molecular level

Photosynthetic down-regulation is characterised at the biochemical and leaf levels by reduced chlorophyll (Chl) content, reduced RuBPCO content and activity, limitations in RuBP and P_i regeneration, higher leaf mass/leaf area ratios, and decreased leaf nitrogen content on a leaf mass basis (Sage 1994, Tissue *et al.* 1995). These down-regulation responses are often associated with increased accumulation of saccharides in leaves that may cause feedback inhibition of the photosynthesis. At the molecular level, hexoses initiate changes in mRNA transcripts (Sheen 1994) and suppress the transcription of important photosynthetic genes (Strain and Thomas 1995). Especially, the genes encoding D1 and D2 proteins of PS2 core, cytochrome *f*, RuBPCO small subunit and RuBPCO activase, and carbonic anhydrase are the most affected (for reviews, see Webber *et al.* 1994, Strain and Thomas 1995).

For example, using the immuno-detection method, all cytochrome *f*, D1, and D2 proteins demonstrated decrease in *Prunus avium* grown in EC (Besford *et al.* 1998). Contrariwise, the abundance of genes coding for ADP-glucose pyrophosphorylase (van Oosten and Besford 1995) was increased in tomato plants, *i.e.* genes involved in synthesis of saccharides were down-regulated, whereas genes for utilisation of saccharides were up-regulated if the assimilates exceeded the rate of their utilisation. Recently, Martin *et al.* (2002) demonstrated in *Arabidopsis* seedlings that the low nitrogen to carbon ratio rather than saccharide status alone predominantly regulates the gene expression.

Primary physiological responses

The main plant physiological roles of CO_2 are as (*1*) a substrate and activator for photosynthetic carbon assimilation, with concomitant effects upon (*2*) photorespiration and (*3*) dark respiration processes, and (*4*) an environmental variable determining stomatal aperture.

Photosynthesis: Photosynthetic acclimation to EC is defined as any adjustment that may develop over time in plants grown continuously in EC to control [CO_2] (Kramer 1981, Sage 1994, Saralabai *et al.* 1997, Wolfe *et al.* 1998, Yordanov *et al.* 2000). Photosynthesis pathway variation is the most obvious example of a genotypic factor affecting acclimation to EC. Potential benefit from EC is less (photorespiration losses are less) in C_4 and CAM species compared to C_3 species, because the Calvin cycle is spatially (C_4) or temporally (CAM) isolated. Within the frame of this review, impacts of EC on the photosynthesis of C_3 species are discussed exclusively.

On the base of the co-limitations in a biochemical model (Farquhar *et al.* 1980, Caemmerer 2000) describing the photosynthetic sensitivity to [CO_2], it was inferred that plant growth in EC should result in a relative reduction of RuBPCO and in an enhancement of RuBP regeneration (Sage 1990). The initial assessments predicted an increase (*i.e.* up-regulation) of P_N (maximal values of P_N-PAR response; P_{Nmax}) and a decrease (*i.e.* down-regulation) of the assimilation capacity (maximal values of P_N-C_i response; P_{Nsat}) (Pearcy and Björkman 1983). Experimental findings confirmed the increase of P_{Nmax} but rejected the speculation that P_{Nsat} is always decreased (Sage *et al.* 1989, Ceulemans and Mousseau 1994, Luo *et al.* 1999b, Medlyn *et al.* 1999).

Primary events in photosynthetic acclimation to EC occur at the molecular level in leaf mesophyll cells. However, final growth response involves acclimation

responses associated with the photosynthate partitioning among plant organs in relation to resources limiting growth (Eamus and Jarvis 1989, Wolfe *et al.* 1998, Idso 1999, Luo *et al.* 1999a,b), and requires evaluation at both spatial (from molecular to ecosystem) and temporal (from seconds to centuries) scales.

The primary stimulation (short-term effect; period of days to weeks) of CO_2 enrichment is mediated by the increase in CO_2 diffusion gradient, reduction in the oxygenase component of RuBPCO (*i.e.* suppressed photorespiration), and insufficiency to saturate RuBPCO activity by the current atmospheric [CO_2] (Stitt 1991, Long and Drake 1992). Also indirect effects such as increase of water use efficiency (Pospíšilová and Čatský 1999) can increase P_N. However, after a long-term (period of months to years) exposure to EC some species reduced the CO_2 assimilation capacity as a result of acclimation depression, referred also as a downward regulation (*e.g.* Kramer 1981, Sage *et al.* 1989, Marek *et al.* 1995, Saralabai *et al.* 1997). The degree of responses is very variable, depending on species, mineral nutrition status, duration of CO_2 enrichment, and/or synergetic influence of other stresses (Ceulemans 1997, Poorter and Pérez-Soba 2001). Thereby, it led to the formation of new alternative hypotheses over the literature to explain responses of P_{Nsat} as follows.

(*1*) Redistribution and/or shortage of inorganic phosphate. An increased accumulation of phosphorylated sugar intermediates in the pathway of sucrose synthesis, occurring within minutes, can lead to a shortage of inorganic phosphate (P_i) in the chloroplast for ATP synthesis and subsequently RuBP regeneration, and thus constrain the stimulatory effects of EC on CO_2 assimilation (Sage and Reid 1994, Webber *et al.* 1994).

(*2*) Decrease of RuBPCO amount and/or activity. Increased contents of specific saccharides (*e.g.* glucose, sucrose), repressing the expression of genes transcribing for RuBPCO or other photosynthetic enzymes (Jang and Sheen 1994, Sheen 1994, Griffin and Seemann 1996) over the time scale of hours to days. Portis (1990) reported that local phosphorus deficiency corresponds to RuBPCO de-carboxylation owing to reduced activity of RuBPCO activase. Increase of intercellular limitations to CO_2 diffusion (Besford *et al.* 1998, Urban and Marek 1999) is usually caused by a decrease in carbonic anhydrase activity (Porter and Grodzinski 1984, Webber *et al.* 1994), facilitating diffusion of CO_2 from the intercellular air space to RuBPCO, *i.e.* indirect decrease of RuBPCO activity *via* the carbamylation process.

(*3*) Mechanical damages of thylakoids resulting from excessive starch accumulation. On the time-scale of hours to days, excessive formation of large starch grains may lead to the shading of light reaching the photosynthetic reaction centres or may lead to the mechanical damage of thylakoid membranes (DeLucia *et al.* 1985, Sasek *et al.* 1985). However, some authors consider these cases as extreme, occurring only in strongly sink-limited species (Ceulemans 1997, Wolfe *et al.* 1998), whereas the stimulation of starch synthesis functions as an important carbon sink (Stitt 1991, Wolfe *et al.* 1998). Marek *et al.* (1995) reported increases of starch content from 15 up to 167 % in Norway spruce needles during the three years of double [CO_2] influence. Linder (1995) demonstrated that most of the seasonal variation in nutrient concentrations could be explained by variations in the amount of starch (up to 30 % of needle dry mass) stored in the needles. The breakdown of the starch reserve is in good agreement with bud-break and coincides with the start shoot extension growth (Linder and Murray 1998), when the daily demand for saccharides normally exceeds net assimilation (Linder 1995).

(*4*) Inhibition of photosynthetic genes. Inhibition of transcription of photosynthetic genes (*e.g.* mRNA, RuBPCO, carbonic anhydrase) by end-product (hexoses; Jang and Sheen 1994, Webber *et al.* 1994, Strain and Thomas 1995) or low N/C ratio (Martin *et al.* 2002) occurs on the time-scale of hours to days.

(*5*) Dilution and/or redistribution of nitrogen (N) at organ and whole-plant levels. Results of experiments where both CO_2 and N supply were varied often show larger downward acclimation of photosynthesis in low compared to high N environments (Ceulemans and Mousseau 1994, Linder and Murray 1998, Peterson *et al.* 1999, Dyckmans *et al.* 2000). Many papers have reported decrease of N content under EC treatment (Marek *et al.* 1995, Linder and Murray 1998, Makino and Mae 1999, Stitt and Krapp 1999). Correlation between reduction of leaf N and activity of photosynthetic enzymes (Conroy 1992) was expected since RuBPCO alone comprises 25-50 % of leaf N (Eichelmann and Laisk 1999). Reduction in RuBPCO content leads to the establishment of new co-limitation of photosynthesis between RuBPCO and RuBP regeneration (Eamus and Jarvis 1989, Webber *et al.* 1994, Urban and Marek 1999) that represents physiological re-optimalisation of the N distribution. Therefore, photon-saturated photosynthesis (P_{Nmax}) is a function of N (*e.g.* Medlyn *et al.* 1999). Also a decrease of Chl synthesis is one of the main symptoms of N deficiency (Linder 1980, Besford *et al.* 1998, Stitt and Krapp 1999). Urban *et al.* (2003) reported lower reallocation of N away from lower parts of the canopy in EC and this leads to the long-term stimulation of assimilation rate, and capacity in shade-adapted spruce needles (Kalina *et al.* 2001, Marek *et al.* 2001).

(*6*) Changes in the harvesting of photons. Decrease of contents of Chls and carotenoids (Cars) under EC has been reported *e.g.* for barley and wheat plants (Sicher and Bunce 1997) and two forest tree species (Wullschleger *et al.* 1992). Norway spruce trees treated in open-top chambers (Janouš *et al.* 1996) manifested decreases of total Chls and Cars up to 45 and 35 %, respectively, whereas Chl *a/b* ratio was increased only by 5 % in this experiment (Kalina *et al.* 1997). Moreover, a significant accumulation of inactive PS2 reaction centres

and diminution of light-harvesting complexes was observed (Kalina *et al.* 1997, Špunda *et al.* 1998).

(*7*) Differences in the new sink-source status of the plant. Over days to weeks, photosynthesis and growth responses to EC depend on the plant ability to develop new sinks or expand the storage capacity or growth rate of existing sinks (Vu *et al.* 1989, Stitt 1991, Luo *et al.* 1994, Ceulemans 1997). There is usually an adjustment of leaf area (Lee *et al.* 1998, Pritchard *et al.* 1999) so that whole-plant growth and C and N partitioning responses alter the rate of production and utilisation of photosynthate, and by feedback affect C metabolism and gene expression at the leaf or chloroplast scale (Wolfe *et al.* 1998). Therefore, source-sink interactions are key determinants of photosynthetic acclimation to EC at both the whole-plant (Luo *et al.* 1994) and ecosystems (Luo *et al.* 1999a) scales. Thus, Overdieck *et al.* (1998) and Tissue *et al.* (2001) reported that EC accelerates the natural decline of photosynthetic performance in older needles, resulting from the lack of an active basal meristem. Therefore, response of evergreen trees to EC depends, moreover, on the needle age distribution within the canopy and the ability to produce new foliage flushes (Lee *et al.* 1998, Tissue *et al.* 2001).

(*8*) Space structure of a canopy affects sink capacity. Space arrangement of the canopy leads to the formation of different types of assimilation apparatus, sun- and shade-adapted, with different assimilation capacities. Mutual interactions among trees evoke the stimulation of growing sinks, predominantly in the apical zones (Wolfe *et al.* 1998). Therefore, the C sink capacity varies for individual plants and plants with mutual interactions.

Priwitzer *et al.* (1998) demonstrated larger reduction of assimilation by the lower [CO_2] in the chloroplasts and larger proportion of free assimilation capacity in shade-adapted spruce needles that was rapidly saturated after the sudden application of EC. Moreover, Kubiske and Pregitzer (1997) suggested well-developed plasticity in partitioning of N between light-harvesting and carbon reduction pools. Therefore, it was speculated (Kerstiens 1998, Hättenschwiler 2001), that there would be stronger stimulation of assimilation rate by EC in foliage adapted to low irradiance. The phenomenon was observed especially in deciduous tree species (Kerstiens 2001) and in evergreen coniferous trees (Gielen *et al.* 2000, Hättenschwiler 2001, Marek *et al.* 2002) after redistribution of N between upper (sun-adapted) and lower (shade-adapted) parts of the crown.

Luo *et al.* (1999b) presented a general model presuming that the balance between biochemical (CO_2 fixation by RuBPCO, RuBP regeneration, and P_i regeneration) and morphological (saccharide storage, leaf thickness, and mesophyll cell number per unit of leaf area, or new additional sink formation) regulation under the impact of EC may significantly control photosynthetic capacity. This idea is experimentally supported by the seasonal periodicity of photosynthetic down regulation (Urban and Marek 1999), tissue age dependence of down regulation (Gielen *et al.* 2000, Tissue *et al.* 2001), and long-term development of assimilation depression according to changes in N redistribution between sun and shade acclimated parts of a canopy (Urban *et al.* 2003).

Photorespiration results from the oxygenase reaction catalysed by RuBPCO. In this reaction glycollate-2-phosphate is produced and subsequently metabolised in the photorespiratory pathway to form the Calvin cycle intermediate glycerate-3-phosphate (*e.g.* Wingler *et al.* 2000). During this metabolic process, CO_2 and NH_3 are produced and ATP and reducing equivalents (NADPH) are consumed. However, photorespiration could serve as an energy sink preventing the over-reduction of the photosynthetic electron transport chain and photoinhihition, especially under stress conditions that lead to reduced rates of photosynthetic CO_2 assimilation (Laisk and Oja 1998, Wingler *et al.* 2000). Furthermore, photorespiration provides metabolites for other metabolic processes, *e.g.* glycine for the synthesis of glutathione, which is also involved in stress protection.

CO_2 and O_2 compete for the active sites on RuBPCO (*e.g.* Farquhar *et al.* 1980, Laisk and Oja 1998). This CO_2/O_2 specificity is influenced by numerous factors such as temperature, activating metal ions, and amino acid substitutions. A reduction in photorespiration, resulting from an increased ratio of CO_2 to O_2 and increased availability of CO_2 at the chloroplasts, also results in an enhanced availability of NADPH and ATP and this may have significant stimulatory effects on photosynthesis (Eamus and Jarvis 1989, Laisk and Oja 1998).

Mortensen (1983) showed that EC caused a significant reduction (up to 45 %) in photorespiration of *Picea abies*. Similarly, fifteen-year-old Norway spruce trees exposed to double CO_2 concentration in open-top chambers demonstrated significant decrease of photorespiration by 45 % after four months, estimated by the post-irradiation burst method (Marek *et al.* 1995). Suppression of photorespiration is regarded a main reason of increases in CO_2 assimilation of higher plants under the short-term (hours to days) exposure to EC (Stitt 1991, Webber *et al.* 1994).

Respiration processes: Studies of the direct effects of EC on plant respiration have become increasingly important as attempts are made to scale the physiological effects of EC from the biochemical to the whole-plant scale (Cannell and Thornley 2000). Gonzàlez-Meler and Siedow (1999) point out that a doubling of atmospheric [CO_2] results in an average 15–20 % reduction in mitochondrial respiration that varies both within and among species (some crops may show as much as a 20 % increase in respiration). The direct effects of [CO_2] on respiration also vary among leaves of different ages (Thomas and Griffin 1994) and between developmental (pre- *ver-*

sus post-reproductive) stages (Griffin *et al.* 1999). Maintenance respiration, which is related to the N content within the tissue, is more sensitive to temperature, CO_2 concentration, protein turnover, and water stress than to growth respiration (Ryan 1991). The exact mechanisms that account for inhibition of respiration by EC have not been fully elucidated, in part because responses have been highly variable. Possible mechanisms include a decrease in the activity of cytochrome *c* oxidase, an enzyme involved in the electron transport of the respiration chain (Gonzàlez-Meler *et al.* 1996), and reduced activity of other enzymes in response to dissolved inorganic carbon (Amthor 1991).

Both Ryan (1991) and Amthor (1991) assess the possible mechanisms responsible for the different results in the literature, *i.e.* (*1*) changes in contents of non-structural saccharides, (*2*) changes in growth rate and structural phytomass, (*3*) composition of phytomass, (*4*) direct chemical interaction between CO_2 and respiratory enzymes, (*5*) direct chemical interaction between CO_2 and other cellular components, (*6*) dark CO_2 fixation, and (*7*) ethylene biosynthesis. Furthermore, Gonzàlez-Meler and Siedow (1999) provide evidence that other mechanisms besides inhibition of mitochondrial enzymes may be associated with reductions of respiration in response to EC.

It is unlikely that root respiration is directly affected by atmospheric [CO_2] (Ceulemans 1997), since roots already grow in air of high CO_2 concentration (*ca.* 3 %). However, some studies have shown an increase in root activity of trees grown in EC, including respiration, enhanced exudation and mycorrhizal development (Ceulemans and Mousseau 1994), as a result of enhanced translocation of assimilates below ground (*e.g.* Wang *et al.* 1998). Modelling studies with young birch trees (Wang *et al.* 1998) showed that only about half of enhanced photosynthetic production was realised in growth. The other half was utilised in larger fine-root production, and by symbiotic mycorrhizas and other below-ground processes. Total respiration of non-leaf tissues accounted for a similar fraction of total P_N in both treatments of this experiment (Wang *et al.* 1998).

Stomatal regulation: The stomata close in response to the frictional resistance to water flow through the tree from root rhizodermis to leaf epidermis. Stomatal conductance (g_s) decreases with the decrease of bulk water potential below a threshold value, in response to increasing peristomatal transpiration, or in response to lower water absorption by roots. Moreover, abscisic acid (ABA) is the main signal messenger in regulating stomatal closure. Zhu *et al.* (1998) suggested that the stomatal response to [CO_2] be mainly linked to the changes in guard cell zeaxanthin accumulation.

g_s declines when plants are exposed to a transient increase in atmospheric [CO_2] (for review, see Pospíšilová and Čatský 1999). While numerous field experiments have shown typical reduction of g_s by 20–60 % for both C_3 and C_4 species (Ceulemans and Mousseau 1994, Hsiao and Jackson 1999), there are large differences among studies, species, and used exposure technique (Field *et al.* 1995, Curtis and Wang 1998). Thirteen long-term (> 1 year) field-based studies on European forest tree species indicated a significant decrease (*ca.* 21 %) in g_s. The response was significantly stronger in (*1*) young trees compared to old ones, (*2*) deciduous compared to coniferous trees, and (*3*) water stressed compared to nutrient stressed trees (Medlyn *et al.* 2001). Growth in EC reduced sensitivity of g_s to vapour pressure deficit (VPD; Heath 1998), reduced sensitivity to drought (Heath and Kerstiens 1997), and reduced sensitivity to atmospheric [CO_2] (Šantrůček and Sage 1996). Moreover, Tognetti *et al.* (1998) reported higher decreases in g_s induced by EC for *Quercus ilex* and *Q. pubescens* in the morning than in the afternoon. Also for *Picea abies*, higher stomatal reactivity was observed in the morning hours (Janouš *et al.*, unpublished) that could be caused by higher relative water content in the assimilation tissues after the night time (Yordanov *et al.* 2000).

Woodward and Bazzaz (1988) formulated hypothesis that stomatal density (*i.e.* the number of stomata per unit of leaf area) should decrease with increasing [CO_2]. Woodward (1987) attributed *ca.* 40 % decrease in stomatal density (*i.e.* the number of stomata per unit of leaf area) in the leaves of herbarium specimens of tree species collected over the last 200 years to the rise in atmospheric [CO_2]. This decrease was associated with a fall in the stomatal index (*i.e.* ratio of stomatal to epidermal cells) showing that the changes were caused by changes in stomatal initiation (Woodward 1987). Also, direct laboratory experiments have illustrated decreased stomatal densities (Woodward and Bazzaz 1988).

However, the main factor reducing stomatal conductance at EC is the reduction in the stomatal aperture (Paoletti and Gellini 1993). Because of no changes in stomatal density and/or stomatal index as a result of doubling [CO_2] by many tree species (Körner 1988, Ceulemans and Mousseau 1994, Ceulemans 1997), the predominant reason for the reduction of g_s is a response to the increase of intercellular [CO_2].

Secondary physiological responses

Photosynthate concentration and translocation: Sucrose and starch content generally increase in the leaves of plants grown in EC (DeLucia *et al.* 1985, Long and Drake 1992, Marek *et al.* 1995). This in part explains the decrease of leaf N content—a dilution effect (Long and Drake 1992, Ceulemans and Mousseau 1994). Carbon assimilation *via* the Calvin cycle and starch synthesis occurs in the chloroplast, while sucrose is synthesised in the cytosol. The latter requires triose phosphate export from the chloroplast in a strict counter-exchange for P_i import into the chloroplast (Wolfe *et al.* 1998). This exchange is translocated by the phosphate translocator

(Flügge and Heldt 1991). Triose phosphates are converted to sucrose in the cytosol, releasing P_i, which returns to the chloroplast in exchange for more triose phosphate.

In the absence of external sinks, sucrose accumulates in the cytosol and may inhibit the key enzyme in sucrose synthesis, sucrose phosphate synthase. The mechanism for the inhibition involves phosphorylation and de-phosphorylation of a serine residue on the protein, leading to the inactivation and activation of the enzyme (Huber and Huber 1992). An unbalance between sink and source capacities can also lead to the accumulation of fructose-2,6-bisphosphate that acts as an inhibitor of fructose-1,6-bisphosphatase (Stitt and Quick 1989).

In this way a slower rate of sucrose synthesis results in accumulation of P_i in the cytosol and its inaccessibility within the chloroplast (Webber *et al.* 1994). The depletion of ATP (result of a low P_i) leads to an accumulation of glycerate-3-phosphate within the chloroplast. Consequently, an increase of the glycerate-3-phosphate to P_i ratio activates ADP-glucose pyrophosphorylase—the key enzyme of the starch synthesis pathway (Stitt and Quick 1989), and leads to an increased starch accumulation within the chloroplast.

Contrariwise to the conclusions of DeLucia *et al.* (1985), some authors suppose that a stimulation of starch synthesis can function as an acclimation response: *e.g.* (*1*) the P_i released within the chloroplast protects the plant from the initial end-product inhibition of photosynthesis (Stitt 1991), and (*2*) the accumulated starch temporarily expands the carbon sink capacity (Wolfe *et al.* 1998).

As mentioned above, the expansion or the formation of new alternative sinks, *i.e.* C and N partitioning, is crucial for the integration of photosynthetic acclimation to CO_2 at the whole-plant level (Eamus and Jarvis 1989, Ceulemans and Mousseau 1994, Wolfe *et al.* 1998, Luo *et al.* 1999b). Plant hormones (long-distance signals) modulate growth of various sink organs and thereby play an important role in regulating partitioning and root/shoot ratio (Brenner 1987, Pritchard *et al.* 1999). However, whether EC and plant saccharide status can directly alter the synthesis, transport function of plant hormones is still unknown. Sucrose is not only the transporter of C, but it is involved in the control of expression of genes regulating sink organ growth (Sheen 1994, Tissue *et al.* 1995). Huber *et al.* (1992) suggest that supply of sucrose and other saccharides can alter growth by affecting activity of key enzymes in C and N metabolism, *e.g.* sucrose phosphate synthase and nitrate reductase, by a reversible post-translational mechanism involving protein phosphorylation, rather that their production (Webber *et al.* 1994, Koch 1996).

Increasing water use efficiency (WUE) in EC can also alter partitioning patterns (Stitt and Schulze 1994, Field *et al.* 1995, Amthor 1999). For example, Spollen and Sharpe (1991) showed that maintenance of root growth, *i.e.* carbon flux, in water-stressed conditions is the result of a combination of regulation of osmotic potential and cell wall elasticity.

Plant water status: Many studies have shown that regulation of stomata by guard cells can be directly affected by CO_2 concentration (*e.g.* Zhu *et al.* 1998, Pospíšilová and Čatský 1999) and leads to a reduction of stomatal conductance of C_3 plants (see above). Moreover, increased epicuticular wax deposition in some tree species grown in EC was reported (Paoletti *et al.* 1998). These responses may lead to a decrease of the transpiration rate (reported in *ca.* 65 % studies) per unit leaf area (Saralabai *et al.* 1997, Pospíšilová and Čatský 1999). However, enlarged leaf area under EC may be the reason for increased transpiration rate per plant (Ceulemans and Mousseau 1994, Scarascia-Mugnozza and De Angelis 1998) or even whole ecosystem evapotranspiration (Lauber and Körner 1997, Granier *et al.* 2000).

An increase (up to 170 %) of WUE (the ratio between the rates of photosynthesis and transpiration) is the most common positive effect (reported in *ca.* 90 % studies) (Ceulemans and Mousseau 1994, Scarascia-Mugnozza and De Angelis 1998, Pospíšilová and Čatský 1999). In the absence of a change in g_s, the increase in WUE is entirely the result of the stimulation of photosynthesis (Gunderson *et al.* 1993) which may consequently promote osmotic adjustment (Hsiao and Jackson 1999).

On the base of direct measurements, reduced sap flow has been found for *Quercus ilex* (Tognetti *et al.* 1998), *Pinus sylvestris* (Kellomäki and Wang 1998), or *Picea abies* (Pokorný *et al.* 2001). Tognetti *et al.* (1999a) showed that in *Quercus pubescens* water flux (relative to cross-sectional area) was reduced in trees grown near a natural CO_2-spring. However, they found that transpiration responses to EC have strong seasonal effects, and the beneficial effects of EC were lowest during the most severe periods of drought (*i.e.* periods of highest vapour pressure deficit). Furthermore, Tognetti *et al.* (1999b) reported that there were inter-specific differences in the hydraulic conductivity of Mediterranean tree species, but trees of the same species did not differ in their responses at a CO_2-spring site compared to a non-CO_2-spring one. These results indicate that long-term exposure to EC may not result in differentiation of xylem hydraulic properties. Atkinson and Taylor (1996) attributed increased hydraulic conductance to increased number of vessels per stem and total vessel lumen cross-sectional area per stem. Pataki *et al.* (1998) found that water flux per unit sapwood did not vary between trees of *Pinus taeda* grown in ambient and EC, but EC increased absolute water loss by increasing leaf and sapwood areas.

These processes enable plants in EC to maintain higher leaf water potential and ameliorate the negative effects of drought stress (Tolley and Strain 1985). Leaf growth by cell enlargement is extremely sensitive to water stress (Hsiao and Jackson 1999), and therefore, plants grown in EC may survive higher drought stress

and this may lead to the extension of current biotopes and changes in biodiversity (Amthor 1999, Luo *et al.* 1999a).

Tertiary whole plant responses

Functionally, developing leaves produce a hormonal signal (*i.e.* auxins) which stimulates differentiation of xylem. Thus, the amount of leaf area dictates production of stem area (Taylor *et al.* 1994, Atkinson and Taylor 1996), assuring functional equilibrium between leaves and stems. Rates of leaf development, leaf area duration, and leaf efficiency (a function of anatomy, ultrastructure, and biochemistry) throughout the growing season dictate the rate of canopy closure and the yearly canopy production index (CPI; annual production of wood per unit of leaf area). An understanding of how increasing [CO_2] will impact the dynamics of leaf initiation, morphogenesis, histogenesis, and phenology is necessary to (*1*) determine the impact of EC on leaf and whole plant function, (*2*) determine the effects of these functional shifts on ecosystem processes and physiognomy, and (*3*) more accurately link vegetation processes with global carbon models and budgets (Luo *et al.* 1999a,b, Pritchard *et al.* 1999).

Generally, EC stimulates production of most C_3 plants. Growth in EC alters plant structure through its effects on both primary and secondary meristems of shoots and roots. Existing literature (Ceulemans and Mousseau 1994, Lee *et al.* 1998, Idso 1999, Pritchard *et al.* 1999, Rogers *et al.* 1999) suggests that cell division, cell expansion, and cell patterning may be affected, driven mainly by increased substrate (*i.e.* sucrose) availability and differential expression of genes involved in cell cycling (*e.g.* cyclins) or cell expansion (*e.g.* xyloglucan endotransglycolase). Furthermore, plants often show larger growth responses to EC when other resources such as nutrients and water are not limiting (Curtis and Wang 1998, Rogers *et al.* 1999).

Increased growth of plants in EC (reported in *ca.* 70 % of studies) is primarily the result of stimulation of cell division within shoot apices. The molecular mechanism of this stimulation may be (*1*) direct—based on insufficiency of the nucleus to govern functions of large expanding cells (Jacobs 1997), (*2*) indirect—based on increasing expression of cyclin genes by auxins and cytokinins that facilitate the transition of cells from G0 to G1 of the cell cycle, *i.e.* chemical signals (Soni *et al.* 1995), or (*3*) a combination of the both. Also, increased sucrose concentration has been reported as a chemical control of cyclin activity (Kinsman *et al.* 1997). Because of increase in the quantum yield efficiency of C_3 species grown in EC (Long and Drake 1992, Marek *et al.* 1997, Medlyn *et al.* 1999), relatively higher growth enhancements are generally observed under low irradiance (up to 400 $\mu mol\ m^{-2}\ s^{-1}$). These irradiances are the most frequent in the natural environment, and thus shaded leaves may produce relatively larger amounts of assimilates (Marek *et al.* 2001, 2002). Furthermore, increased tissue water availability and increasing root proliferation could contribute to increased cell expansion resulting from increased cell turgor pressure (Sasek and Strain 1988, Hsiao and Jackson 1999).

However, cell proliferation may be stimulated to different extents throughout different meristematic regions, and this, perhaps, accounts for observed increases in nodal elongation relative to branch initiation, and other shifts in whole plant architecture (Pritchard *et al.* 1999). Sionit *et al.* (1985) observed taller trees of *Pinus taeda* grown in EC, but no changes in total number of branches, *i.e.* the growth and development of cells and tissues below the site of lateral organ formation were stimulated to a larger extent than the formation of organ primordia at the shoot tip. Kinsman *et al.* (1997) partly explained these results by a decrease in the ratio of cell doubling time (cdt) in the pith rib meristem to cdt in the central and peripheral zones.

Observed increases in leaf expansion may be explained by the cellular mechanisms driving increased cell expansion (Taylor *et al.* 1994). This is caused by (*1*) increasing cell wall extensibility, (*2*) reduction of cell wall yield turgor, and (*3*) significant increase of xyloglucan endotransglycolase activity.

Although node number appears rather insensitive to EC, several studies have reported that branch initiation and number have been stimulated. For example, Conroy *et al.* (1990) reported higher branch numbers, resulting from more branches per whorl in *Pinus radiata*, even though whole tree height decreased possibly because of reduced apical dominance (Pritchard *et al.* 1999). Reduced apical dominance may result from altered hormonal production and/or transport as a result of effects on apical meristem function, *e.g.* apical bud necrosis (Mousseau and Enoch 1989), or from alterations in whole plant carbon allocation (Dyckmans *et al.* 2000).

Initial stimulation of growth in response to EC may diminish over time, possibly because of down-regulation of photosynthesis, modifications in biomass allocation, and/or phenology. For example, Jach and Ceulemans (1999) observed an enhancement of relative growth rates during the first season of exposure of three-year-old *Pinus sylvestris* to EC, but reported similar growth rates to control plants during the next season (Jach and Ceulemans 2000).

Plants are generally thought to allocate biomass to structures that are involved in limiting uptake of resources. Thus, relative limitation by nitrogen and other soil nutrients in EC was initially predicted to increase the allocation of biomass to the roots (Brenner 1987, Opluštilová and Dvořák 1997, Saralabai *et al.* 1997, Luo *et al.* 1999b, Dyckmans *et al.* 2000). An increase in the root/shoot biomass ratio was found in *ca.* 90 % of studies (*e.g.* Ceulemans and Mousseau 1994, Pritchard *et al.* 1999, Rogers *et al.* 1999), and also, increases in number of roots, root length, root growth, and fine root mass were

substantially higher under EC. Accordingly, the roots alter production and transport of hormones (*e.g.* cytokinins, ABA) to shoots, thereby modulating the activity of meristematic tissues above the ground, as well as the expression of genes coding for photosynthetic enzymes, *e.g.* phosphoenolpyruvate carboxylase, carbonic anhydrase, and the small RuBPCO subunit (Strain and Thomas 1995, Aiken and Smucker 1996). The flow of carbon through the roots into the soil is one of the key processes for the formation of soil carbon pools and understanding the functioning of forest ecosystem (Ceulemans and Mousseau 1994, Luo 1999) in an EC environment.

Root N uptake capacity is highly modulated by the plant growth rate, *i.e.* fast-growing species show a greater N uptake capacity compared to the slow-growing species. Short-term CO_2 enrichment often stimulates plant growth rate (*e.g.* Lee *et al.* 1998, Pritchard *et al.* 1999), but there are evidences that the magnitude of the response is greater in fast- than in slow-growing species. Therefore, it is reasonable to expect that N uptake responses to EC be mediated by changes in the relative growth rate (Zerihun and Bassirirad 2001).

EC can alter plant phenology (*i.e.* development rate) and time to senescence at both leaf and whole-plant scales. Jach and Ceulemans (1999) observed that in *Pinus sylvestris* needle fall occurred earlier in EC than in current ambient [CO_2], and the authors attributed this to possible changes in transpiration rate or earlier translocation of nutrients away from the leaves. In contrast, delayed leaf senescence response to EC was found in *Glycine max* (Hardy and Havelka 1975). Leaves of *Eriophorum vaginatum* maintained high P_N later in the season when grown in EC than if grown in current ambient [CO_2] (Tissue and Oechel 1987).

Interactions with other environmental stresses

Many papers and reviews describe interactions between EC and environmental variables (*e.g.* Curtis and Wang 1998, Luo *et al.* 1999a, Makino and Mae 1999). An understanding of how environmental variables regulate or limit carbon assimilation is essential to make predictions about the consequences of environmental change.

Poorter and Pérez-Soba (2001) presented a methodology of the minimal experimental design to analyse [CO_2] *versus* environment interactions. It requires an orthogonal combination of two CO_2 concentrations (ambient and elevated) and two levels of other environmental variables (optimal and non-optimal for growth). The first parameter is an indicator of the stimulating effect of EC on total plant biomass calculated as the ratio of plant biomass in EC and ambient [CO_2], *i.e.* biomass enhancement ratio (BER). The second parameter is an indicator of the stress experienced by plants resulting from a non-optimal level of the environmental variable under study. Evaluation of the stress conditions is based on the slope of the line connecting these two BER values. A negative slope indicates that at a given non-optimal level of the interacting variable, the relative growth response to EC is smaller than under the optimal conditions.

According to the theory of the model of photosynthesis (Farquhar *et al.* 1980, Caemmerer 2000), the maximum RuBP-saturated rate indicates the maximal carboxylation rate (V_{Cmax}), which is proportional to the amount or activity of RuBPCO in needles. The V_{Cmax} value may change during long-term treatments for various reasons, including changes in nitrogen nutrition, irradiance regime, and/or temperature (Overdieck *et al.* 1998, Medlyn *et al.* 1999). Moreover, the capacity to regenerate RuBP depends on the amount of photons intercepted by the LHCs, on the activity of the electron transport chain and the Calvin cycle enzymes, excluding RuBPCO. At saturating irradiance (>1 300 μmol m^{-2} s^{-1}), properties of both the thylakoid membranes and the Calvin cycle reactions limit the rate of RuBP regeneration, and determine the temperature-dependence of maximal electron transporte rate (J_{max}). Overdieck *et al.* (1998) reported that acclimation processes to elevated temperature and EC mainly involve changes in heat stability of the thylakoids and in the activity of RuBPCO. Hence temperature alone and in the combination with CO_2 enhances heat tolerance, but CO_2 treatment alone increases cold tolerance (Harley and Baldocchi 1995, Overdieck *et al.* 1998).

Up-scaling and ecosystem modelling of the CO_2 responses

Scaling-up studies of leaf photosynthesis have been generated by the need to quantify the global terrestrial carbon sink and assess regional-scale carbon sources and sinks (Field 2001, Scholes and Noble 2001). Two general problems occur during extrapolation from leaf physiology to the global change prediction: environmental variability and biological diversity (Luo 1999). Identifying scaleable parameters, that characterise intrinsic properties of the system and have minimal variability associated with environmental heterogeneity and genetic diversity, is probably the most effective approach to reduce the uncertainties. For example, extrapolation of the quantum yield of CO_2 uptake can predict the global distributions of C_3 and C_4 plants (Luo *et al.* 1999a) or photosynthetic sensitivity of leaf to rising [CO_2] can be used to predict the increment of global gross primary production (Watson *et al.* 2000).

Luo (1999) presented four supplementary studies that led to the following conclusions: (*1*) Photosynthesis sensitivity intrinsically oscillates, between light- and enzyme-limited processes, in natural ecosystems according to light availability. (*2*) g_s has a negligible effect on sensitivity and global estimation of additional C influx. (*3*) Fluctuation in instantaneous temperature results in spatial and temporal variation in the *L* function (a leaf-level function denoting the normalised leaf photosynthetic

response to one unit of atmospheric [CO_2] change) and does not allow simple extrapolation of the function from the leaf to the globe. (*4*) Photosynthetic acclimation may be regulated by N availability, but the regulation was significant in field studies across diverse ecosystems.

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