

Photosynthetic down-regulation in *Larrea tridentata* exposed to elevated atmospheric CO₂: interaction with drought under glasshouse and field (FACE) exposure

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ABSTRACT

The photosynthetic response of *Larrea tridentata* Cav., an evergreen Mojave Desert shrub, to elevated atmospheric CO₂ and drought was examined to assist in the understanding of how plants from water-limited ecosystems will respond to rising CO₂. We hypothesized that photosynthetic down-regulation would disappear during periods of water limitation, and would, therefore, likely be a seasonally transient event. To test this we measured photosynthetic, water relations and fluorescence responses during periods of increased and decreased water availability in two different treatment implementations: (1) from seedlings exposed to 360, 550, and 700 $\mu\text{mol mol}^{-1}$ CO₂ in a glasshouse; and (2) from intact adults exposed to 360 and 550 $\mu\text{mol mol}^{-1}$ CO₂ at the Nevada Desert FACE (Free Air CO₂ Enrichment) Facility. FACE and glasshouse well-watered *Larrea* significantly down-regulated photosynthesis at elevated CO₂, reducing maximum photosynthetic rate (A_{max}), carboxylation efficiency (CE), and Rubisco catalytic sites, whereas droughted *Larrea* showed a differing response depending on treatment technique. A_{max} and CE were lower in droughted *Larrea* compared with well-watered plants, and CO₂ had no effect on these reduced photosynthetic parameters. However, Rubisco catalytic sites decreased in droughted *Larrea* at elevated CO₂. Operating C_i increased at elevated CO₂ in droughted plants, resulting in greater photosynthetic rates at elevated CO₂ as compared with ambient CO₂. In well-watered plants, the changes in operating C_i , CE and A_{max} resulted in similar photosynthetic rates across CO₂ treatments. Our results suggest that drought can diminish photosynthetic down-regulation to elevated CO₂ in *Larrea*, resulting in seasonally transient patterns of enhanced carbon gain. These results suggest that water status may ultimately control the photosynthetic response of desert systems to rising CO₂.

Key-words: *Larrea tridentata* (Creosotebush); A–C_i response; drought; elevated CO₂; FACE; Mojave Desert; NDFF; photosynthetic down-regulation; transient CO₂ response.

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INTRODUCTION

It is increasingly important to examine the responses of terrestrial plants to changing levels of atmospheric CO₂ in light of current predictions of a doubling in CO₂ concentration that may occur within the next century (Bazzaz 1990). Increasing atmospheric CO₂ overcomes biochemical limitations by enhancing CO₂ concentration at the site of carboxylation in C₃ plants and by reducing the oxygenase activity of Rubisco, resulting in increased rates of carbon fixation (Sharkey 1985). Thus, for plants exposed to elevated CO₂, leaf photosynthetic rates initially increase (Bowes 1991). However, for many C₃ species, long-term growth at elevated CO₂ eventually results in photosynthetic rates that are only marginally enhanced, or even lower than those observed prior to elevated CO₂ exposure (Sage 1994). This long-term, down-regulation response generally results in a reduced leaf nitrogen content and a decreased amount of protein associated with the photosynthetic apparatus (Sage, Sharkey & Seemann 1989; Kuehny *et al.* 1991; Sage 1994; Jacob, Greitner & Drake 1995). The mechanisms underlying this long-term downward adjustment of photosynthesis to elevated CO₂ are not well understood, and the developed hypotheses are based on data collected from glasshouse-grown, potted plants. Data from intact plants in field conditions may provide crucial insights into the mechanisms associated with photosynthetic down-regulation and growth in elevated CO₂ (Jacob *et al.* 1995; Lewis, Tissue & Strain 1996).

Large increases in leaf carbohydrate amounts (up to two times for starch in tobacco) occur in plants exposed to elevated CO₂ (Long & Drake 1992), and carbohydrate accumulation in source tissues is often associated with inhibition of photosynthesis (Azcon-Bieto 1983; Tissue, Thomas & Strain 1993). Increased carbohydrate metabolism in source leaves may produce a regulatory signal that results in decreased photosynthetic gene expression readjusting source–sink imbalance (Van Oosten & Besford 1996). When sink activity is enhanced, such as when root growth restrictions are removed (typical characteristics associated with the down-regulation response), photosynthetic acclimation typically decreases (Thomas & Strain 1991). Therefore, the inability

of sink tissues to maintain a sufficient demand for use of photosynthetic end products potentially explains the down-regulation response of photosynthesis to elevated CO₂ (Stitt 1991). Additionally, environmental conditions such as drought may reduce both sink activity and leaf water status to such low levels that accumulation of photosynthetic end products does not occur, resulting in a decrease in photosynthetic down-regulation (Oechel *et al.* 1995). Data from intact plants exposed to elevated CO₂ in the field may provide additional insight on the mechanisms of long-term photosynthetic response to elevated CO₂, as plants grown in the field provide a normal relationship between sink and source tissues.

Seasonal patterns of photosynthetic down-regulation may have been affected by environmental conditions in *Pinus taeda* and *Scirpus olneyi* grown at elevated CO₂ in open-top chambers (Jacob *et al.* 1995; Lewis *et al.* 1996). Environmental stress may impose modifications on the responses of terrestrial plants to increased CO₂ (Field *et al.* 1992; Curtis 1996; Sage 1996). Because sink strength can interact with the stimulation of photosynthesis to regulate whole plant responses to elevated CO₂ (Thomas & Strain 1991; Barrett & Gifford 1995) and water availability can be quite important in regulating sink strength (Krizek *et al.* 1985), the relationship between water availability and photosynthetic response to elevated CO₂ is especially important to evaluate. Drought may reduce the carbohydrate build-up in leaves, and in one study eliminated increased photosystem II efficiency associated with growth at elevated CO₂ (Roden & Ball 1996b). In water-limited systems, sink demands are driven by highly variable seasonal precipitation patterns, and photosynthetic responses to elevated CO₂ may be directly or indirectly controlled by variation in water availability (Oechel *et al.* 1995).

Because the down-regulation of photosynthesis may be a key physiological process that limits increases in productivity of a high CO₂ world, we evaluated the pattern of photosynthetic down-regulation to elevated CO₂ in a glasshouse and in the field during periods of seasonal water stress and increased water availability in the Mojave Desert shrub *Larrea tridentata* Cav. We expected that drought would result in reductions in the degree of photosynthetic down-regulation to elevated CO₂ as compared with non-droughted conditions in both glasshouse and field conditions. This follows from the influence of drought, which can reduce photosynthetic rates and leaf carbohydrate accumulation to levels such that feedback inhibition of photosynthesis may not occur (Oechel *et al.* 1995). In addition, we expected the glasshouse-grown plants to exhibit a greater down-regulation response to elevated CO₂ than their field-grown counterparts as a result of the restriction on root growth imposed by pots. To test these hypotheses, we evaluated gas exchange and fluorescence characteristics under droughted and well-watered conditions on fully established adult plants in a Free Air Carbon Dioxide Enrichment (FACE) system and seedlings in a glasshouse. This approach allows for a comparison of results from two CO₂ treatment implementation techniques that may lead to different source-sink aspects under contrasting moisture conditions.

MATERIALS AND METHODS

Study species

Larrea tridentata Cav. (Zygophyllaceae) is a dominant evergreen, distributed throughout the warm deserts of the arid New World (Smith, Monson & Anderson 1997). The distribution of *Larrea* is a primary determinant of the boundaries of the North American warm deserts. *Larrea* has been classified as a true drought-resisting species that remains metabolically active during dry seasons and has the potential to exhibit opportunistic flowering and growth in response to rainfall at any time of the year (Oechel, Strain & Odening 1974; Bowers & Dimmitt 1994). *Larrea* can reach a relatively high rate of positive net photosynthesis as compared with most desert evergreens and shows remarkable photosynthetic acclimation to temperature variation (Mooney, Bjorkman & Collatz 1978). The seedlings used in this study were taken as seed from a population on the Nevada Test Site, near the location of the Nevada Desert FACE Facility (NDFF). Adult plants at NDFF were undisturbed during construction of the FACE facility and remain intact.

CO₂ treatment facilities

Larrea seedlings were exposed to three CO₂ treatment levels (ambient – 360 μmol mol⁻¹; 1.5 × ambient – 550 μmol mol⁻¹; and 2 × ambient – 700 μmol mol⁻¹) in a controlled-environment glasshouse at the University of Nevada, Las Vegas for a year prior to plant sampling (ambient CO₂ concentration fluctuated between 360 and 390 μmol mol⁻¹ in this urban area). The seedlings were planted in 15 cm diameter by 1 m tall PVC pipes in a 20:80 silt:sand mix. All *Larrea* received 400 cm³ 1:40 Hoaglands solution every 2 weeks until 2.5 months prior to sampling, when water and Hoagland's solution were withheld from randomly selected individuals in each CO₂ treatment. The glasshouse facility received natural light (maximum photosynthetic photon flux density (PPFD) ≈ 1600 μmol m⁻² s⁻¹) and roughly tracked external temperature with limits of 0 °C and 45 °C.

Mature *Larrea* shrubs were exposed to either ambient (360 μmol mol⁻¹) or 1.5 × ambient (550 μmol mol⁻¹) CO₂ at NDFF beginning in April 1997. NDFF is located on the Nevada Test Site (36°49' N, 115°55' W, 970 m), an environmental research park operated by the US Department of Energy. This area is a pristine portion of the Mojave Desert, which has been free from grazing for more than 40 years. The average precipitation is 138 ± 62 mm, falling mostly as rain during winter months (Hunter 1994), but the Mojave Desert can receive unpredictable rain during any portion of the year (Bowers 1987). NDFF consists of nine ringed plots (491 m²), each encompassing ≈ 12 *Larrea*. Three plots are maintained at 550 μmol mol⁻¹ CO₂, while the remaining six rings are controls for CO₂ enhancement and application assembly. The NDFF maintains continuous CO₂ enrichment except when the 5 min wind speed average exceeds 6.0 m s⁻¹, resulting in elevated CO₂ treatment conditions occurring greater than

95% of the time. The plants are accessed from an overhead moveable walkway system that allows sampling without disturbing soil in the plots. A more extensive facility description is available in Jordan *et al.* (1998).

Plant sampling

Larrea were organized into three blocks within each CO₂ treatment room in the glasshouse, and individuals within each block were randomly assigned a droughted or well-watered condition. Two to three individuals in each block for each treatment combination were randomly selected for sampling. The block (a mean of these two to three individuals) was used as the replicate for statistical analysis. Similarly, one to three individuals were randomly selected from each treatment ring at the FACE site, and the ring was used as the replicate for statistical analysis. This organization led to a sample size of three for each CO₂ treatment/water status combination at both the glasshouse and FACE site. *Larrea* were sampled at the FACE site over 1 week in June 1997 during a pronounced drought, and well-watered individuals were measured in October 1997 following a series of late summer rainfall events. All plants were sampled between 0900 and 1200 h, a period that has the least diurnal fluctuation in gas exchange and fluorescence patterns. All measurements were performed on the terminal newest expanded leaves. For gas exchange, leaf area was determined by regressions between leaf number, stem diameter and length, and leaf area from harvested individuals.

The A–C_i (assimilation rate–internal CO₂ concentration) response of *Larrea* was determined with a programmable, open-flow gas exchange system (Li 6400, LiCOR Inc., Lincoln, NE, USA). Block temperature was held at 30 °C and relative humidity at 20%, typical of environmental conditions in the field. All measurements were made with a red light source at PPFD values of 1500 μmol m⁻² s⁻¹. The CO₂ concentration of the cuvette (C_a) was initially maintained at 200 μmol mol⁻¹ for 5 min to stimulate stomatal opening and then reduced to 75 μmol mol⁻¹. C_a was incrementally increased to 150, 250, 350, 550, 700, 800, 900, 1200, and 2000 μmol mol⁻¹, and gas exchange properties were logged at each C_a once the system had reached a pre-determined stability point (coefficient of variation = 1%). The data from the A–C_i response was fitted to a non-linear least-squares regression using an exponential model from which maximum photosynthetic rate (A_{max}) and carboxylation efficiency (CE) were determined (Jacob *et al.* 1995).

Chlorophyll fluorescence was used to assess the photochemical efficiency of photosystem II (F_v/F_m), non-photochemical (QNP) and photochemical (QP) quenching. Terminal shoots were dark adapted in aluminium foil for 1 h, and then fluorescence was measured with a pulse-amplitude-modulated (PAM) fluorimeter (Model MFMS/2S, Hansatech Instruments Ltd, King's Lynn, UK) under the control of a personal computer. Initial fluorescence (F₀) was taken to be the average of 20 s fluorescence upon exposure to a low level light source (583 nm; 2 μmol m⁻² s⁻¹). Maximal fluorescence (F_m) was deter-

mined by exposing the shoots to 0.5 s pulses of saturating pulse-modulated light (8500 μmol m⁻² s⁻¹ PPFD; 1 Hz). F_v/F_m was determined where F_v = F_m – F₀. QNP was determined by 1 – F_v'/F_v where F_v' = F_m' – F₀' (F_m' is maximum fluorescence following an initial pulse of 8500 μmol m⁻² s⁻¹ and F₀' is initial fluorescence under actinic light). QP was calculated as 1 – QNP.

Leaf samples for chlorophyll content and Rubisco analysis were taken concurrent with A–C_i measurements from shoots adjacent to gas exchange measurements, and immediately preserved in liquid nitrogen. Stems and dead leaves were removed prior to grinding in a mortar. Approximately 0.15 g of leaves were ground in 1.5 cm³ of buffer containing 100 mM Bicine pH 7.8, 5 mM MgCl₂, 1 mM ethylene diamine tetraacetic acid (EDTA), 5 mM dithiothreitol (DTT), 0.1% (v/v) Triton X-100, 25 mM KCl, and 20% (w/w) polyvinylpyrrolidone. Extracts were filtered through Miracloth and centrifuged for 1 min at 10 000g. The chlorophyll content was determined following extraction with ethanol (Wintermans & DeMots 1965). Rubisco catalytic sites were determined by immunoprecipitation as described by Evans & Seemann (1984).

Xylem water potential (Ψ_{xylem}) was determined with a Scholander-type pressure chamber (Soil Moisture Stress Inc, Santa Barbara, CA, USA) on terminal shoots taken at midday from the *Larrea* on which the A–C_i response was determined.

Statistical analysis

A multivariate analysis of variance (MANOVA, Statistica, StatSoft Inc., Tulsa, OK, USA) was used to compare the overall responses of *Larrea* to different CO₂ treatment techniques, CO₂ growth concentrations, and drought. Initially the 700 μmol mol⁻¹ treatment was excluded so that the design was factorial; inclusion of the 700 μmol mol⁻¹ growth condition was applied to the glasshouse data independently using a MANOVA so that pairwise comparisons could be constructed. Six response variables were used in the MANOVA, including A_{max}, CE, Ψ_{xylem}, F_v/F_m, QNP, and QP. Post-hocs were Newman–Kuels tests at α = 0.05. MANOVA data distribution assumptions were met by arcsin square root transformation of the data.

The effect of CO₂ treatment, drought, and their interaction on leaf chlorophyll content and Rubisco catalytic sites were analysed separately for the FACE and glasshouse plants with a two factor ANOVA and Newman–Kuels tests as post-hocs, with significance at α = 0.05.

RESULTS

When the six response variables (A_{max}, CE, Ψ_{xylem}, F_v/F_m, QNP, and QP) in the MANOVA model were considered, the effects of exposure to elevated CO₂ and drought were significant for both the glasshouse and FACE treatment techniques (Table 1). In addition, the interactions between drought and fumigation technique, as well as drought and CO₂ growth condition, were significant. There was no

Factor	Wilks' lambda	d.f.	Significance
Fumigation technique	0.581	6, 11	—
CO ₂ concentration	0.235	6, 11	*
Drought	0.036	6, 11	*
Fumigation × CO ₂	0.618	6, 11	—
Fumigation × drought	0.091	6, 11	*
Drought × CO ₂	0.360	6, 11	*
Fumigation × drought × CO ₂	0.448	6, 11	—

A_{\max} , maximum photosynthetic rate; CE, carboxylation efficiency; Ψ_{xylem} , xylem water potential; QNP, non-photochemical quenching; QP, photochemical quenching; FACE, Free Air CO₂ Enrichment.

interaction between the fumigation technique and the effect of CO₂ growth environment upon the response variables, suggesting that the pattern of response to elevated CO₂ was consistent. There were no significant differences in the patterns of *Larrea* response for the plants grown in the glasshouse as compared with the plants exposed at the FACE site.

Overall, elevated CO₂ had little effect on the response of midday Ψ_{xylem} . Ψ_{xylem} in *Larrea* varied from -6 MPa during drought to -2 MPa when soil moisture was high at the FACE site. Ψ_{xylem} in droughted FACE plants were much more negative than droughted glasshouse plants, and their well-watered counterparts were less negative than well-watered glasshouse plants (Fig. 1). There was a CO₂-by-drought interaction for plants grown in the glasshouse, leading to an amelioration of drought in the $2 \times$ ambient CO₂ concentration. There were no pairwise significant differences in Ψ_{xylem} in FACE plants with respect to growth at elevated CO₂.

Well-watered *Larrea* grown in glasshouse conditions showed a progressive decline in the initial slope of the $A-C_i$ response (CE), along with a decrease in the asymptote (A_{\max}) when grown exposed to $1.5 \times$ and $2 \times$ ambient CO₂ as compared with those grown in ambient conditions, and the degree of response appeared to be a gradient with

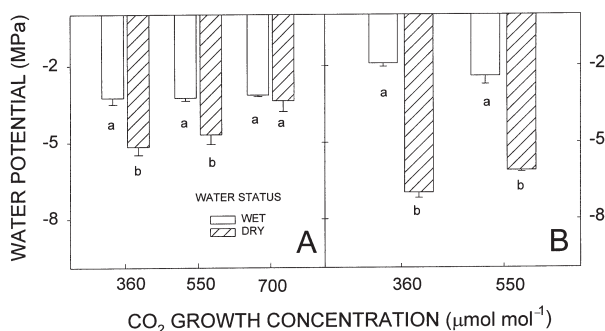


Figure 1. Midday xylem water potentials (Ψ_{xylem}) of *Larrea tridentata* exposed to elevated levels of CO₂ under wet (well-watered) and dry (droughted) conditions in a glasshouse (a) and at a FACE (Free Air CO₂ Enrichment) facility (b). Letters indicate significant differences among treatments within panels at $\alpha = 0.05$.

Table 1. Results of a general three-way MANOVA with six response variables (A_{\max} , CE, Ψ_{xylem} , F_v/F_m , QNP, and QP) and main factors of fumigation technique (glasshouse versus FACE), drought (well-watered versus droughted), and CO₂ concentration ($360 \mu\text{mol mol}^{-1}$ versus $550 \mu\text{mol mol}^{-1}$). Significance (*) is at $\alpha = 0.05$

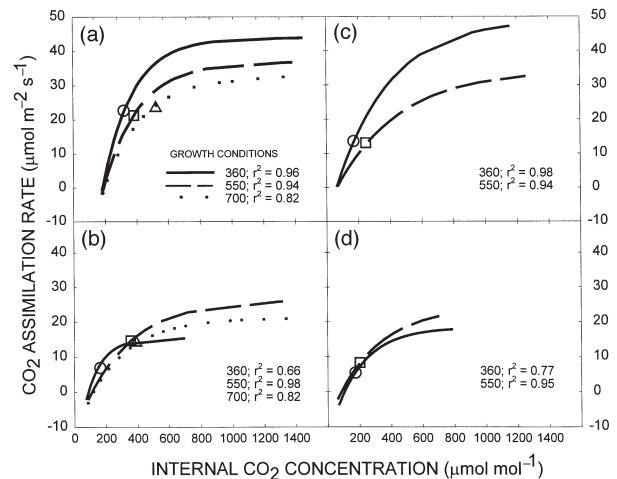


Figure 2. Net assimilation rate (A) versus internal CO₂ concentration (C_i) for *Larrea tridentata* exposed to various levels of CO₂ in a glasshouse (a & b) and at a FACE (Free Air CO₂ Enrichment) facility (c & d) under wet (well-watered; a & c) and dry (droughted; b & d) conditions. Curves plotted are the outputs of a non-linear least-squares regression model fitted to the actual data (Jacob *et al.* 1995). Open circles upon the regression line indicate operational C_i for ambient-grown *Larrea*, while open squares are for $1.5 \times$ ambient and open triangles are for $2 \times$ ambient CO₂ *Larrea*. Coefficients of determination are indicated within each plot, identified by CO₂ environment concentration.

CO₂ concentration (Fig. 2a). Drought diminished this response, and the $A-C_i$ curves from greenhouse plants overlapped regardless of the CO₂ growth conditions (Fig. 2b). *Larrea* exposed to $1.5 \times$ ambient CO₂ in the FACE facility showed the same decline in initial slope and asymptote under periods of favourable water status (Fig. 2c), and drought imposed a similar response as in glasshouse conditions (Fig. 2d). While the droughted response from the FACE site was very similar to the glasshouse droughted response to increased CO₂ concentration, the well-watered FACE *Larrea* exposed to $1.5 \times$ ambient CO₂ exhibited a greater down-regulation response as compared with glasshouse counterparts. These patterns had the effect of manipulating the operational C_i of *Larrea* at their respective C_a . Well-watered glasshouse and FACE

Larrea maintained C_i in such a manner that A_{net} was consistent between CO₂ growth environments (Fig. 2a & c). In droughted *Larrea*, the increase in C_i resulted in greater increases in A_{net} in elevated plants as compared with ambient plants (Fig. 2b & d).

A_{max} was reduced by 25% in non-water-stressed plants exposed to elevated CO₂ compared with ambient CO₂ non-water-stressed plants for both treatment facilities. Drought was a significant factor in determining A_{max} , regardless of

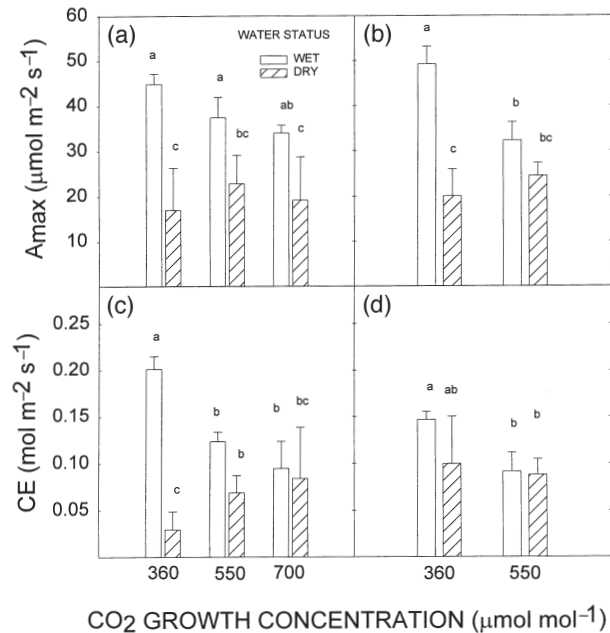


Figure 3. Maximum CO₂ assimilation rate (A_{max}) and carboxylation efficiency (CE) calculated from the non-linear least-squares regression models from Fig. 2 for *Larrea tridentata* exposed to ambient and elevated CO₂ under wet (well-watered) and dry (droughted) conditions in a glasshouse (a & c) and at a FACE (Free Air CO₂ Enrichment) facility (b & d). Letters indicate significant treatment differences within panels at $\alpha = 0.05$.

CO₂ growth concentration, typically reducing A_{max} by $\approx 50\%$ (Fig. 3). Therefore, A_{max} was significantly higher in well-watered plants exposed to ambient CO₂ as compared with the A_{max} of well-watered plants exposed to elevated CO₂, and both were higher than the A_{max} of all droughted plants. CE exhibited a similar pattern as A_{max} in glasshouse-grown plants, in that drought led to a decline in CE (Fig. 3). An interaction between drought and CO₂ concentration resulted in the largest CE values in the ambient CO₂, well-watered condition (Fig. 3). In FACE-exposed plants, the pattern was similar, but there were no pairwise differences between the wet and dry plants but rather a strong effect of CO₂, leading to a 40% decrease in CE at elevated CO₂ in *Larrea* (Fig. 3).

The chlorophyll content of *Larrea* leaves responded to changes in water availability (Table 2). There was a $\approx 10\%$ increase from drought to well-watered conditions. The number of Rubisco catalytic sites decreased by 25% with increasing CO₂ concentration, similar to the decrease in CE for well-watered plants in both treatment facilities (Table 2). However, for droughted plants, the number of Rubisco catalytic sites did not differ between ambient and elevated CO₂ treatments at NDFF, but the greenhouse plants did show a decrease in Rubisco catalytic sites even in droughted plants (Table 2). It is important to note that chlorophyll content and Rubisco active sites were determined on a fresh mass basis, and interpretation should be made with caution. However, the fresh mass to dry mass ratio of *Larrea* leaves from glasshouse-grown conditions increased by only 14% from droughted to well-watered conditions and relative water content increased by less than 10% (data not shown). The direction of change in water content with increasing drought and CO₂ environment suggests that the differences reported here in chlorophyll content and Rubisco active sites represent a conservative estimate of actual differences associated with elevated CO₂.

Exposure to elevated CO₂ increased the overall quantum yield of photosystem II (F_v/F_m) by 10%, regardless

Sample	Treatment	Chlorophyll ($\mu\text{g g}^{-1}$ FW)	Rubisco protein (nmol catalytic sites g^{-1} FW)
FACE	Dry	360	620 ± 125^a
		550	839 ± 100^a
	Wet	360	1402 ± 125^a
		550	1397 ± 198^a
Glasshouse	Dry	360	822 ± 38^a
		550	590 ± 88^b
		700	557 ± 21^b
	Wet	360	875 ± 60^a
		550	1189 ± 156^b
		700	780 ± 198^a
			31.2 ± 2.9^a
	6.4 ± 1.2^b		
	4.8 ± 0.8^b		
	35.2 ± 3.5^a		
	26.5 ± 3.3^b		
	13.7 ± 5.1^c		

Table 2. Chlorophyll content and Rubisco active sites extracted from leaves of *Larrea tridentata* exposed to elevated or ambient CO₂ at a FACE facility and in a glasshouse under three levels of CO₂ fumigation. Data are means \pm SD. Letters indicate significant treatment differences within fumigation technique and water treatment at $\alpha = 0.05$

FACE, Free Air CO₂ Enrichment; SD, standard deviation; FW, fresh weight.

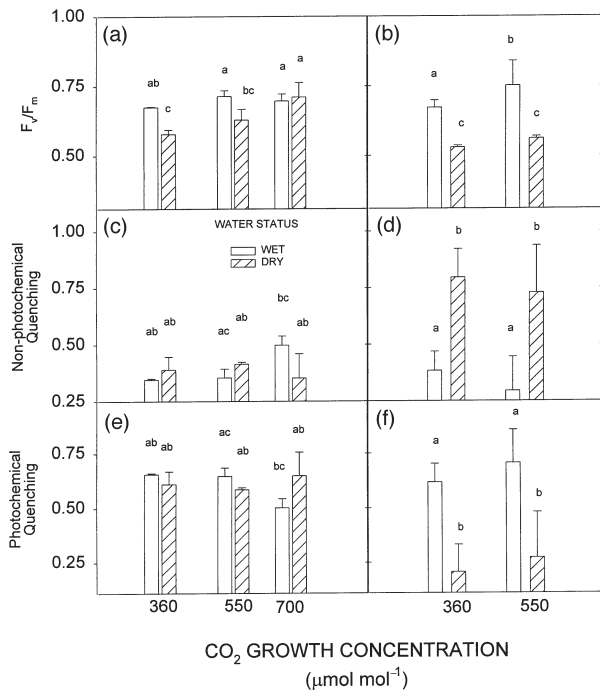


Figure 4. Quantum yield of photosystem II (F_v/F_m), non-photochemical quenching (QPN) and photochemical quenching (QP) of individuals of *Larrea tridentata* exposed to elevated levels of atmospheric CO₂ under wet (well-watered) and dry (droughted) conditions in a glasshouse (a, c & e) and at a FACE (Free Air CO₂ Enrichment) facility (b, d & f). Letters indicate significant differences within panels at $\alpha = 0.05$.

of drought or fumigation technique. For plants exposed to greater than ambient CO₂ and drought in the glasshouse, F_v/F_m increased by over 20% (Fig. 4). Well-watered *Larrea* from FACE showed a $\approx 12\%$ increase in F_v/F_m upon exposure to elevated CO₂ (Fig. 4). Additionally, the droughted *Larrea* from FACE had very low values of F_v/F_m that were not significantly different, suggesting that growth in elevated CO₂ does not offset a stress response with respect to photochemistry. For QNP and QP, there was an overall change in the relative importance of these two parameters for plants grown at FACE with respect to drought, but no changes between CO₂ treatments. QNP represented greater than 50% of the quenching during periods of water stress, while QP dominated during periods of water availability (Fig. 4). In plants grown in the greenhouse, there were pairwise differences in QP and QNP associated with growth in elevated CO₂. QNP increased slightly in well-watered plants exposed to 2 \times ambient CO₂. In this regard, there was a slight decrease in QP with an increase in CO₂ growth concentration. These changes in fluorescence measurements may have had a relationship to the differences in chlorophyll concentrations in different CO₂ and fumigation treatments (Table 2), where NDFP typically had plants with greater

leaf chlorophyll contents, which increased from droughted to well-watered treatments.

DISCUSSION

The photosynthetic responses of *L. tridentata* to elevated CO₂ indicate that this long-lived, relatively slow-growing Mojave Desert shrub adjusts resource investment to photosynthesis when exposed to elevated CO₂ under conditions of potentially optimum resource availability, either in the field or under controlled glasshouse conditions. However, this down-regulation of photosynthesis to elevated CO₂ diminishes following exposure to drought. In fact, drought converged all values of A_{max} in each treatment combination to very low levels. Similarly, the chaparral shrub *Adenostoma fasciculatum* exhibits down-regulation of photosynthesis upon exposure to elevated CO₂ in controlled-environment conditions, but drought diminishes the down-regulation response (Oechel *et al.* 1995). *Adenostoma* exposed to elevated CO₂ and drought experienced some degree of increased instantaneous photosynthetic rates and biomass production as compared with those grown in ambient conditions, even though there was still some reduction in A_{max} in elevated CO₂-grown plants (Oechel *et al.* 1995). Currently, there is no known mechanism that explains the drought-induced loss of down-regulation of photosynthesis to elevated CO₂. Drought may act to reduce A_{max} to such a level that the amount of photosynthate supplied to the plant is insufficient to invoke a form of end product inhibition or feedback response of photosynthesis (Oechel *et al.* 1995). Therefore, the idea that down-regulation may be a general response to elevated CO₂ in suboptimal environments (Curtis 1996) may need to be re-evaluated. In this respect, inter- and intra-annual patterns of water availability may be very important in integrating photosynthetic and productivity patterns of desert systems in future climate scenarios, including elevated CO₂.

While down-regulation of photosynthesis occurs, growth at elevated CO₂ improves the overall quantum yield of photosystem II (F_v/F_m) in all conditions. This was primarily due to the increase in F_v/F_m in elevated CO₂ during drought, suggesting that elevated CO₂ may partially offset stress to photosystem II associated with growth in droughted conditions. Similarly, F_v/F_m increased at elevated CO₂ for *Arbutus unedo*, presumably associated with amelioration of drought (Jones *et al.* 1995). In *Eucalyptus*, when plants were exposed to elevated CO₂, high temperature and light, there was a decrease in the quantum yield of photosystem II, presumably related to a down-regulation of electron transport associated with increased photosynthetic end products (Roden & Ball 1996a). These differences observed in the current experiment and others invoking temperature- and light-induced photoinhibition coupled with increases in CO₂ do not lead to mutually exclusive conclusions concerning the effects of elevated CO₂ on plants, but highlight the importance of considering the state of photosynthetic down-regulation when stress

treatments are implemented in conjunction with changes in CO₂ environment.

The down-regulation response of *Larrea* in the field was rather rapid considering the plants were exposed to elevated CO₂ for only 5 months. To date, the down-regulation response to elevated CO₂ has taken several to many growing seasons to occur in other field studies (Korner & Diemer 1994; Jacob *et al.* 1995). Predictions of plant responses based on functional types suggest that *Larrea* should not have shown a down-regulation response over such a short time period. This is based on the slow growth rate and high stress tolerance demonstrated by this long-lived desert plant. However, *Larrea* photosynthesis and growth respond rapidly to the return of optimal conditions following drought (Oechel *et al.* 1974; Brisson & Reynolds 1994), suggesting that a rapid response to changing CO₂ concentration should not be unexpected. Indeed, analysis by gas exchange 1 month prior to the rainfall event, which saturated soils and improved water availability, indicated no down-regulation response (T. E. Huxman, unpublished results). In the glasshouse, watering of droughted *Larrea* increases A_{\max} rapidly (2–4 d) in all CO₂ conditions, and the typical decreased A–C_i asymptotes associated with photosynthetic down-regulation occurs within several days in elevated as compared with ambient CO₂-grown *Larrea* (T. E. Huxman, unpublished results).

In glasshouse conditions, *Larrea* exhibits changes in root growth upon exposure to elevated CO₂. Total root system biomass increased when exposed to elevated CO₂, and the proportion and absolute amount of fine roots to coarse roots decreased, resulting in a smaller number of fine roots (BassiriRad, Reynolds & Viginia 1997). However, there appear to be no differences in the ability of root systems of *Larrea* to take up nutrients (BassiriRad *et al.* 1997) or to conduct water to stems (Huxman, Smith & Neuman 1998) in elevated versus ambient CO₂. Combining results showing down-regulation of photosynthesis with no significant change in the potential rates of nutrient and water delivery to the shoot (albeit from different studies) suggest that the pattern of photosynthetic readjustment may be adaptive in terms of linking above- and below-ground processes, ensuring long-term productivity. However, this idea is based upon the assumption that root characteristics in the field do not change with changing CO₂ concentration, which remains to be validated. Whole plant nitrogen and phosphorus budgets have yet to be determined under field conditions at elevated CO₂, which may also help our understanding of the significance of the down-regulation response to whole plant function.

While it appears from these data that the seasonal course of drought is quite important in determining plant gas exchange response to elevated CO₂ for arid and semi-arid systems, typified by *Larrea* and *Adenostoma*, respectively, temperature may also be important and potentially help to explain the results from the FACE *Larrea* in the current experiment. The relative increases in photosynthetic responses of *Pinus taeda* to elevated CO₂ were

much higher during summer periods as compared with winter (Lewis *et al.* 1996). This may be due to the counteractive effects of increasing temperature and CO₂ on photorespiration (Long 1991). Increasing temperature reduces the specificity of Rubisco for CO₂ by enhancing the oxygenase activity of Rubisco more than the carboxylase activity, thus increasing the proportion of potential photosynthesis lost to photorespiration (Brooks & Farquhar 1985). Increasing CO₂ overrides oxygenase activity, thus providing relief for plants growing in elevated atmospheric CO₂ from the inefficient use of Rubisco active sites. Because deserts are typified by extreme seasonality in temperature and episodic precipitation patterns, the photosynthetic response of desert plants to elevated CO₂ may be more difficult to predict, and the changes in temperature between field measurements in this study may help to explain the patterns observed. In spite of this, the data from greenhouse-grown *Larrea*, which remove the temperature differences between field sampling dates, suggest that drought is still important.

Taken together, these results suggest that the photosynthetic response of *L. tridentata* to increasing CO₂ concentration may be controlled by seasonal patterns of resource availability. Specifically, the down-regulation response in *Larrea*, and perhaps in other desert perennials, may be seasonally transient, occurring primarily in wet seasons but largely absent during the extensive dry seasons that typify the Mojave Desert. Therefore, the potential for interaction between drought-controlled and nutrient-controlled down-regulation response may exist, as periods of high water availability are often concurrent with periods of nitrogen limitation on plant growth in North American deserts (Smith *et al.* 1997). Because the down-regulation response may be seasonally transient, future research will be designed to evaluate the potential for seasonal patterns of photosynthetic adjustment in *Larrea* and other desert perennials of the Mojave Desert FACE site. Many ecosystem-level processes are affected by the characteristics of carbon fixation and soil resource utilization in plants, suggesting the pattern of down-regulation response throughout a year may be very important in terms of understanding how plants adjust to the new resource levels exhibited in a future high CO₂ world. Because the down-regulation response of *Larrea* is related to water availability and deserts are systems that are driven by episodic patterns of precipitation, the pattern of down-regulation over a season may be extremely important. Specifically, if the down-regulation response occurs in tandem with precipitation patterns, the accumulation of carbon-based end products of photosynthesis and dilution of leaf nitrogen by carbon may also occur seasonally, influencing litter quality, decomposition rates, and nutrient cycling in the desert. In addition, because we have observed no evidence of stomatal acclimation to elevated CO₂ (Hamerlynck, unpublished results), the down-regulated photosynthetic rates may reduce the usually observed benefit of increased water-use efficiency associated with growth in elevated CO₂.

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