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Plant responses to elevated CO₂ concentration at different scales: leaf, whole plant, canopy, and population

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Abstract Elevated CO₂ enhances photosynthesis and growth of plants, but the enhancement is strongly influenced by the availability of nitrogen. In this article, we summarise our studies on plant responses to elevated CO₂. The photosynthetic capacity of leaves depends not only on leaf nitrogen content but also on nitrogen partitioning within a leaf. In *Polygonum cuspidatum*, nitrogen partitioning among the photosynthetic components was not influenced by elevated CO₂ but changed between seasons. Since the alteration in nitrogen partitioning resulted in different CO₂-dependence of photosynthetic rates, enhancement of photosynthesis by elevated CO₂ was greater in autumn than in summer. Leaf mass per unit area (LMA) increases in plants grown at elevated CO₂. This increase was considered to have resulted from the accumulation of carbohydrates not used for plant growth. With a sensitive analysis of a growth model, however, we suggested that the increase in LMA is advantageous for growth at elevated CO₂ by compensating for the reduction in leaf nitrogen concentration per unit mass. Enhancement of reproductive yield by elevated CO₂ is often smaller than that expected from vegetative growth. In *Xanthium canadense*, elevated CO₂ did not increase seed production, though the vegetative growth increased by 53%. As nitrogen concentration of seeds remained constant at different CO₂ levels, we suggest that the availability of nitrogen limited seed production at elevated CO₂ levels. We found that leaf area development of plant canopy was strongly

constrained by the availability of nitrogen rather than by CO₂. In a rice field cultivated at free-air CO₂ enrichment, the leaf area index (LAI) increased with an increase in nitrogen availability but did not change with CO₂ elevation. We determined optimal LAI to maximise canopy photosynthesis and demonstrated that enhancement of canopy photosynthesis by elevated CO₂ was larger at high than at low nitrogen availability. We also studied competitive asymmetry among individuals in an even-aged, monospecific stand at elevated CO₂. Light acquisition (acquired light per unit aboveground mass) and utilisation (photosynthesis per unit acquired light) were calculated for each individual in the stand. Elevated CO₂ enhanced photosynthesis and growth of tall dominants, which reduced the light availability for shorter subordinates and consequently increased size inequality in the stand.

Keywords Allocation · Carbon fixation · Competition · Nitrogen availability · Nitrogen use · Scaling up

Introduction

During the last 200 years, atmospheric CO₂ concentration has increased from a pre-industrial level of 280 μmol mol⁻¹ to 370 μmol mol⁻¹ (in 2004). It is still increasing at a rate of 1.5 μmol mol⁻¹ per year and may reach 700 μmol mol⁻¹ at the end of this century (IPCC 2001). Because CO₂ is a substrate for photosynthesis, an increase in atmospheric CO₂ concentration stimulates photosynthetic rates in C₃ plants (Kimball 1983; Cure and Acock 1986; Bazzaz 1990; Poorter 1993; Sage 1994; Curtis 1996; Ward and Strain 1999). However, the effect of elevated CO₂ on growth and reproduction is often much weaker than that predicted by the photosynthetic response. It also differs considerably between species and between plants grown under different conditions

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(Bazzaz 1990; Arp 1991; McConnaughay et al. 1993; Sage 1994; Farnsworth and Bazzaz 1995; Makino and Mae 1999; Ward and Strain 1999; Jablonski et al. 2002). Nutrient availability has been considered one of the key factors for the variation in plant responses to elevated CO₂ (e.g. Ziska et al. 1996; Lutze and Gifford 1998; Kim et al. 2001; Stitt and Krapp 1999; Kimball et al. 2002).

Nitrogen is one of the most important mineral nutrients that limit plant growth in many natural and managed ecosystems (Aerts and Chapin 2000). Since a large fraction of leaf nitrogen is in the photosynthetic apparatus, a strong correlation holds between photosynthetic capacity and nitrogen content of leaves (Evans 1989; Hikosaka 2004). Higher photosynthetic rates at high CO₂ concentrations may lead to an imbalance of carbon and nitrogen in the plant body because carbon acquisition is stimulated relative to nitrogen uptake at elevated CO₂. Accumulated carbohydrates sometimes cause a feedback limitation of photosynthesis (Peterson et al. 1999; Medlyn et al. 1999; Stitt and Krapp 1999).

Plants respond to the availability of limited resources by altering their physiological and morphological characteristics to ameliorate the resource imbalance. At low nutrient availability, for example, plants allocate more biomass to roots, which compensates for low nutrient uptake rates per unit root mass (Brouwer 1962). This contributes to balancing carbon and nitrogen uptake and to the maximisation of relative growth rates (Hirose 1987, 1988; Hilbert 1990). CO₂ responses of plants may also involve adaptive acclimation, which potentially increases plant growth and reproduction at elevated CO₂ levels. Optimality models may be useful to assess the adaptability of plant responses (Anten et al. 2000).

In the GCTE-TEMA program, we studied plant responses to elevated CO₂ at different scales: leaf, whole-plant, canopy, and population. Nitrogen was considered as a key factor to analyse the variation in the CO₂ responses. In this article, we summarise our findings.

Leaf

The photosynthetic rate of a leaf is determined by the amount of leaf nitrogen and its partitioning between photosynthetic and non-photosynthetic proteins, and among the various photosynthetic components (Hikosaka 2004). We studied nitrogen use in leaves grown at elevated CO₂ both theoretically and experimentally.

Nitrogen partitioning in the photosynthetic apparatus in leaves grown at elevated CO₂ concentrations: importance of seasonal acclimation

Light-saturated rates of photosynthesis are limited either by RuBPCase (ribulose-1,5-bisphosphate carboxylase/oxygenase) activity or by the RuBP regeneration process (Farquhar et al. 1980). The former tends to limit pho-

tosynthesis at lower CO₂ concentrations while the latter does so at higher CO₂ concentrations. The capacity of the two processes is considered to co-limit at around the current CO₂ concentration (Wullschlegel 1993). Therefore, under future higher CO₂ concentrations, it is possible that only the RuBP regeneration process will limit photosynthesis. Both RuBP carboxylation and RuBP regeneration processes need a substantial amount of nitrogen to maintain high photosynthetic capacity (Evans and Seemann 1989; Hikosaka 1997). To use nitrogen efficiently, nitrogen should be reallocated from non-limiting to limiting processes (Evans 1989; Hikosaka and Terashima 1995). It has been suggested that nitrogen reallocation from RuBP carboxylation to the RuBP regeneration processes would increase photosynthetic nitrogen use efficiency at elevated CO₂ (Sage 1994; Webber et al. 1994; Medlyn 1996). Using a theoretical model of nitrogen partitioning in the photosynthetic apparatus, Hikosaka and Hirose (1998) suggested that nitrogen reallocation to RuBP regeneration in the doubled CO₂ level would increase photosynthesis by 20%. This prediction was supported by an experimental study using a transgenic rice plant with a reduced amount of RuBPCase (Makino et al. 1997, 2000). When compared at the same nitrogen content, the transgenic rice had greater amounts of proteins in the RuBP regeneration process and higher photosynthetic rates than the wild type at high CO₂ concentrations. In normal plants, however, nitrogen allocation between RuBP carboxylation and regeneration processes does not seem to be influenced by CO₂ concentrations at which plants are grown (Medlyn et al. 1999).

Recent studies, however, have found that growth temperature affects the balance between RuBPCase and the RuBP regeneration process. Hikosaka et al. (1999) demonstrated that *Quercus myrsinaefolia* leaves grown at a low temperature had a higher ratio of RuBP regeneration capacity (expressed as the maximum electron transport rate, J_{\max}) to carboxylation capacity (V_{cmax}) than those grown at a high temperature, and consequently that photosynthesis was more sensitive to CO₂ in plants acclimated to low temperature. A similar trend was found by Wilson et al. (2000), who reported that autumn leaves had a higher ratio of J_{\max}/V_{cmax} than summer leaves in several deciduous tree species from temperate forests. These results suggest that seasonal changes in temperature alter the balance between RuBP carboxylation and RuBP regeneration, which will affect the extent of CO₂ stimulation of photosynthesis.

We tested the hypothesis that seasonal changes in air temperature affect the balance and modulate the CO₂ response of photosynthesis (Onoda et al. 2005). V_{cmax} and J_{\max} were determined in summer and autumn for leaves of *Polygonum cuspidatum* grown at two CO₂ concentrations. The elevated CO₂ concentration reduced both V_{cmax} and J_{\max} without changing the J_{\max}/V_{cmax} ratio. Seasonal environment, on the other hand, altered the ratio such that the J_{\max}/V_{cmax} ratio was higher in autumn than summer leaves. This alteration made the

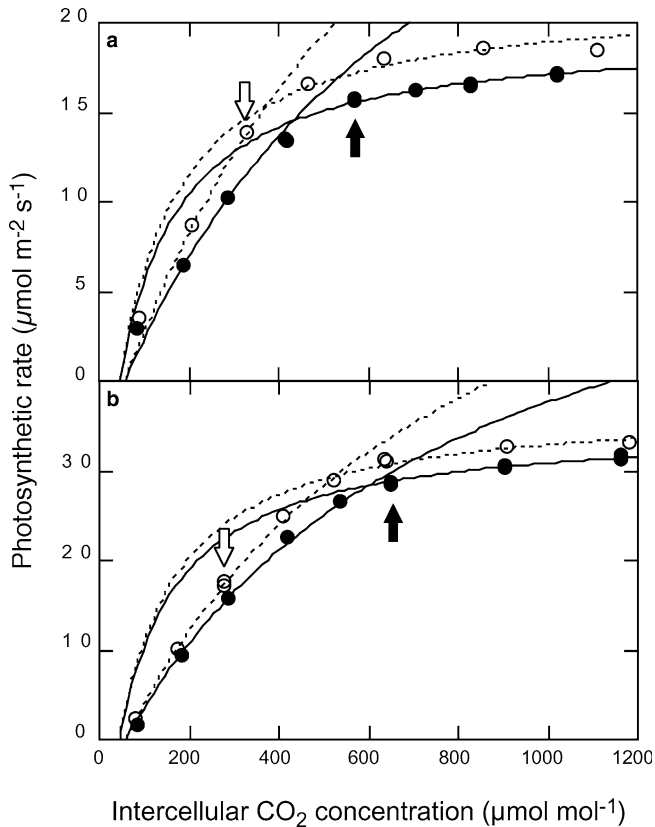


Fig. 1 Photosynthetic rate versus intercellular CO_2 concentration of *Polygonum cuspidatum* grown either at ambient CO_2 ($370 \mu\text{mol mol}^{-1}$, open symbols) or at elevated CO_2 ($700 \mu\text{mol mol}^{-1}$, closed symbols) in August (a) and October (b). The model of Farquhar et al. (1980) was fitted to the observations. Arrows indicate the photosynthetic rate at growth CO_2 concentration

photosynthetic rate more dependent on CO_2 concentration in autumn leaves (Fig. 1). Therefore, when photosynthetic rates were compared at growth CO_2 concentration, the stimulation in photosynthetic rate was larger in autumn than summer leaves. Across the two seasons and the two CO_2 concentrations, V_{cmax} was strongly correlated with RuBPCase and J_{max} with cytochrome *f* content. These results suggest that seasonal changes in climate influence the relative amount of photosynthetic proteins, which in turn affects the CO_2 response of photosynthesis.

Whole plant

Photosynthates acquired by leaves are used for the production of leaves, stems, roots, and reproductive organs. Increase in allocation to the leaf would be beneficial for photosynthesis, but may reduce other functions such as nutrient uptake and reproduction. We studied changes in biomass allocation with CO_2 elevation with respect to the balance between enhanced photosynthesis and other functions.

Maximisation of relative growth rate at elevated CO_2 concentrations

Plants respond to an alteration of nitrogen availability by changing their root/shoot (R/S) ratio. Brouwer (1962) and Davidson (1969) proposed the “functional equilibrium” hypothesis; i.e. the R/S ratio changes to maintain the activity ratio between the shoot and root. According to this hypothesis, any environmental changes that increase root activity would decrease the R/S ratio and any environmental changes that increase shoot activity would increase the R/S ratio. As elevated CO_2 increases photosynthetic activity of the leaf, the functional equilibrium predicts an increase in the R/S ratio and a reduction in leaf mass ratio (LMR, the fraction of plant mass in the leaf) in plants growing at elevated CO_2 . However, LMR in actual plants does not necessarily respond to elevated CO_2 as expected (Stulen and den Hertog 1993; Luo et al. 1999). While some studies showed a reduction in LMR at elevated CO_2 as expected (e.g. Larigauderie et al. 1988; Wilson 1988), others showed that LMR did not change with CO_2 elevation (e.g. Pettersson et al. 1993; Curtis and Wang 1998).

Many studies have shown that leaf mass per unit area (LMA) consistently increases under elevated CO_2 (Poorter et al. 1996; Yin 2002). LMR and LMA are important parameters to describe plant growth. Relative growth rate (RGR, growth rate per unit plant mass) is factorised into three components: $\text{RGR} = \text{NAR} \times \text{LMR}/\text{LMA}$, where NAR is net assimilation rate (growth rate per unit leaf area). This equation indicates that an increase in LMA reduces RGR. Increase in LMA at elevated CO_2 has been ascribed to accumulation of non-structural carbohydrates as a result of a source-sink imbalance (Poorter et al. 1997). However, Luo et al. (1994) suggested a possible advantage of increasing LMA under elevated CO_2 , because it would contribute to increasing leaf nitrogen content per unit area (N_{area}): $N_{\text{area}} = N_{\text{mass}} \times \text{LMA}$, where N_{mass} is leaf nitrogen concentration per unit mass. The decrease in N_{mass} as a result of elevated CO_2 may be compensated for by an increase in LMA to maintain a high N_{area} (Luo et al. 1994; Peterson et al. 1999). However, the effect of increased LMA on whole-plant growth has not been studied (but see Hirose 1987). Hilbert et al. (1991) studied the optimal biomass allocation under elevated CO_2 , but did not consider the effect of LMA.

To test the hypothesis that an increase in LMA at elevated CO_2 benefits plant growth by maintaining a high N_{area} , we raised *P. cuspidatum* at ambient and elevated CO_2 concentrations with three levels of nitrogen availability (Ishizaki et al. 2003). Elevated CO_2 significantly increased LMA but the effect on LMR was small. The increased LMA compensated for the lowered N_{mass} , leading to similar N_{area} between ambient and elevated CO_2 conditions. The effect of change in LMA on RGR was investigated by means of a sensitivity analysis: LMA

values observed at ambient and elevated CO_2 were substituted into a steady-state growth model to calculate RGR. In this model, NAR was assumed to be a function of N_{area} . Allocation of more biomass to roots increased N_{mass} via increased nitrogen uptake, but decreased leaf mass. An increase in LMA increased N_{area} but decreased leaf area. At ambient CO_2 , substitution of a high LMA (observed at elevated CO_2) did not increase RGR, compared with RGR for a low LMA (observed at ambient CO_2), whereas at elevated CO_2 the RGR values calculated for the high LMA were always higher than those calculated for the low LMA. The optimal combination of LMR and LMA to maximise RGR was determined for different CO_2 and nitrogen availabilities (Fig. 2). The optimal LMR was nearly constant, while the optimal LMA increased with CO_2 elevation, and decreased at higher nitrogen availabilities. These results suggest that the increase in LMA contributes to growth enhancement under elevated CO_2 . The changes in LMR of actual plants may be a compensation for the limited plasticity of LMA.

Reproductive growth at elevated CO_2

Although vegetative growth is enhanced by elevated CO_2 , it is not always reflected by an increase in reproductive yield (final mass of the reproductive part). From more than 150 reports on the effect of elevated CO_2 on the reproductive yield of both crop and wild species, Jablonski et al. (2002) found a mean relative yield increase of 12% in fruits and 25% in seeds. These responses were smaller than the response of total plant mass (31%). In some cases, elevated CO_2 even reduced reproductive yield, though vegetative mass was increased (Larigauderie et al. 1988; Fajer et al. 1991; Farnsworth and Bazzaz 1995). Thus, the increase in reproductive yield is not parallel to that in plant growth, and the enhancement of vegetative growth is not a reliable predictor of enhancement of reproductive yield (Ackerly and Bazzaz 1995).

The difference in responses to elevated CO_2 between vegetative growth and reproductive yield should be explained by factors involved in the process of reproductive growth. Reproductive growth is determined not only by biomass production but also by biomass allocation to the reproductive part. We analysed reproductive growth under elevated CO_2 using a simple growth model (Kinugasa et al. 2003). Reproductive mass was expressed as the product of (1) the duration of the reproductive period, (2) the rate of biomass acquisition in the reproductive period, and (3) the fraction of acquired biomass allocated to the reproductive part (Sugiyama and Hirose 1991; Shitaka and Hirose 1998). We raised *Xanthium canadense*, an annual, under ambient and elevated CO_2 concentrations with two nitrogen availabilities. Elevated CO_2 increased reproductive yield at high nitrogen availability, but this increase was caused by increased capsule mass without a significant increase in seed production (Fig. 3). The

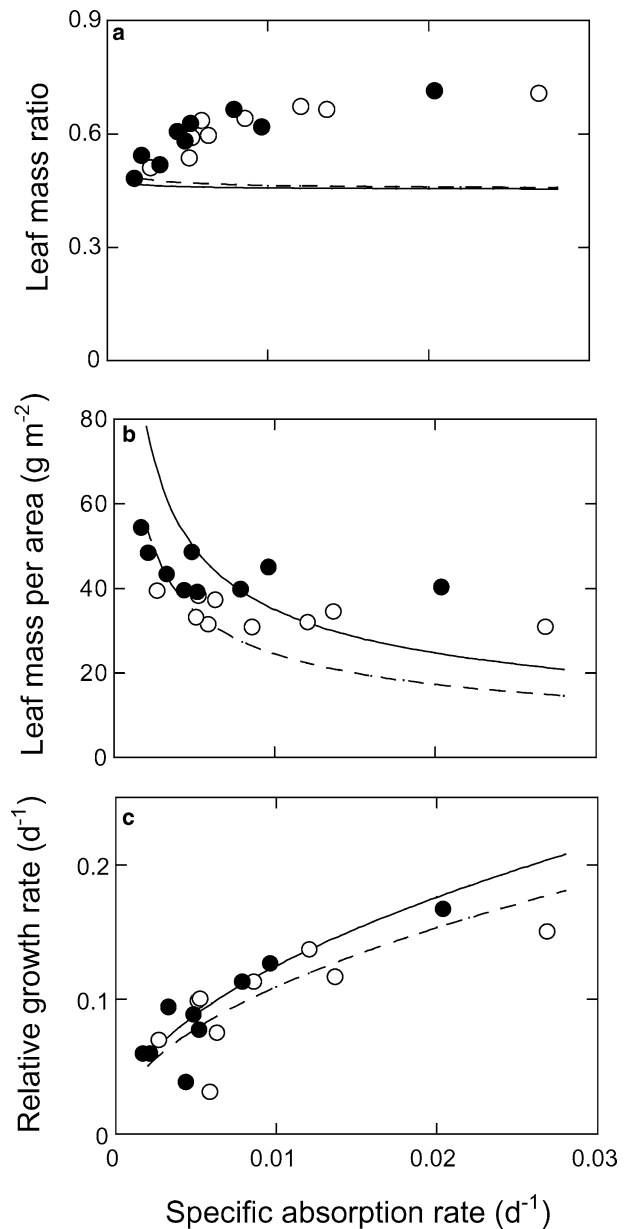


Fig. 2 The optimal leaf mass ratio (a) and leaf mass per unit area (b) that maximise the relative growth rate (c), plotted against specific absorption rate of nitrogen per unit root mass. Lines are the theoretical optimum calculated from the model for 370 (dashed) and 700 (solid) $\mu\text{mol mol}^{-1}$ CO_2 and symbols are data observed for *P. cuspidatum* grown at 370 (open) and 700 (closed) $\mu\text{mol mol}^{-1}$ CO_2 . Redrawn from Ishizaki et al. (2003)

increase in total reproductive mass was due mainly to an increase in the rate of biomass acquisition in the reproductive period, with a delay in leaf senescence. This positive effect was partly offset by a reduction in biomass allocation to the reproductive part at elevated CO_2 . The duration of the reproductive period was not affected by elevated CO_2 .

Seed production was strongly constrained by the availability of nitrogen for seed growth. The nitrogen concentration in seeds was very high in *X. canadense*

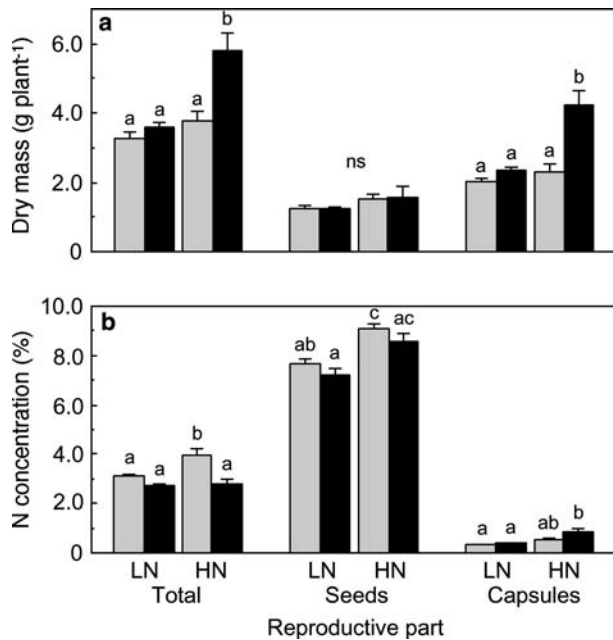


Fig. 3 Dry mass (a) and N concentration (b) of the reproductive part (total, seeds, and capsules) of *Xanthium canadense*. Different letters above columns indicate a significant difference between treatments ($P < 0.05$, Tukey–Kramer method). White bars 360 $\mu\text{mol mol}^{-1}$, black bars 700 $\mu\text{mol mol}^{-1}$ CO₂. LN and HN represent low and high nitrogen availability (12 and 24 mM N), respectively. Redrawn from Kinugasa et al. (2003)

and did not decrease at elevated CO₂ (Fig. 3). On the other hand, capsule production seems to be less constrained by nitrogen availability. Capsules had very low nitrogen concentration and elevated CO₂ increased capsule mass at high nitrogen availability. Interestingly Kimball et al. (2002) reported that the boll (seed + lint) yield of cotton was increased 40% by elevated CO₂ while the lint fiber portion of the yield increased even more, by about 54%. In soybean, elevated CO₂ increased the pod wall mass more than seed yield (Allen et al. 1988). It seems that elevated CO₂ leads to a greater increase in the mass of a reproductive structure with a low nitrogen concentration than a structure with a high nitrogen concentration. This may be one of the reasons for a large variation in CO₂ response of reproductive yield.

Canopy

Leaf canopy is a unit of photosynthesis at the ecosystem level. It is a collection of leaves that are exposed to a large gradient of light availability and have different photosynthetic characteristics depending on their microclimate. An important question is whether enhancement of canopy photosynthesis at elevated CO₂ is solely ascribed to enhanced leaf photosynthetic rate or also involves alteration in canopy structure.

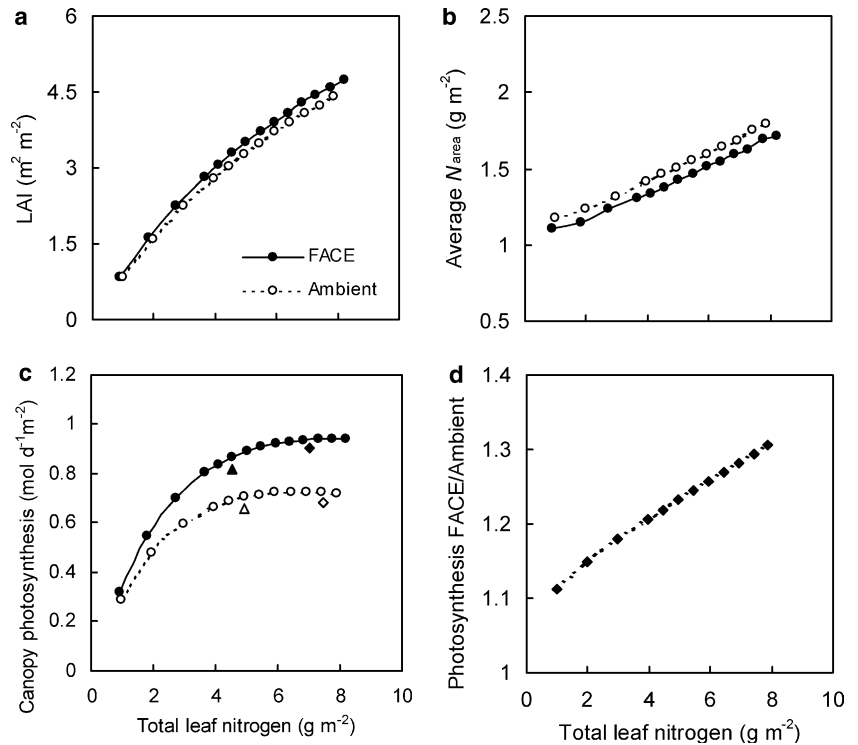
Effect of elevated CO₂ on canopy photosynthesis: does leaf area index respond to growth CO₂?

Reviewing studies on canopy photosynthesis, Drake and Leadly (1991) showed that elevated CO₂ increased canopy photosynthesis in almost all cases. The extent to which canopy photosynthesis increases, however, depends on species and on the availability of other resources (Bazzaz 1990; Arp 1991; McConnaughay et al. 1993). The rate of canopy photosynthesis is affected not only by photosynthetic rates in leaves but also by leaf area index (LAI, leaf area per unit ground area) in the canopy. There are disagreements about the effect of elevated CO₂ on leaf area development: LAI increased with elevated CO₂ in the canopy of perennial ryegrass (Nijs et al. 1988), soybean (Campbell et al. 1990), and rice (Rowland-Bamford et al. 1991), while it remained the same in artificial tropical forest ecosystems (Körner and Arnone 1992) and experimental stands of annuals (Hirose et al. 1996).

Leaf area development is strongly determined by nitrogen availability (Anten et al. 1995). Hirose et al. (1996) found a strong correlation between LAI and aboveground plant nitrogen, regardless of growth CO₂ levels in annual stands, suggesting that an increase in LAI at elevated CO₂ will occur only if plants simultaneously take up more nitrogen, through increased root growth and/or through increased root activity. However, Harz-Rubin and DeLucia (2001) found that vegetation stands under elevated CO₂ had greater LAI even when compared at the same nitrogen uptake. Kim et al. (2001) also found LAI for a given nitrogen uptake to be greater for plants under elevated CO₂, but only when nitrogen uptake itself was high, and not when it was low.

Although an increase in LAI enhances canopy photosynthesis due to increased light interception, when nitrogen in the canopy is limited an increase in LAI reduces leaf nitrogen per unit leaf area, leading to a decline in the photosynthetic capacity of leaves. There exists an optimal LAI at which the canopy photosynthetic rate for a given canopy nitrogen is maximised (Anten et al. 1995; Hirose et al. 1997). It has been shown that predicted LAI values are strongly correlated with measured LAIs (Anten et al. 2000). Anten et al. (2004) applied the concept of optimal LAI to stands of rice grown under free air CO₂ enrichment (FACE). In this experiment, LAI increased with increasing nitrogen availability but was not affected by elevated CO₂. Elevated CO₂ did not affect total plant nitrogen in the stand, but slightly reduced leaf nitrogen per unit ground area due to reduced allocation of nitrogen to leaves. These results indicate that elevated CO₂ increases LAI when compared at the same leaf nitrogen levels, which is consistent with the model prediction (Fig. 4a, b). However, the increase in LAI by elevated CO₂ was only 6–8%, both in the experiment and the prediction, suggesting that nitrogen availability is the most important factor for leaf development even under elevated CO₂.

Fig. 4 The predicted relationship between leaf area index (LAI), canopy photosynthesis and leaf nitrogen in rice stands grown at FACE (free air CO₂ enrichment, ambient plus 200 μmol mol⁻¹ CO₂) and ambient conditions. **a** Optimal LAI for maximum carbon gain as a function of total amount of leaf nitrogen in the canopy (N_{canopy}), **b** associated optimal average leaf nitrogen content (optimal $N_{\text{area}} = N_{\text{canopy}} / \text{optimal LAI}$), and **c** net daily canopy carbon gain. Canopy photosynthesis at actual N_{canopy} is also given in **c**: open symbols ambient CO₂; closed symbols FACE; triangles standard nitrogen (9 g N m⁻²); diamonds high nitrogen (15 g N m⁻²). **d** Ratio of canopy photosynthesis in elevated CO₂ to that in ambient CO₂ (FACE: ambient). Redrawn from Anten et al. (2004)



Anten et al. (2004) further analysed the canopy photosynthetic rates in the rice stands. There are clear indications that the positive effect of elevated CO₂ on canopy carbon gain increases with nitrogen availability (Fig. 4c, d). So far, this interactive effect of CO₂ and nitrogen has been attributed to two mechanisms. First, inhibition of leaf photosynthesis by carbohydrate accumulation at elevated CO₂ tends to be stronger under low than under high nitrogen availability (Rogers et al. 1996). Second, nitrogen uptake increases under elevated CO₂ only when nitrogen availability is high, and not when it is low (Stitt and Krapp 1999). Anten et al. (2004) proposed a mechanism of an interactive effect of nitrogen and CO₂ that is independent of the above two factors. When nitrogen availability is low, the canopy is relatively open and most leaves receive relatively high light. Under these conditions, the effect of elevated CO₂ on canopy photosynthesis will be predominantly through its effect on the light-saturated rate of photosynthesis in the leaves. But as nitrogen availability increases, the canopy becomes denser and lower leaves become increasingly shaded. Under these conditions the enhanced quantum yield under elevated CO₂ will have an increasingly positive effect on canopy photosynthesis (see Fig. 5e in Anten et al. 2004).

Population

Plant population consists of individuals varying in size. Competition for light has been suggested as an important factor for the development of size inequality

(Weiner 1990). Using even-aged, monospecific stands of an annual herb, we studied the effect of elevated CO₂ on competition between individuals and the mechanism of development of size inequality.

Effects of elevated CO₂ on size distribution of individuals in a monospecific stand

Competition among individuals in plant populations are categorised with respect to symmetry in competition: symmetric and asymmetric competition (Weiner 1990). Symmetric competition indicates that individuals in a stand acquire resources in proportion to their size, while in asymmetric competition large individuals acquire more than proportional amounts of resources. It has been suggested that competition for light is asymmetric (Ford and Diggle 1981; Weiner 1986; Jurik 1991; Nagashima 1999; Hikosaka et al. 1999), while that for nutrients is more symmetric (Weiner et al. 1997; Hikosaka and Hirose 2001). The mode of competition is critical to the development of size inequality in the stand. Size inequality is assessed with the coefficient of variation (CV) (Weiner 1990). Symmetric competition, where plant growth is proportional to the size, does not alter size inequality, while asymmetric competition increases size inequality in the stand.

Since diffusion of CO₂ within plant stands is very fast, competition for CO₂ is unlikely to occur among individuals (Jones 1992). Even though elevated CO₂ benefits all individuals in the stand, the enhancement of growth by elevated CO₂ may indirectly alter the mode of competi-

tion (Wayne and Bazzaz 1997). There are two alternative hypotheses in this respect. One is that elevated CO_2 makes the competition more asymmetric and increases size inequality in the stand. It occurs when enhanced growth of larger individuals suppresses light acquisition of smaller individuals. The other is that elevated CO_2 decreases the degree of asymmetry in competition and, consequently, size inequality. This is because the end-product inhibition of photosynthesis due to elevated CO_2 (Stitt and Krapp 1999) may be stronger in larger plants exposed to high light, and because the reduction of the light compensation point of photosynthesis at elevated CO_2 will benefit the smaller shaded individuals more than the larger ones (Osborne et al. 1997).

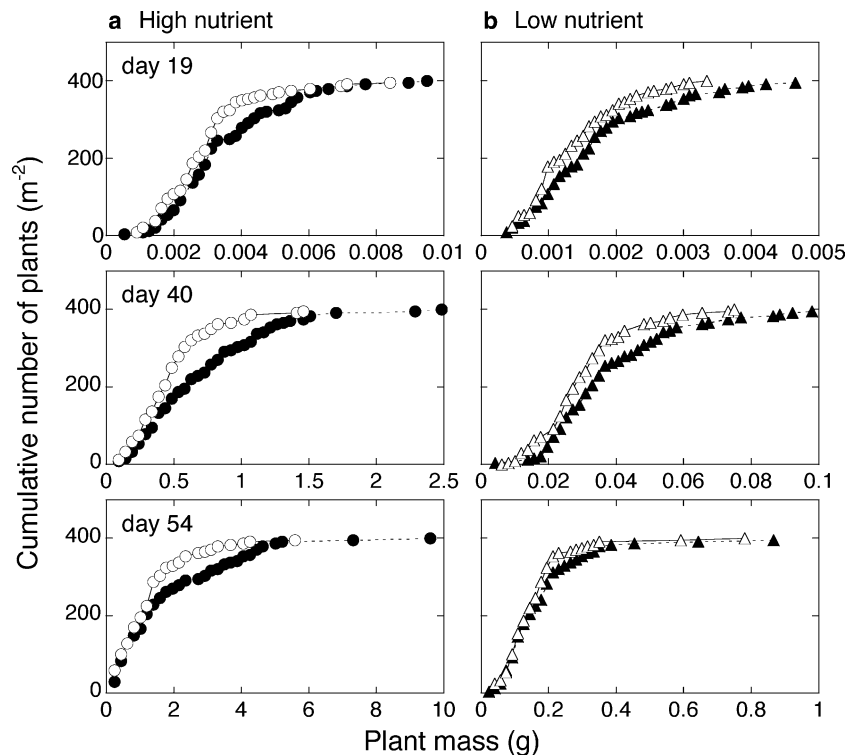
To test these hypotheses, we established even-aged monospecific stands of an annual, *Chenopodium album*, at ambient and double CO_2 levels, with high and low nutrient availabilities in open-top chambers (Nagashima et al. 2003). The growth of individual plants was monitored non-destructively every week until flowering. Elevated CO_2 significantly enhanced plant growth at high nutrient levels, but did not at low nutrient levels. The size inequality represented by CV tended to increase at elevated CO_2 . Size structure of the stands was analysed by the cumulative frequency distribution of plant size (Fig. 5). At early stages of plant growth, CO_2 elevation benefited all individuals and shifted the whole size distribution of the stand to large size classes. At later stages, dominant individuals were still larger at elevated than at ambient CO_2 , but the difference in small subordinate individuals between the two CO_2 levels became smaller. Although these tendencies were found at both

nutrient availabilities, the difference in size distribution between CO_2 levels was larger at high nutrients. The CO_2 elevation did not significantly enhance the growth rate as a function of plant size except for the high nutrient stand at the earliest stage, indicating that the higher biomass at elevated CO_2 at later stages in the high nutrient stand was caused by the larger size of individuals at the earliest stage. Thus, elevated CO_2 seems to increase size inequality in vegetation stands and this effect becomes stronger at high nitrogen availability.

Effects of elevated CO_2 on light competition: an individual-based analysis of light acquisition and utilisation

We then investigated the physiological factors that underlie the effects of elevated CO_2 on the competitive interactions between plants. As mentioned above, difference in size structure results from different size-dependent growth rates of individuals in the stand. In a dense stand, large, dominant individuals have an advantage in capturing light because they place their leaves in the highest, most illuminated parts of the canopy. Small, subordinate individuals, on the other hand, may have an advantage because they need less investment of biomass in support tissues to maintain leaves at lower positions (Givnish 1982). As a result, they can allocate relatively more biomass to leaf area growth, and this can mitigate the negative effects of shading (Anten and Hirose 1998). To indicate the efficiency of biomass-use to capture light, Hirose and

Fig. 5 Comparisons of cumulative frequency distributions of biomass in the even-aged stand of *Chenopodium album* between ambient ($360 \mu\text{mol mol}^{-1}$, open symbols) and elevated ($700 \mu\text{mol mol}^{-1}$, closed symbols) CO_2 concentrations, at high (a) and low (b) nutrient levels (3.6 and $0.36 \text{ g N m}^{-2} \text{ week}^{-1}$, respectively). These results are shown for 19, 40, and 54 days after emergence. Redrawn from Nagashima et al. (2003)

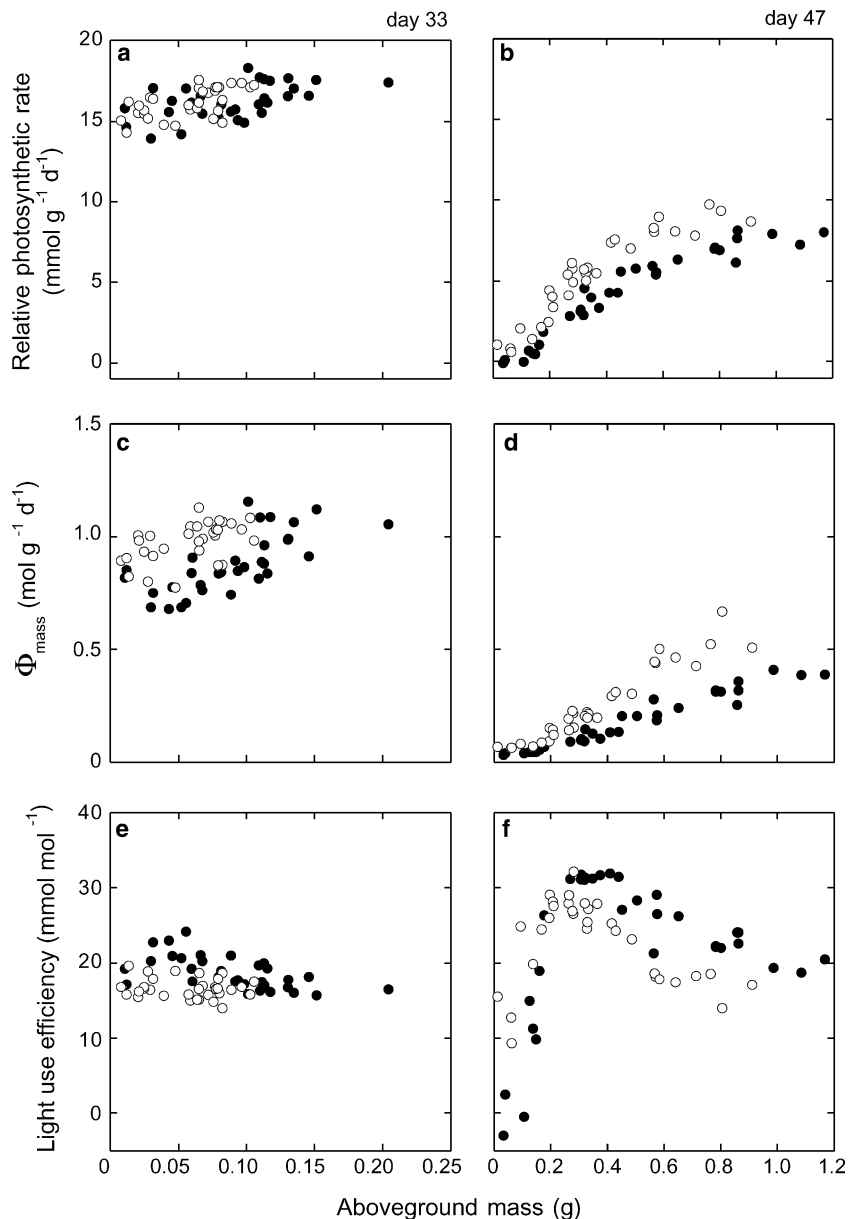


Werger (1995) introduced the parameter Φ_{mass} , defined as photon flux captured per unit above-ground mass. They suggested that Φ_{mass} might not differ between dominant and subordinate species in multispecies systems. However, plant growth is determined not only by the amount of acquired resources, but also by the efficiency of resource use (growth per unit amount of resource acquired). Hikosaka et al. (1999) defined light-use efficiency of photosynthesis (LUE) as photosynthesis per unit photon interception, and described the photosynthesis of individuals as the product of Φ_{mass} and LUE: $\text{RPR} = \Phi_{\text{mass}} \times \text{LUE}$, where RPR is the relative photosynthetic rate (photosynthetic rate per unit above-ground mass). Provided that plant growth is proportional to leaf photosynthesis, RPR is closely related to the relative growth rate (RGR). With a modification of

the canopy photosynthesis model of Hirose and Werger (1987), Hikosaka et al. (1999) estimated the photosynthetic rate of individuals in a natural, monospecific stand of an annual, *X. canadense*. They found that Φ_{mass} was higher in larger individuals, while LUE was highest in intermediate individuals. As a consequence, RPR was high in intermediate and larger individuals, and lowest in smaller individuals.

The model described above was then applied to monospecific stands growing at ambient and at elevated CO_2 (Hikosaka et al. 2003). As in the previous study (Nagashima et al. 2003), we established even-aged stands of an annual, *C. album*, at two CO_2 levels in open-top chambers with sufficient nutrient supply. The whole-plant photosynthesis of every individual in the stand was calculated from (1) the distribution of light

Fig. 6 Relative photosynthetic rates (RPR, whole-plant photosynthetic rate per unit above-ground mass; **a, b**), Φ_{mass} (photon flux captured per unit above-ground mass; **c, d**), and light use efficiency (LUE, photosynthesis per unit captured photon; **e, f**), as a function of above-ground dry mass at 33 (**a, c, e**) and 47 (**b, d, f**) days after emergence. $\text{RPR} = \Phi_{\text{mass}} \times \text{LUE}$. Open circles ambient; closed circles elevated CO_2 ($360 \mu\text{mol mol}^{-1}$ and $700 \mu\text{mol mol}^{-1}$, respectively). Redrawn from Hikosaka et al. (2003)



and leaf nitrogen, and (2) the relationships between photosynthetic parameters and leaf nitrogen content per area. Elevated CO₂ increased light-saturated rates of photosynthesis by 10–15% and the initial slope of the light-response curve by 11%, but had no effect on dark respiration. The relative rate of photosynthesis (RPR, the rate of photosynthesis per unit above-ground mass) was analysed as the product of light capture (Φ_{mass} , the photon flux captured per unit above-ground mass) and light-use efficiency (LUE, plant photosynthesis per unit photon capture) (Fig. 6). At an early stage of stand development (33 days after germination), RPR was nearly constant and no difference was found between ambient and elevated CO₂. However, CO₂ elevation influenced the components of RPR differently. Elevated CO₂ reduced Φ_{mass} , which offset the increase in LUE. Later (47 days), RPR was positively correlated with plant mass at both CO₂ concentrations. When compared at an equal plant mass, RPR was lower at elevated CO₂, which was caused by a reduction in Φ_{mass} despite some compensation by higher LUE. We conclude that elevated CO₂ increases size inequality of a stand through enhanced photosynthesis and growth of dominants, which reduce the light availability for subordinates and consequently increase size inequality in the stand.

Conclusion

Elevated CO₂ enhances photosynthetic rates. The enhanced photosynthesis, however, does not directly lead to increased plant growth and reproduction. As nitrogen uptake is not stimulated as much as carbon uptake, CO₂ elevation alters the C/N balance in the plant body. Plants respond to elevated CO₂ by changing biomass allocation to mitigate the altered C/N balance. Increase in LMA compensates for lowered leaf nitrogen concentration per unit mass to maintain a certain level of leaf nitrogen per unit area. In plants with protein-rich seed, reproductive growth is limited by nitrogen rather than by carbon. Elevated CO₂ does not increase reproductive yield as much as vegetative growth. Proportionate allocation of biomass to reproduction decreases when reproductive growth is limited by nitrogen rather than by carbon. Effects of elevated CO₂ at canopy and population levels are manifested through interactions between light and nitrogen availability, and also through interactions among individuals. In a leaf canopy, leaf area increases with CO₂ elevation when nitrogen uptake is simultaneously increased. If dominant plants increase their leaf area, they will reduce light availability in the lower layers of the canopy and thus the growth of plants there, which makes competition among individuals more asymmetric. Interaction among individuals makes responses to elevated CO₂ fairly sensitive to nitrogen availability in the soil. Integrating these responses would be indispensable for understanding functioning of plants in a high-CO₂ world.

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