

Growth and Photosynthetic Responses of One C₃ and Two C₄ Chenopodiaceae Plants to Three CO₂ Concentration Conditions

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ABSTRACT: Growth and photosynthetic responses of one C₃ and two C₄ plants in the family Chenopodiaceae in three CO₂ concentration ([CO₂]) conditions—low (about 243 μmol mol⁻¹, LC), present (about 378, PC), and high (about 465, HC)—were investigated in open top chambers. The relative growth rate (RGR) and net assimilation rate in the C₃ plant, *Chenopodium album*, increased with increasing [CO₂], though the RGR was not enhanced significantly in the HC condition. The leaf area ratio and leaf weight ratio of the C₃ plant drastically decreased with increasing [CO₂], suggesting that the C₃ plant invests more biomass to leaves in lower [CO₂] conditions. The two C₄ plants, *Atriplex glauca* and *A. lentiformis*, showed relatively small changes in those growth parameters. These photosynthetic-pathway-dependent responses suggest that growth patterns of C₃ and C₄ plants have been altered by past increases in atmospheric [CO₂] but that there will be relatively little further alteration in the future high-CO₂ world.

Key words: C₃ plant, C₄ plants, Growth analysis, High CO₂, Low CO₂, Net photosynthetic rate

INTRODUCTION

The atmospheric CO₂ concentration fluctuates over time: it was only 160 to 200 μmol mol⁻¹ at the Last Glacial Maximum, about 20,000 years ago (Delmas et al. 1980, Barnola et al. 1987), whereas in the preindustrial age, the concentration was about 280 μmol mol⁻¹ and since then it has been steadily increasing (Nefel et al. 1985), reaching a concentration of about 379 μmol mol⁻¹ in 2005 (IPCC WG I 2007). This increase is likely to continue due to human activities and, even under the most optimistic scenario, it is predicted that the atmospheric CO₂ concentration will be at least 450 μmol mol⁻¹ by the end of the 21st century (IPCC WG I 2007). Many studies on the effects of high growth CO₂ concentration ([CO₂]) on plants have been performed on crop plants and horticultural species, which are mainly C₃ species, and aimed to predict the effects of increased CO₂ on food availability in the future high-CO₂-world (e.g. Acock and Allen 1985, Cure 1985). Effects of high [CO₂] on wild C₃ plants have also been studied to estimate vegetation changes in the future (e.g. Bazzaz et al. 1985, Oechel and Strain 1985, Bazzaz 1990, Ceuleman and Mousseau 1994, Raschi et al. 1997). Most previous studies have shown that, without water and nutrient deficits, plant biomass and net photosynthetic rates will increase with increasing [CO₂], although there is large interspecific variation in degree of the increase. On the other hand, only a limited number of studies have examined responses of C₄ species

to high [CO₂] (but see Poorter 1996, Ward and Strain 1997, Wand et al. 1999).

It is often suggested that C₄ photosynthesis evolved under low [CO₂] conditions in the glacial age (7 million years ago) and that C₄ plants had an advantage over C₃ plants under past low [CO₂] condition (e.g. Ehleringer et al. 1991, Ehleringer et al. 2005). This speculation is derived from a general observation that, compared with C₃ plants, C₄ plants have higher net photosynthetic rates at a lower CO₂ saturation concentration of around 100 μmol mol⁻¹ intercellular concentration (e.g. Ehleringer et al. 1991). However, as there have been only a limited number of comparative studies on the growth responses of C₃ and C₄ plants to low [CO₂] (but see Polley et al. 1992, Polley et al. 1993, Dippery et al. 1995, Tissue et al. 1995, Ward and Strain 1997, Cowling and Sage 1998, Wand et al. 1999), the growth responses of both C₃ and C₄ plants to various [CO₂] values must be clarified to investigate the advantage of C₄ over C₃ photosynthesis under historical atmospheric [CO₂] regimes and to understand the evolution of C₄ photosynthesis (e.g. Ehleringer 2005).

The objective of the present study was to clarify differences between C₃ and C₄ plants in growth responses to various [CO₂] growth conditions to investigate the proposed advantage of C₄ photosynthesis under historical [CO₂]. The relative performances of C₄ and C₃ plants were examined in growth experiments under three different [CO₂] regimes approximating atmospheric conditions in the preindustrial period, in the present and in the predicted future.

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Many previous studies have made comparisons among plant species belonging to different families, making direct comparisons of growth responses difficult, as there are often large differences of morphology between plants belonging to different families, and this innate difference may affect the results of growth analysis. Thus, for a more straightforward comparison of growth responses, I employed herbaceous plant species belonging to the same family in this study. Responses to variation in $[\text{CO}_2]$ were investigated in the plant seedling stage because the earliest stage in the plant life cycle is the most sensitive to environmental factors. Plant individual growth in this stage is one of the most important of the factors that determine whole plant population dynamics and thus plant productivity (Harper 1977).

MATERIALS AND METHODS

Plant Materials

Three plant species in the family Chenopodiaceae were used for the experiment: *Chenopodium album* L., a C_3 annual cosmopolitan herb, and *Atriplex glauca* and *Atriplex lentiformis*, C_4 perennial herbs. Seeds of *C. album* were collected from an experimental field in the National Institute for Environmental Studies, Tsukuba, Japan ($140^\circ 07' \text{ E}$ and $36^\circ 04' \text{ N}$) and dried and kept frozen at -30° C until use. Seeds of *A. glauca* and *A. lentiformis* were imported from a seed company (Heritage Seed, NSW, Australia) and kept frozen at -30° C until use.

Seeds were sown in plastic trays ($15 \times 10 \text{ cm}$ and 3 cm height) filled with peat, and set in an artificially lit (fluorescent tubes) incubator (MLR350, SANYO, Japan) with $25/15^\circ \text{ C}$ (day/night) air temperature and a photoperiod of 12 h with $150 \mu\text{mol m}^{-2} \text{ s}^{-1}$ photosynthetically active photon flux density (PPFD). After about three weeks, each of the established plants was transplanted into a plastic pot (10 cm height and 10 cm diameter) filled with vermiculites and placed in the experimental field in University of Tsukuba ($140^\circ 07' \text{ E}$ and $36^\circ 04' \text{ N}$). These plant materials were cultivated under full sunlight condition and irrigated with 1/2000 strength Hyponex solution (Hyponex licensee, Japan) every three to four days to achieve maximum soil-water content. The Hyponex solution contains about 2.3 mM nitrogen (0.3 mM ammonium nitrogen and 2.0 mM nitrate-formed nitrogen), 0.2 mM water-soluble phosphate, 0.96 mM potassium and trace amounts of micronutrients.

Open Top Chamber System

In this study the plant materials were exposed to three different $[\text{CO}_2]$ by enclosing each of the plants individually in a 45 cm high open top chamber (OTC) made of a transparent plastic sheet with 3.6 L internal space. Thus, the number of plant replications was

equal to the number of chamber replications. Each of the OTCs was aerated continuously at about 1.8 L min^{-1} , so estimated flushing time was 2.0 minutes.

For the low CO_2 treatment (LC), the CO_2 concentration of the inlet air was reduced by soda-limes. For the high CO_2 treatment (HC), the inlet air was sampled from a transparent plastic container (about 70 L) filled with about 60 L mixed litter of *Castanea crenata* and *Quercus dentata*. The litter was irrigated once a week with tap water. For the present CO_2 treatment (PC), the external air was supplied directly. For each of the treatments, CO_2 concentrations inside the OTCs were measured using a gas analyzer system (KIP8510, Koito, Japan) by sampling the air at 20 cm height inside of two to five randomly-selected OTCs per treatment. The CO_2 concentrations were measured in the daytime period (12:00 to 18:00 JST, mainly around 13:00 JST) every day. The average $[\text{CO}_2]$ in the HC was $465 \pm 44 \text{ SD } \mu\text{mol mol}^{-1}$, whereas that in the PC was 378 ± 32 , and that in the LC was 243 ± 49 in the daytime during the experimental period.

I monitored the air temperatures at 20 cm height inside three OTCs per each treatment using copper-constantan thermocouples (0.1 mm diameter) covered with aluminum shelters. The PPFD outside the OTCs was measured with a quantum sensor (IKS-25, Koito, Kanagawa, Japan), which was set at 1 m height. These environmental factors were monitored and stored in a data logger (Thermocac-EF, Etoh, Tokyo, Japan). Almost all of the days during the experimental period (15 days in late summer) were clear and the light interception of the transparent sheet of the OTCs was negligible. Thus, I estimated the average daily PPFD inside the OTCs to be same as that of the outside: $27 \pm 7 \text{ SD mol m}^{-2} \text{ day}^{-1}$. Average air temperatures in the OTCs during the experimental period were $26 \pm 8 \text{ SD } ^\circ \text{ C}$ and did not differ significantly among the treatments (post-hoc test by Scheffe's S). The main sources of variation in the environmental factors were diurnal and day-to-day variation.

Growth Experiments

Each of the plant materials was enclosed in the OTC for 15 days and irrigated with about 250 mL of 1/2000 strength Hyponex solution every day. The experimental period was set as 15 days because the study species are fast-growing and thus growth analysis must be applied in the early stages of plant growth to avoid effects of self-shading as much as possible. The OTC systems were placed in the experimental field at the University of Tsukuba, Japan ($140^\circ 07' \text{ E}$ and $36^\circ 04' \text{ N}$) under full sunlight conditions. Plants harvested at the beginning and the end of the exposure experiments were cut into roots, stems and leaves and were weighed after being oven-dried for a week at 70° C . Total leaf area of individual plants was measured before drying.

Growth analysis was conducted using data obtained from the initial and final harvests. Relative growth rates (RGR, g g⁻¹ day⁻¹) for whole plants, net assimilation rates (NAR, g m⁻² day⁻¹) and leaf area ratios (LAR, m² g⁻¹) were calculated using the following equations:

$$\text{RGR} = (\ln(W_f) - \ln(W_i)) / T_m \quad (1)$$

$$\text{NAR} = (W_f - W_i) (\ln(L_f) - \ln(L_i)) / (L_f - L_i) / T_m \quad (2)$$

$$\text{LAR} = (L_f / W_f + L_i / W_i) / 2 \quad (3)$$

where W and L are plant total dry weight and leaf area at the initial (i) and the final harvesting (f) respectively, and T_m is the growth period in days (15 days in the present study). Specific leaf area (SLA, m² g⁻¹) and dry weight ratios of roots, stems and leaves to the total dry weight were also calculated from the data obtained at the final harvesting.

The relationship between RGR, NAR and LAR is expressed by the following equation:

$$\text{RGR} \approx \text{NAR} \cdot \text{LAR} \quad (4)$$

Furthermore, LAR is divided into two parameters:

$$\text{LAR} \approx \text{SLA} \cdot \text{LWR} \quad (5)$$

where LWR is the leaf weight ratio, the ratio of leaf dry weight to total dry weight.

NAR is divided into five physiological components (Iwaki, 1958):

$$\text{NAR} = p - r_f - r_c / \text{SLA} \cdot C/F \quad (6)$$

where p and r_f are the amounts of photosynthesis and leaf respiration per unit leaf area, respectively, r_c is the respiration of non-photosynthetic organs per unit dry weight, and C/F is the dry weight ratio of the photosynthetic organ to non-photosynthetic organ. I define the C/A ratio, the ratio of the weight of non-photosynthetic organs to leaf area as follows:

$$C/A = 1 / \text{SLA} \cdot C/F \quad (7)$$

Then, equation (6) is rewritten as:

$$\text{NAR} = p - r_f - r_c \cdot C/A \quad (6')$$

Measurement of Maximum Photosynthetic Rate

Leaf samples (about 1 to 10 cm² each) were harvested from just-matured leaves of the plant materials on the last day of the growth experiment and were used for the measurement of maximum pho-

tosynthetic rate (P_{max}) using a leaf disc oxygen electrode (LD2, Hansatech Instruments Ltd., Norfolk, UK). The measurement was performed at 30°C leaf temperature, 1,500 μmol m⁻² s⁻¹ PPFD and 5~7% CO₂ concentration.

All statistical treatments were done using Super ANOVA (Abacus Concept Inc., Berkeley, CA, USA), and values of minimum significance level (P) were calculated. If these values are lower than 0.05, the differences between the mean values are statistically significant.

RESULTS

Growth Analysis

The initial and final total dry weights of the plant materials are summarized in Table 1. Although the experimental period of 15 days was relatively short compared to the life cycles of the plant materials (one year or more), total dry weights at the final harvesting were 3 to 5 times higher than those at the initial harvesting. Thus, growth analysis for plant initial growth could be applied to the present experimental results.

Responses of RGR, NAR and LAR to the three [CO₂] treatments are shown in Fig. 1. Over all, RGRs in the three plants in LC were significantly lower than those in PC (ANOVA, P < 0.0001) and the RGR values in the LC were not different among the three species (post-hoc test by Scheffe's S). However, compared to the value in the PC, the percentage of reduction of RGR in the LC in the C₃ plant, *C. album* (21%), was higher than that of the two C₄ plants, *A. glauca* and *A. lentiformis* (14% and 18%, respectively). On the

Table 1. Total dry weights in the three species at initial and final harvest. The average growth CO₂ concentrations were 243 (LC) 378 (PC), and 465 (HC) μmol mol⁻¹. In each treatment for each species, mean values with a symbol (*) are significantly different from initial dry weight (ANOVA with Bonferroni Test, P < 0.001). Standard deviations are shown in parentheses. n = 6 to 13.

	Total dry weight (g)			
	Initial harvest	Final harvest		
		LC	PC	HC
<i>Chenopodium album</i> (C ₃)	0.32 (0.15)	1.44* (0.31)	2.67* (0.47)	2.90* (0.45)
<i>Atriplex glauca</i> (C ₄)	0.18 (0.07)	0.61* (0.31)	0.90* (0.46)	0.84* (0.41)
<i>Atriplex lentiformis</i> (C ₄)	0.21 (0.13)	0.67* (0.22)	1.00* (0.36)	1.12* (0.44)

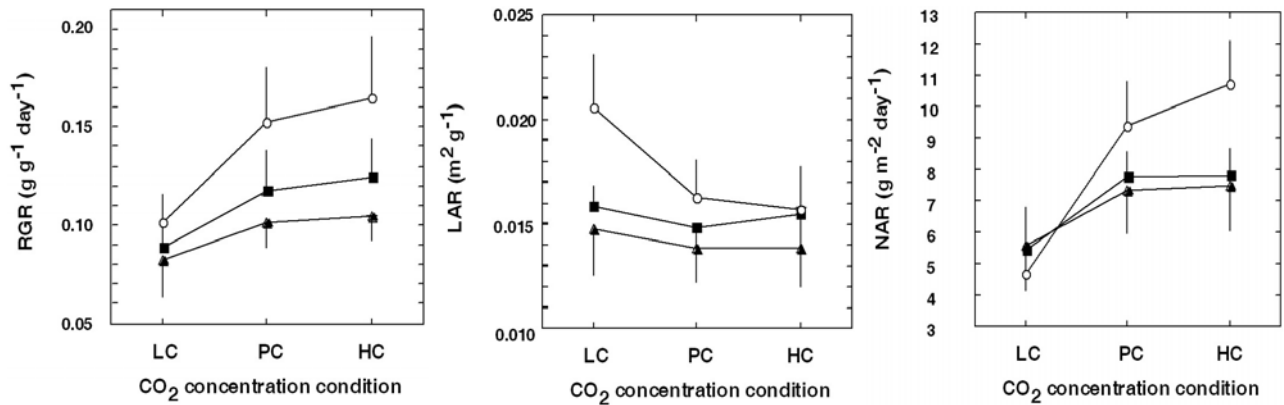


Fig. 1. Responses of relative growth rate (RGR, upper), leaf area ratio (LAR, middle), and net assimilation rate (NAR) of *Chenopodium album* (C₃, ○), *Atriplex glauca* (C₄, ▲) and *A. lentiformis* (C₄, ■) to three growth CO₂ concentration conditions. The average CO₂ concentrations were 243 (LC), 378 (PC), and 465 (HC) $\mu\text{mol mol}^{-1}$. Vertical bars represent standard deviations. $n = 6$ to 13.

other hand, no significant enhancement of RGR by the HC was detected in neither the C₃ nor the C₄ plants (post-hoc test by Scheffe's S).

In *C. album*, NAR increased significantly with increasing [CO₂] (ANOVA, $P < 0.0001$), and that in the LC was only half of that in the PC (post-hoc test by Scheffe's S, $P < 0.0001$). The NAR value in the HC was 1.1 times that in the PC, though this difference is not statistically significant (post-hoc test by Scheffe's S, $P < 0.034$). In each of the two C₄ plants, NAR values in the LC were much lower than those in the PC and HC (post-hoc test by Scheffe's S, $P < 0.0006$) and those in the PC and the HC were not significantly different (post-hoc test by Scheffe's S).

In *C. album*, the response pattern of LAR to [CO₂] was completely different from that of NAR. LAR in this C₃ plant decreased with increasing [CO₂] (ANOVA, $P < 0.0001$) and LAR in the LC was about 1.3 times higher than that in the PC (post-hoc test by

Scheffe's S, $P < 0.0001$). On the other hand, in each of the two C₄ plants, LAR values did not differ among the three treatments.

Enhanced LWR was observed in *C. album* in the LC, together with reductions in SWR and RWR (ANOVA, $P < 0.0001$) (Fig. 2). The two C₄ plants did not show any significant difference in dry weight allocation among the three treatments.

The SLA in the LC in *C. album* was significantly higher than that in the higher [CO₂] treatments (post-hoc test by Scheffe's S, $P < 0.0003$) (Fig. 3), and the C/A ratio in *C. album* increased with increasing [CO₂] (ANOVA, $P < 0.0001$). The two C₄ species showed little difference in SLA and C/A ratio among the three treatments.

Maximum Photosynthetic Rate

C. album showed no significant difference in P_{max} among the three treatments (post-hoc test by Scheffe's S), although the value

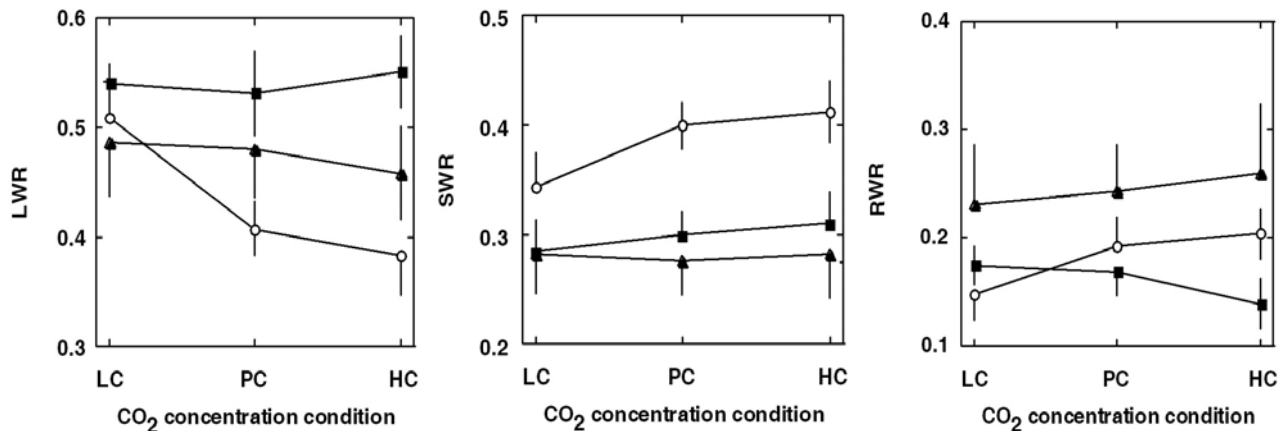


Fig. 2. Responses of leaf weight ratio (LWR, upper), shoot weight ratio (SWR, middle) and root weight ratio (RWR, lower) of *Chenopodium album* (C₃, ○), *Atriplex glauca* (C₄, ▲) and *A. lentiformis* (C₄, ■) to three growth CO₂ concentration conditions. The average CO₂ concentrations were 243 (LC), 378 (PC), and 465 (HC) $\mu\text{mol mol}^{-1}$. Vertical bars represent standard deviations. $n = 6$ to 13.

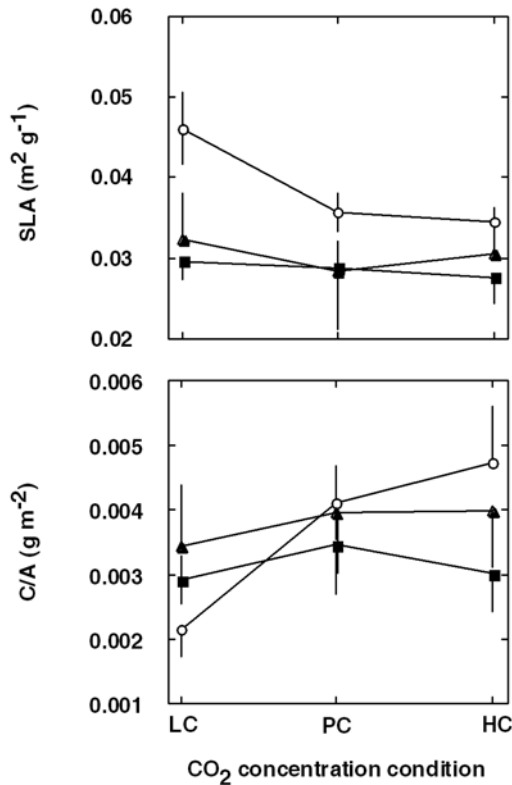


Fig. 3. Responses of specific leaf area (SLA, upper) and C/A ratio (lower) of *Chenopodium album* (C₃, ○), *Atriplex glauca* (C₄, ▲) and *A. lentiformis* (C₄, ■) to three growth CO₂ concentration conditions. The average CO₂ concentrations were 243 (LC), 378 (PC), and 465 (HC) μmol mol⁻¹. Vertical bars represent standard deviations. *n* = 6 to 13.

in the LC was slightly lower (Fig. 4). The two C₄ species also showed no significant difference in P_{\max} among the three treatments. P_{\max} values in the two C₄ species were always higher than those in the C₃ plant, *C. album*, independent of the treatment (ANOVA, $P < 0.0001$).

DISCUSSION

Past increases in global [CO₂] must have already affected the morphology and growth activity of C₃ plants by altering parameters such as RGR, NAR, LAR, LWR, SLA and C/A ratios in the plants' initial growth stage. The high [CO₂] conditions predicted to occur in the future, which were estimated to peak [CO₂] at about 465 μmol mol⁻¹ in this study, will also affect growth parameters, as the increase in NAR and the decreases in LAR and LWR of the C₃ plant seedlings in this study were significant, though the changes may be relatively small. On the other hand, the results of this study suggest that it is unlikely that the increase in global [CO₂] in the future will

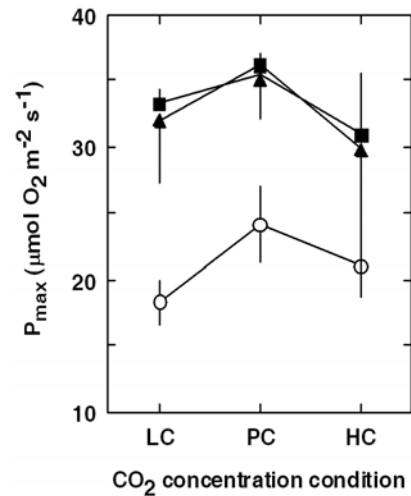


Fig. 4. Responses of maximum photosynthetic rate (P_{\max}) of *Chenopodium album* (C₃, ○), *Atriplex glauca* (C₄, ▲) and *A. lentiformis* (C₄, ■) to three growth CO₂ concentration conditions. The average CO₂ concentrations were 243 (LC), 378 (PC), and 465 (HC) μmol mol⁻¹. Vertical bars represent standard deviations. *n* = 4 to 6.

have significant effects on the morphology, growth and photosynthetic activities of C₄ plants in the initial growth stage. My results suggest that the advantage of C₄ plants over C₃ plants under past low [CO₂] may result from the relatively small reduction of NAR (Fig. 1) and their ability to maintain high photosynthetic capacity under low [CO₂] conditions (Fig. 4), which together kept the reduction of RGR relatively small in the initial growth stage (Fig. 1). This interpretation is also supported by the large reduction of RGR in the LC and the reduction of NAR in the C₃ plant seedlings.

However, the enhancement of LAR (Fig. 1), LWR (Fig. 2) and SLA (Fig. 3) in *C. album* (C₃) in the LC suggests that the physiological disadvantage of C₃ plants under low [CO₂] may be compensated for by morphological changes to some extent. Compensation for decreasing NAR under low [CO₂] by morphological adjustment was also found in *Abutilon theophrasti*, a C₃ annual (Dippery et al. 1995). However, the generality of such morphological adjustments by C₃ plants under low [CO₂] is still unclear because of the quite limited number of studies.

It is often supposed that the low [CO₂] in the glacial and preindustrial periods favored C₄ plants because of their high photosynthetic rate under low [CO₂] (Ehleringer et al. 1991, Ehleringer et al. 1997, Ehleringer et al. 2005). However, the results of this study suggest that, at the whole-plant level, the advantage of C₄ plants over C₃ plants under low [CO₂] is not large.

As shown in equation (5), LAR is composed of two parameters, SLA and LWR. The enhancement of LAR in *C. album* in the LC

(Fig. 1) was due to increases in LWR (Fig. 2) and SLA (Fig. 3). In many C_3 plants, LAR and SLA decreases and LWR remains unchanged at about $700 \mu\text{mol mol}^{-1}$ [CO_2] (Poorter et al. 1996, Poorter and Navas 2003). These results suggest that the reduction of LAR and the increase of SLA with increasing [CO_2] are general features of C_3 plants. On the other hand, as few studies have been conducted of responses to low [CO_2], an increase of LWR under low [CO_2] has only been reported for *Abutilon theophrasti* (Dippery et al. 1995). The generality of the LWR response to low [CO_2] should be confirmed in other C_3 plants.

It is unlikely that the reduction of NAR in *C. album* in the LC (Fig. 1) was derived from the alternation of photosynthetic activity by the low [CO_2], because there was no significant change in P_{max} (Fig. 4). From equation (6'), I infer that r_c may have been enhanced by the low [CO_2], because the C/A ratio was reduced in the LC (Fig. 3), and $p-r_f$ can be related to P_{max} , which did not change significantly (Fig. 4). Some studies of photosynthetic responses of C_3 plants to low [CO_2] show reduced P_{max} as well as a reduced apparent photosynthetic rates (Overdieck 1989, Sage and Reid 1992, Tissue et al. 1995). The differences between my results and the results of previous studies suggest that photosynthetic responses to low [CO_2] may vary among species. Substantial interspecific variation in plant respiratory responses to high [CO_2] has also been reported (Amthor 1991, Ceuleman and Mousseau 1994) and little is known about the effects of low [CO_2] on plant respiration. Thus, further studies should be done to clarify how respiratory and photosynthetic responses to various [CO_2] are related, considering the mitochondrial response summarized in Drake et al. (1999).

In many C_3 species, including herbaceous (Poorter 1993) and some woody species (Poorter 1993, Callaway et al. 1994, Farnsworth et al. 1996, Koike et al. 1996), high [CO_2] (about $700 \mu\text{mol mol}^{-1}$) causes significant reductions in LAR, which cancel out the increases in the NAR, resulting in no significant increase in RGR. Although I did not detect statistically significant differences in the responses of NAR and LAR to HC (about $465 \mu\text{mol mol}^{-1}$) vs. other conditions in *C. album*, these results suggest that morphological adjustment is a general feature of C_3 plants' responses to high [CO_2]. Higher [CO_2], such as $700 \mu\text{mol mol}^{-1}$, which has often been used in the previous studies, may result in greater increases of biomass in C_3 species if the reduction in LAR is not greater than the increase in NAR.

It is unlikely that C_4 photosynthesis has a significant advantage over C_3 photosynthesis under [CO_2] higher than the present atmospheric concentration, because there was no significant increase in RGR and NAR in the two C_4 plants in the HC, and the values were much lower than those of the C_3 plant (Fig. 2). Although P_{max} values of the C_4 plants were much higher than those of the C_3 plant

(Fig. 5), these did not result in higher RGR or NAR in the C_4 plants in the HC (Fig. 1). These results are consistent with previous reports that high [CO_2] had little effect on growth parameters in C_4 plants (Acock and Allen, 1985, Polley et al. 1992, Tissue et al. 1995, Poorter et al. 1996; Wand et al. 1999, Poorter and Navas 2003).

The reduction in relative leaf quantity with increasing [CO_2] in *C. album* (Fig. 2) suggests that changing [CO_2] may alter the competitive relationships between C_3 and C_4 plants by changing the shading ability of C_3 species. Shading by leaves is one of the critical factors determining the result of competition in plants (e.g. Tremmel and Bazzaz 1993, Tremmel and Bazzaz 1995).

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