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Combined Effects of CO_2 Enrichment and Drought Stress on Growth and Energetic Properties in the Seedlings of a Potential Bioenergy Crop *Jatropha curcas*

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Abstract The rapid growth of worldwide energy demands has led to mounting concerns about energy shortages and has promoted the development of biofuels, which are susceptible to climate change. To evaluate the effects of future environmental changes such as CO_2 enrichment and water stress on the growth and biodiesel production of bioenergy plants, we exposed *Jatropha curcas* to two levels of CO_2 concentration (ambient and elevated) and three watering regimes (well-watered, moderate drought, and severe drought) to study its biomass accumulation and allocation, energy cost-gain properties, and photosynthetic response. Elevated CO_2 enhanced biomass accumulation of *J. curcas* by 31.5, 25.9, and 14.4 % under well-watered, moderate drought, and severe drought treatments, respectively, indicating that the stimulating effect was greater under optimum water conditions than in water-deficit conditions. Drought stress significantly increased the biomass allocation to roots, especially the fine roots. CO_2 enrichment also increased the root mass fraction, though not significantly. CO_2 enrichment significantly enhanced the photosynthetic rate measured under growth CO_2 concentration (A_{growth}) and decreased foliar N content and therefore construction cost irrespective of watering conditions. Under elevated CO_2, *J. curcas* employed a quicker return energy use strategy indicated by the higher photosynthetic energy use efficiency and lower payback time. There was a pronounced downregulation in the light-saturated photosynthetic rate under the common CO_2 concentration ($P_{\max}$) under long-term CO_2 exposure, due to a decrease in the initial and total ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activities and partially lower foliar N content. The significant interaction of CO_2 enrichment and watering regimes implied that the stimulation of plant growth by CO_2 enrichment may be negated by soil drought in the future. Long-term field experiments manipulating multiple factors simultaneously are needed to explore how the ecophysiological traits measured for *J. curcas* translate into bioenergy production.

Keywords CO_2 enrichment · Drought stress · Energy gain-cost analysis · *Jatropha curcas* · Photosynthetic acclimation

Introduction

The rise in atmospheric carbon dioxide (CO_2) concentration is one of the best documented global changes of the past half century. CO_2 concentration, currently 360 ppm, is approximately 30 % higher than the preindustrial level and is conservatively projected to double by the end of the 21st century, strongly dependent on future scenarios of anthropogenic emissions (IPCC 2007). Enormous research efforts have been undertaken to understand how plants and ecosystems, both natural and managed, will respond to rising CO_2 (Long and others 2004; Norby and others 2010; Reich and others 2006). The primary and instantaneous responses for plants to an increase in CO_2 are an increased rate of photosynthesis and a decreased rate of transpiration at the leaf level, followed by growth stimulation (Gonzelez-Meller and others 2004; Ainsworth and Long 2005; Korner 2006; Ainsworth and Rogers 2007). In the absence of

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photosynthetic downregulation, increasing CO_2 should result in a 35–60 % stimulation in photosynthetic carbon fixation, primarily due to a reduction in photorespiration because the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) carboxylation reaction is favored in these conditions (Farquhar 1980; Zhang and others 2008).

However, in the long term this initial stimulation of carbon uptake cannot be sustained and is often followed by an acclimatory downregulation in photosynthetic capacity (Ainsworth and Long 2005; Norby and others 2010). In a meta-analysis of 15 field-based experiments on European forest tree species, photosynthesis measured at a common CO_2 concentration was 10–20 % lower in trees grown at elevated CO_2 (Medlyn and others 1999). A variety of factors have been proposed that contribute to the downregulation, including limited sink capacity, end-product limitation, excess accumulation of nonstructural carbohydrates, or decreased Rubisco content and activation state caused by decreased N concentrations (Mooney and others 1999; Luo and others 2004; Ainsworth and Long 2005).

The responses of stimulated photosynthesis and growth to elevated CO_2 can be weakened by most natural environmental stress factors, including nutrition and water deficit (Xu and others 2007; Langley and Megonigal 2010; Ye and others 2011; Lei and others 2012). Drought stress is considered to be one of the most important abiotic factors that can limit plant growth and crop yield (Kramer and Boyer 1995). The impact of either elevated CO_2 or drought stress on plant growth has been studied extensively, but their interactive effects on plant ecophysiological adjustments are still inadequately understood. These two factors will accompany each other strongly in the future, so it is imperative to study whether CO_2 will exaggerate, alleviate, or diminish the deleterious effect of drought stress. Environmental factors often change simultaneously and influence plants interactively, showing opposite, additive, or synergetic effects (Albert and others 2011).

Plant productivity involves both the assimilation and the expenditure of energy (Griffin 1994). Photosynthesis provides just a basic assessment of the ability of a plant to gain energy, whereas construction cost (CC) reflects the energy demand (Nagel and others 2004). These two variables represent a trade-off between potential enhancements and limitations to plant productivity, respectively; so collective consideration of these measures could provide insightful evaluation of plant growth and proliferation (Feng and others 2011; Lei and others 2012). Increasing photosynthetic energy-use efficiency (PEUE), a measure of energy gain via photosynthesis per unit energy investment, and decreasing payback time, the time required for a leaf to amortize CC through photosynthesis, may be conducive to plant growth (Poorter and others 2006; Feng and others 2011).

In the process of rapid industrialization, urbanization, and modernization, China has become the largest energy consumer in the world. In recent years, more people have come to realize that renewable energy can contribute significantly to global climate change mitigation and it is also important for national energy security (Deng and others 2012). Forest-based biodiesel, with the significant advantage of not competing with grain production, has been considered a promising substitute for diesel fuel. Consequently, extracting biodiesel from *Jatropha curcas* has become a growing industry in China. *Jatropha curcas* L. (Euphorbiaceae), a deciduous perennial shrub native to Central America, is now widely introduced and cultivated in the Chinese tropics and subtropics as a potential bioenergy crop for arid areas because its oil seeds are suitable for conversion into biodiesel (Achten and others 2008; Jain and Sharma 2009; Diaz-Lopez and others 2012). Furthermore, *J. curcas* may fulfill environmental functions in degraded ecosystems by helping to avoid soil degradation, improving soil resistance to wind erosion, and enhancing macroaggregate stability to water erosion (Kumar and Sharma 2008; Gimeno and others 2012). Thus, it is imperative to study the effects of environmental changes, including CO_2 enrichment and water stress, on such a versatile plant to predict its growth performance and yield production, and give directions for management practices. We hypothesized that (1) CO_2 enrichment and drought stress would cause morphological adjustment and variations in the biochemical composition of *J. curcas*, (2) under elevated CO_2, *J. curcas* would employ a quicker return energy-use strategy due to increased photosynthetic capacity and reduced energy costs, and (3) the stimulating effect of CO_2 enrichment on the growth of *J. curcas* would be dependent on watering conditions.

Materials and Methods

Plant Materials and Experimental Design

In March 2010, seeds of *Jatropha curcas* were sown into a seedbed in a greenhouse. When the seedlings were about 15 cm tall, similar-sized individual trees were transplanted singly into 10-L pots filled with 6 kg homogenized forest topsoil. After 1 month of growth outside the greenhouse, the seedlings were moved into four closed-top chambers (E-sheng Tech. Co., Beijing, China) that were located outdoors at the Ailaoshan Station for Subtropical Forest Ecosystem Studies (24°32′ N, 101°01′ E, 2,490 m a.s.l.), Jingdong County, Yunnan Province, southwest China. Each chamber consisted of two parts: a cylindrical structure (4.0-m diameter) of eight walls (1.5 × 2 m) as the bottom and a 0.5-m-high hemispherical structure as the top.

The walls were made of 8-mm-thick glass (85 % transparency) and the hemispherical structure was made of 10-mm-thick twin-wall hollow polycarbonate sheets (82 % transparency).

Two of the chambers were supplied with compressed CO_2 gas to obtain a doubled atmospheric CO_2 concentration (CO_2 enrichment treatment, EC), and the other two were used as controls. The CO_2 concentration in the EC chambers was controlled automatically with a computer-controlled CO_2 supply system (LT/ACR-ePLC, E-Sheng Tech. Co., Beijing, China). The CO_2 concentration and temperature in each chamber were recorded at 15-s intervals. Hourly mean CO_2 concentrations were 330–370 and 660–740 μmol mol^{-1} in the control and EC chambers, respectively. Thus, 350 and 700 μmol mol^{-1} CO_2 were used when measuring photosynthesis in chambers with ambient and elevated CO_2 concentrations, respectively.

Sixty similar-sized seedlings were randomly divided into three groups (20 per group) in each chamber. Water and CO_2 treatment began after 1 month of acclimation in greenhouse conditions. Each group for well-watered, moderate drought-treated, and severe drought-treated pots were controlled at 80, 40, and 20 % of relative soil water content. The water status in the substrate was determined daily and the amount of water lost was supplied to each pot to keep the designed soil water content according to Lei and others (2006). Each treatment was divided into four subgroups (five seedlings per subgroup), which were assigned to different parts of each chamber to decrease the potential influence of possible environmental heterogeneity within each chamber. Measurements were taken from four (two individuals from each chamber, one from a subgroup) or eight (four individuals from each chamber, one from each subgroup) individuals per treatment after 4 months of treatments.

Photosynthesis Measurement

Under saturating photosynthetic photon flux density (PPFD) determined by preliminary measurements, photosynthesis was measured on the youngest fully expanded leaf of each sample plant using a Li-6400 Portable Photosynthesis System (LI-COR, Lincoln, NE) in September. The relative humidity of the air in the leaf chamber was controlled at about 70 % and the leaf temperature was controlled at 25 °C. Actual photosynthetic rates under ambient growth CO_2 concentrations (A_{growth}) were measured at 350 and 700 μmol mol^{-1} CO_2 in the reference chamber for plants grown under ambient CO_2 concentration conditions (AC) and EC, respectively. Light-saturating photosynthetic capacity ($P_{\max}$) was measured under common 380 μmol mol^{-1} CO_2 and saturating PPFD. Before measurement, each sample leaf was illuminated with saturating PPFD provided by the LED light source of the equipment for 10–30 min to achieve full photosynthetic induction. No photoinhibition occurred during the measurements.

After the leaf area was determined using a Li-3000C leaf area meter (LI-COR), the leaf samples were oven-dried at 60 °C for 48 h. Specific leaf area (SLA) was calculated as the ratio of leaf area to mass. Leaf N content was determined with a Vario MAX CN Element Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). The same leaf of each sample plant was used for measurements of the above variables. Leaf relative water content (RWC) measurements were made using two leaves of each sample plant and then were weighed immediately to obtain fresh weight (FW), then placed in a beaker filled with water, and left overnight in the dark. They were reweighed the next morning to obtain turgid fresh weight (TW), and then dried at 80 °C for 24 h to obtain the dry weight (DW). RWC was then calculated as $\mathrm{RWC} = [(\mathrm{FW} - \mathrm{DW}) / (\mathrm{TW} - \mathrm{DW})] \times 100$.

Construction Cost and Payback Time

Construction cost was measured using five leaves collected from each sample plant ($n = 4$). The leaves were oven-dried at 60 °C for 48 h and powdered. Leaf ash concentration (Ash) was determined gravimetrically after combusting preweighed leaf powder in a muffle furnace (Vulcan A-550, Vulcan, UK) at 550 °C for 6 h. Heat of combustion (ΔH_c) of the powdered leaves was measured using a Microbomb Calorimeter (SDCM-IIIa; Sundy Instrument, Hunan, China). Leaf construction cost (g glucose g^{-1}) was calculated according to Williams and others (1989) as $\mathrm{CC} = [(0.06968\, \Delta H_c - 0.065)\,(1 - \mathrm{Ash}) + 7.5\,(k\mathrm{N}/14.0067)]/E_G$, where k is the oxidation state of the N absorbed (+5 for nitrate, −3 for ammonium), N is leaf N concentration, and E_G is the growth efficiency [0.87 according to Poorter and others (2006)]. The ratio of nitrate to ammonium in the growth substrate was measured using a flow injection analyzer (QC8000, Lachat, Hach Co., Loveland, CO, USA) and the weighted average k was used in this study.

Photosynthetic energy- and N-use efficiencies were calculated as the ratio of A_{growth} to construction cost and foliar N concentration, respectively. Payback time was calculated using the method of Williams and others (1989), where we assumed that photosynthetic time per day was 12 h, and the mean net photosynthetic rate across day light hours was half of A_{growth}.

Rubisco Activity

Two leaves were collected concurrently with gas exchange measurements from each sample plant ($n = 4$), immediately placed on ice, and quickly transported to an adjacent lab. Leaves were placed into an ice-cold test tube with extraction buffer (1 ml extraction buffer per 50 mg FW), containing 100 mM HEPES (pH 7.5 at 25 °C), 5 mM EDTA, 2 % soluble polyvinylpyrrolidone (w/v), 0.7 % polyethylene glycol (w/v), and 14 mM β-mercaptoethanol. The extract was centrifuged at 9,000 *g* for 2 min in an Eppendorf microcentrifuge, and the supernatant was collected and frozen in liquid nitrogen until analyzed. Rubisco activity was measured at 25 °C by combining 70 μl sample extract with 900 μl Rubisco assay solution, containing 25 mM Tris (pH 8.1), 25 mM $KHCO_3$, 20 mM $MgCl_2$, 3.5 mM ATP, 5 mM phosphocreatine, 80 nkat glyceraldehyde-3-phosphate dehydrogenase, 80 nkat 3-phosphoglyceric phosphokinase, 80 nkat creatine phosphokinase, and 0.25 mM NADH. Rubisco activity was determined spectrophotometrically (UV2550, Shimadzu, Japan) by measuring the rate of disappearance of NADH at 340 nm according to the method of Zhang and others (2008) and Lilley and others (2010). For initial Rubisco activity, 0.5 mM RuBP was added to the assay solution and changes in A_{340} were measured approximately 30 s later when a steady slope was observed. For total (fully activated) Rubisco activity, 0.5 mM RuBP was added after 15 min of complete activation and changes in A_{340} were measured. The activation state of Rubisco was calculated as the ratio of initial activity to total activity.

Growth Measurements

At the end of the experiment in October, the pots were destroyed. Eight seedlings per treatment were harvested; rinsed in tap water with caution to separate stems, leaves, fine (diameter < 1 mm) and coarse roots; oven-dried at 60 °C for 48 h, and weighed. Biomass allocations to leaf, stem, root, and fine root ratios were calculated.

Statistical Analysis

Effects of CO_2 concentrations, watering regimes, and their interactions on determined variables were analyzed using a Split-Plot ANOVA, with CO_2 concentrations as the main factor, watering regimes as a subfactor, and block (section in our study) as a random factor (Ye and others 2011). Data were log-transformed to meet the requirement of ANOVA (normal distribution and homogeneity of variances) when necessary. Differences in each trait among treatments were determined using a one-way ANOVA (Duncan tests). Differences between CO_2 treatments in correlation between each pair of variables (illustrated in Figs. 2 and 4) were tested using a one-way ANCOVA. The above analyses were conducted using SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Biomass Accumulation and Allocation

The ratio of biomass accumulation under elevated and ambient CO_2 was 1.32, 1.26, and 1.14 for well-watered, moderate drought, and severe drought treatments, respectively. This showed that the stimulating effect was greater under optimum watering conditions than under drought stress, which was confirmed by the significant interaction of water and CO_2 effect on biomass accumulation (Table 1). Drought stress decreased only specific leaf area under CO_2 enrichment and decreased relative water content under severe water deficit in *J. curcas*. Elevated CO_2 had little effect on the above two leaf morphological variables (Table 1). As for the pattern of biomass allocation, leaf and stem mass fractions were scarcely affected by water and CO_2 treatments. CO_2 enrichment increased the root mass fraction and fine root ratio, though not significantly, whereas drought stress significantly increased the proportion of biomass partitioned to roots, especially the fine roots (Table 1).

Energy Cost-Gain Analysis

CO_2 enrichment significantly enhanced A_{growth} and the enhancement was greater under well-watered and moderate drought conditions than with severe drought (Fig. 1a). Elevated CO_2 significantly decreased foliar N content (Fig. 1b) and, therefore, construction cost (Fig. 1c) irrespective of watering conditions. Following the changes in A_{growth}, N, and construction cost, a significant increase in PNUE and PEUE and a significant decrease in payback time were observed under elevated CO_2, and they were also more pronounced under well-watered and moderate drought conditions (Fig. 1d–f). Drought stress had little effect on construction cost, caused a decrease in A_{growth}, N content, PNUE, and PEUE, and caused an increase in payback time; these changes were smoother compared with those caused by CO_2 enrichment (Fig. 1).

Biomass was positively correlated with A_{growth}, PNUE, and PEUE and negatively correlated with payback time (Fig. 2). At a given biomass, PNUE and PEUE were greater under elevated CO_2 than under ambient CO_2, opposite the trend for payback time.

Photosynthetic Acclimation

Under long-term CO_2 enrichment fumigation, there was a significant downregulation in $P_{\max}$ (Fig. 3a) due to the decrease in the initial and total Rubisco activities (Fig. 3b, c). Drought stress also decreased $P_{\max}$. Under moderate water deficit, the decrease in $P_{\max}$ was due to merely the

Table 1 Leaf morphology, biomass accumulation, and allocation of *Jatropha curcas* under ambient (AC) and elevated (EC) atmospheric CO_2 concentrations combined with three watering regimes

Variables	Well-watered		Moderate drought		Severe drought		*P* values		
	AC	EC	AC	EC	AC	EC	P_W	P_C	$P_{W \times C}$
Total biomass (g)	19.7 ± 2.67b	25.9 ± 3.97a	15.8 ± 2.52c	19.9 ± 3.53b	7.65 ± 2.44d	8.75 ± 0.82d	***	***	*
Specific leaf area ($cm^2\ g^{-1}$)	198.9 ± 10.8a	194.8 ± 13.6a	186.2 ± 6.05ab	180.9 ± 8.74b	184.7 ± 10.4ab	160.4 ± 20.9c	**	*	ns
Relative water content	81.4 ± 6.22a	81.7 ± 2.62a	78.0 ± 5.45ab	79.3 ± 3.10ab	71.6 ± 4.24b	72.3 ± 3.59b	*	ns	ns
Leaf mass fraction	24.2 ± 3.22a	21.6 ± 2.75a	22.9 ± 2.36a	21.6 ± 2.51a	20.7 ± 2.06a	20.2 ± 1.37a	ns	ns	ns
Stem mass fraction	42.0 ± 6.46a	43.2 ± 1.65a	41.8 ± 4.47a	40.9 ± 2.91a	39.7 ± 3.93a	37.7 ± 2.00a	ns	ns	ns
Root mass fraction	33.8 ± 3.79c	35.2 ± 3.44bc	35.3 ± 3.80bc	37.5 ± 2.48b	39.6 ± 3.56ab	42.0 ± 2.61a	**	ns	ns
Fine root ratio	19.5 ± 3.10c	22.7 ± 1.77bc	24.3 ± 1.64b	26.5 ± 2.99b	31.8 ± 3.71a	33.3 ± 2.71a	**	ns	ns

Values are mean ± standard error ($n = 8$). Different letters in the same line indicate significant differences ($P < 0.05$) according to one-way ANOVA

P_W water effect, P_C CO_2 effect, $P_{W \times C}$ water × CO_2 effect, *ns* not significant

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

lower initial Rubisco activity; when drought became severe, total Rubisco activity also decreased significantly, conducive to the decreased P_{max}. There was no significant change in the Rubisco activation state between ambient and elevated CO_2, whereas moderate and severe drought treatments possessed similar Rubisco activation states, lower than well-watered (Fig. 3d).

A significantly positive relationship was observed between P_{max} and Rubisco initial and total activities, whereas there was no correlation between P_{max} and Rubisco activation state (Fig. 4). At a given P_{max}, Rubisco initial activity was higher under ambient than in elevated CO_2 conditions (Fig. 4a).

Discussion

In our study, *Jatropha curcas* could be considered a drought-tolerant species because it was able to maintain an adequate plant water status under drought stress, as indicated by the relative water content being higher than 70 %, even under severe drought (Table 1). This idea is supported by the results of previous studies (Maes and others 2009; Diaz-Lopez and others 2012) and the fact that treated seedlings showed no leaf shedding or leaf burn injuries (personal observation). In many woody and shrubby plants, the leaf relative water content reaches 40–50 % and occasionally is as low as 20 % during severe drought, which is accompanied by leaf senescence (Galle and others 2007; Warren and others 2011). The relatively high tolerance of *J. curcas* to water-limited conditions could be due to a higher percentage of biomass that was allocated to the roots, especially the fine roots (Table 1). Fine roots have a high surface area-to-mass ratio and are thought to be the site of major resource absorption (Bauhus and Messier 1999; Pregitzer 2002). Moreover, unlike coarse roots, fine roots are ephemeral and can be relatively rapidly deployed in response to changing availability of nutrients such as water and nitrogen (Hodge 2004).

CO_2 enrichment significantly enhanced biomass accumulation of *J. curcas* irrespective of watering regimes (Table 1); this can be explained by the stimulated actual photosynthesis rate under growth CO_2 concentration (A_{growth}) and decreased construction cost (Fig. 1). Given their opposing potential influences on plant productivity, photosynthetic activity and biomass construction cost could be examined together in ecophysiological contexts (Poorter and others 2003; Nagel and others 2004; Lei and others 2012). In our study, doubled CO_2 resulted in a more than 40 % increase in A_{growth} in the three watering treatments, higher than the average of 31 % enhancement in C_3 plants grown in some meta-analyses (Medlyn and others 1999; Ainsworth and Long 2005). This discrepancy might

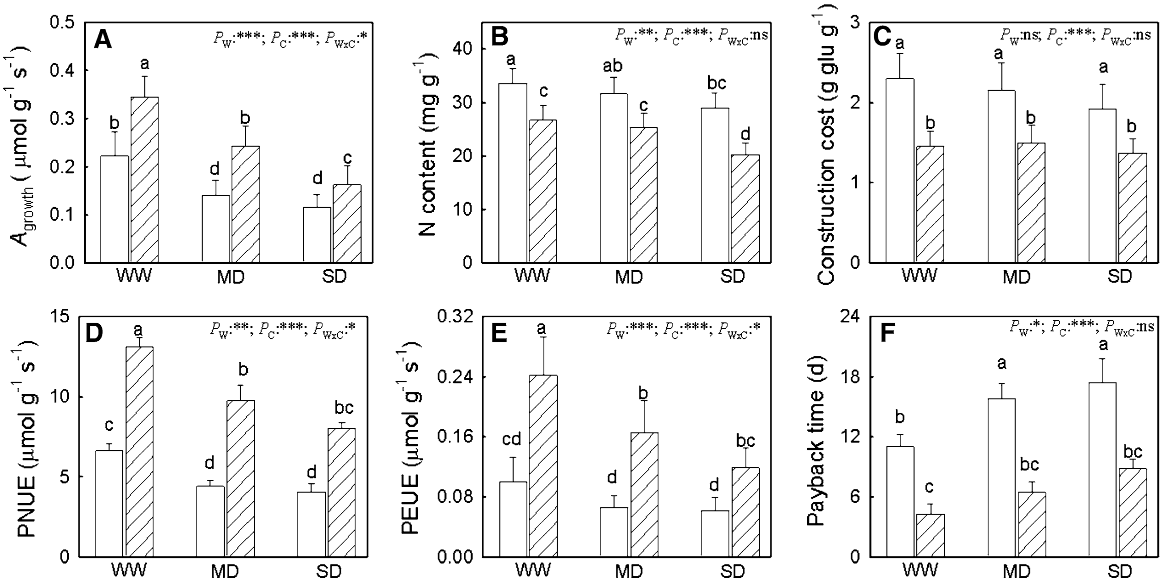
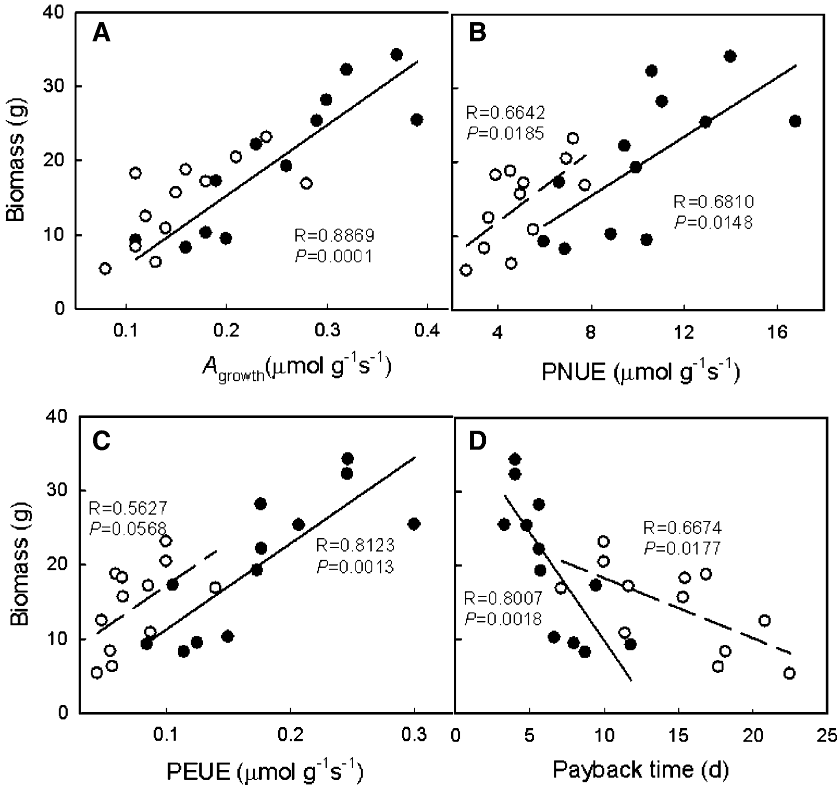


Fig. 1 Actual photosynthetic rate measured under growth CO_2 concentrations (A_{growth}, **a**), foliar N content (**b**), construction cost (**c**), photosynthetic N use efficiency (PNUE, **d**), photosynthetic energy use efficiency (PEUE, **e**), and payback time (**f**) in *Jatropha curcas* as affected by ambient CO_2 (*white bar*) and elevated CO_2 (*hatched bar*), combined with well-watered (WW), moderate drought (MD), and severe drought (SD). Values are the mean ± standard error of eight replicates for A_{growth} and of four for the others. Different letters indicate significant differences ($P < 0.05$) among treatments according to one-way ANOVA. P_W, water effect, P_C CO_2 effect, $P_{W\times C}$ water $\times CO_2$ effect. $^*P$ 0.05, $^{**}P < 0.01$, $^{***}P < 0.001$, *ns* not significant

Fig. 2 Biomass accumulation as a function of A_{growth} (**a**), PNUE (**b**), PEUE (**c**), and payback time (**d**) in *Jatropha curcas* as affected by ambient CO_2 (*white circle*) and elevated CO_2 (*black circle*). *Lines* fitted for ambient (*dashed line*) and elevated (*solid line*) atmospheric CO_2 treatments were given separately, if the difference between treatments was significant according to the result of one-way ANCOVA. Otherwise, only one line was given



be because species with higher growth rates may benefit more from enriched resource availability, including *J. curcas* and some invasive species (Davey and others 2006; Lei and others 2012). Elevated CO_2 also significantly decreased foliar N content, and, therefore, construction cost (Fig. 1) as construction cost is usually positively correlated with the expensive N-containing substances (Feng and others 2011; Lei and others 2012). In our study,

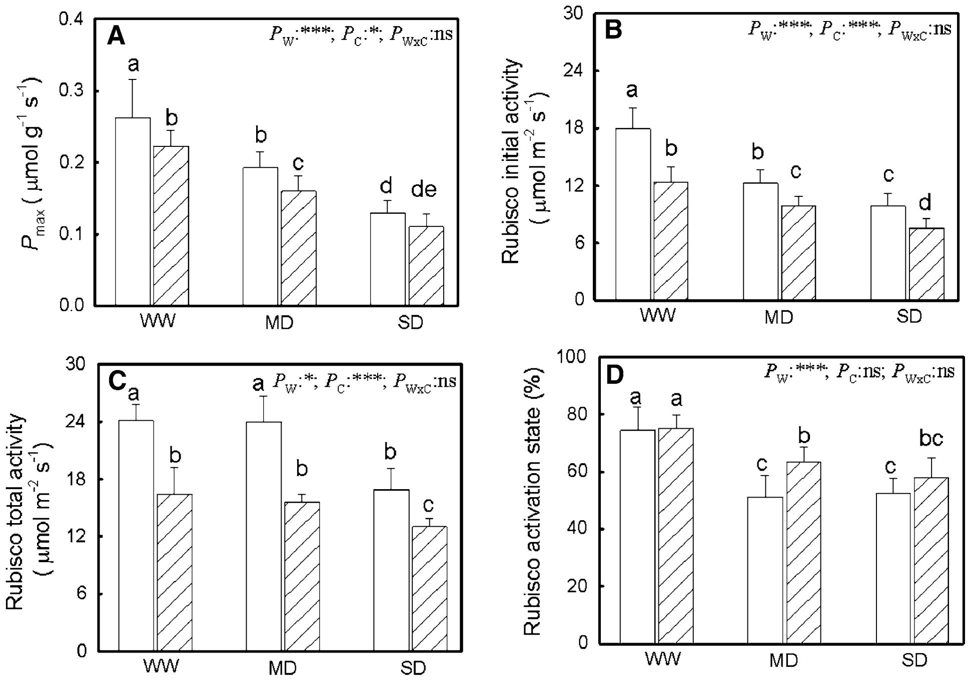


Fig. 3 Light-saturated photosynthetic rate measured under common CO_2 concentrations (P_{max}, **a**), Rubisco initial activity (**b**), Rubisco total activity (**c**), and Rubisco activation state (**d**) in *Jatropha curcas* as affected by ambient CO_2 *(white bar)* and elevated CO_2 (*hatched bar*), combined with well-watered (WW), moderate drought (MD), and severe drought (SD). Values are the mean ± standard error of eight replicates for P_{max} and of four for the others. Different letters indicate significant differences ($P < 0.05$) among treatments according to one-way ANOVA. P_W, water effect, P_C, CO_2 effect, $P_{W \times C}$, water $\times CO_2$ effect. *$P < 0.05$, **$P < 0.01$; ***$P < 0.001$; ns, not significant

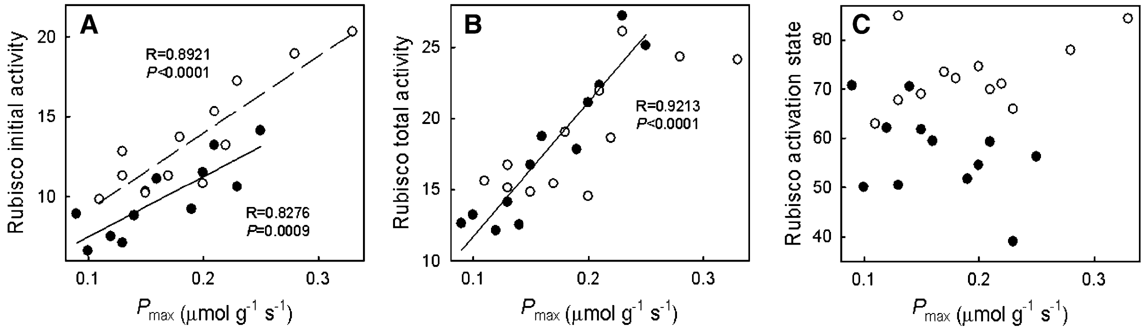


Fig. 4 P_{max} as a function of Rubisco initial activity (**a**), total activity (**b**), and activation state (**c**) in *Jatropha curcas* as affected by ambient CO_2 (*white circle*) and elevated CO_2 (*black circle*). Lines fitted for ambient (*dashed line*) and elevated (*solid line*) atmospheric CO_2 treatments were given separately if the difference between treatments was significant according to the result of one-way ANCOVA. Otherwise, only one line was given

N concentration was decreased by 20.6, 19.9, and 25.4 % under well-watered, moderate drought, and severe drought, respectively, consistent with the results from a literature survey that found a mean decrease of 21 % in tree leaf N in response to growth in an environment where CO_2 concentrations were doubled (McGuire and others 1995). Moreover, increased CO_2 has typically been associated with increases in nonstructural carbohydrate concentrations (Curtis and Wang 1998; Zhang and others 2008), which can also account for the decrease in construction cost. Thus, doubled CO_2 enhanced the ratio of energetic gains to costs at the leaf level, indicated by the decreased payback time and enhanced photosynthetic energy-use efficiency (PEUE), which is a measurement of the total energy gained via photosynthetic activity per unit of energy required to construct photosynthetic (that is, leaf) biomass (Fig. 1). On the other hand, the decreased growth rate under water deficit resulted mainly from the decreased A_{growth}, because drought stress had little effect on construction cost in *J. curcas*. Overall, biomass accumulation was positively correlated with A_{growth}, PNUE, and PEUE and negatively correlated with payback time (Fig. 2).

However, *J. curcas* showed a significant downregulation in P_{max} under elevated CO_2 exposure (Fig. 3), which is consistent with the photosynthetic acclimation of many species grown under long-term CO_2 enrichment (Long and others 2004; Langley and Megonigal 2010; Lei and others 2012; Wang and others 2012). Central to this acclimation response is a reduction in ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) protein activity and content. A decline in Rubisco activity, activation state, and protein content in many CO_2-enriched plants has been shown

(Zhang and others 2008). In our study, despite a much higher growth rate in CO_2-enriched *J. curcas*, there was a 31.1, 19.6, and 31.7 % decrease in in vivo (that is, initial) Rubisco activity and a 31.0, 35.0, and 23.0 % decrease in total Rubisco activity under well-watered, moderate drought, and severe drought stress, respectively; CO_2 enrichment had little effect on the Rubisco activation state (Fig. 3). Sage and others (2008) also reported a 71, 19, and 30 % decrease in Rubisco initial activity, activation state, and Rubisco protein content, respectively, in all five C_3 species studied following long-term exposure to high levels of CO_2. Here, we did not measure the Rubisco protein content; however, from the decrease in foliar N content, we could infer the lower Rubisco content in *J. curcas* under elevated CO_2, because about 30–50 % of leaf N was used for Rubisco construction (Eichelmann and Laisk 1999). Therefore, the acclimatory response of photosynthesis was likely due to both downregulation of Rubisco activity and N dilution. Other studies have suggested that the accumulation of large starch grains, a common phenomenon under CO_2 enrichment, can also contribute to the lower photosynthetic rate through disruption of chloroplast integrity or hindrance of CO_2 diffusion from the intercellular space to the chloroplast stroma (Daniel and Pierre 2006). On the other hand, under moderate water deficit, the decrease in photosynthesis capacity in *J. curcas* was due to merely the lower initial Rubisco activity. When drought became severe, total Rubisco activity also decreased substantially, which was conducive to the decreased P_{max} (Fig. 3). P_{max} was positively correlated with Rubisco initial and total activities, whereas there was no relationship between P_{max} and the Rubisco activation state (Fig. 4).

The magnitude of the response to elevated CO_2 varied between species, growing season, and experimental conditions, especially nitrogen and water status (Poorter and Navas 2003). In studies concerned with the interactive effects of elevated CO_2 and water stress, some found that elevated CO_2 significantly increased plant growth under water deficit, possibly because plants growing in elevated CO_2 have many features in common with those under drought stress, including stomatal closure, increased leaf thickness, and more biomass allocation to roots, and therefore can improve water relations and withstand the drought stress better (Derner and others 2003; Xiao and others 2005). However, some studies demonstrated that growth responses to elevated CO_2 were greater in well-watered plants than in drought-stressed plants (Xu and others 2007). Elevated CO_2 enhanced biomass accumulation of *J. curcas* by 31.5, 25.9, and 14.4 % under well-watered, moderate drought, and severe drought treatments, respectively, which shows that the stimulation of elevated CO_2 cannot be sustained under drought stress (Table 1). This was in accordance with the recent findings that the effect of CO_2 enrichment was strongly related to soil N conditions. Under control and N deposition (NH_4NO_3 addition), elevated CO_2 significantly enhanced the growth of invasive *Eupatorium adenophorum* and native *E. stoechadosmum*, whereas under reduced N through sugar addition, the stimulating effect was diminished, especially for the native species (Lei and others 2012).

In conclusion, stimulation of plant growth by CO_2 enrichment may be negated under soil drought in the future by regulating the biomass allocation, energy gain, and cost balance. Long-term field experiments in which multiple factors are manipulated simultaneously are needed to explore how the ecophysiological traits measured for *J. curcas* translate into bioenergy production in the future.

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References

Achten WMJ, Verchot L, Franken YJ, Mathijs E, Singh VP, Aerts R, Muys B (2008) *Jatropha* bio-diesel production and use. Biomass Bioenerg 32:1063–1084

Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytol 165:351–371

Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. Plant Cell Environ 30:258–270

Albert KR, Ro-Poulsen H, Mikkelsen TN, Michelsen A, van der Linden L, Beier C (2011) Interactive effects of elevated CO_2, warming, and drought on photosynthesis of *Deschampsia flexuosa* in a temperate heath ecosystem. J Exp Bot 12:4253–4266

Bauhus J, Messier C (1999) Soil exploitation strategies of fine roots in different tree species of the southern boreal forest of eastern Canada. Can J For Res 29:260–273

Curtis PS, Wang XZ (1998) A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. Oecologia 113:299–313

Daniel PR, Pierre T (2006) Leaf carbohydrate controls over Arabidopsis growth and response to elevated CO_2: an experimentally based model. New Phytol 172:500–513

Davey PA, Olcer H, Zakhleniuk O, Bernacchi CJ, Calfapietra C, Long SP, Raines CA (2006) Can fast-growing plantation trees escape biochemical down-regulation of photosynthesis when grown throughout their complete production cycle in the open air under elevated carbon dioxide? Plant Cell Environ 29:1235–1244

Deng XZ, Han JZ, Yin F (2012) Net energy, CO_2 emission and land-based cost-benefit analysis of *Jatropha* biodiesel: a case study of the Panzhihua region of Sichuan Province in China. Energies 5:2150–2164

Derner JD, Johnson HB, Kimball BA, Pinter PJ, Polley HW, Tischler CR, Boutton TW, Lamorte RL, Wall GW, Adam NR, Leavitt SW, Ottman MJ, Matthias AD, Brooks TJ (2003) Above- and below-ground responses of C3–C4 species mixtures to elevated CO2 and soil water availability. Global Change Biol 19:452–460

Diaz-Lopez L, Gimeno V, Simon I, Martinez V, Rodriguez-Ortega WM, Garcia-Sanchez F (2012) *Jatropha curcas* seedlings show a water conservation strategy under drought conditions based on decreasing leaf growth and stomatal conductance. Agric Water Manage 105:48–56

Eichelmann H, Laisk A (1999) Ribulose-1,5-bisphosphate carboxylase/oxygenase content, assimilatory charge, and mesophyll conductance in leaves. Plant Physiol 119:179–189

Farquhar GD (1980) Carbon isotope discrimination by plants: effects of carbon dioxide concentrations and temperature via the ratio of intercellular and atmospheric CO_2 concentration. In: Pearnman GI (ed) Carbon dioxide and climate: Australian Research. Australian Academy of Science, Canberra, pp 105–110

Feng YL, Li YP, Wang RF, Callaway RM, Valiente-Banuet A, Inderjit A (2011) A quicker return energy-use strategy by populations of a subtropical invader in the non-native range: a potential mechanism for the evolution of increased competitive ability. J Ecol 99:1116–1123

Galle A, Haldimann P, Feller U (2007) Photosynthetic performance and water relations in young pubescent oak (*Quercus pubescens*) trees during drought stress and recovery. New Phytol 174:799–810

Gimeno V, Syvertsen JP, Simon I, Nieves M, Diaz-Lopez L, Martinez V, Garcia-Sanchez F (2012) Physiological and morphological responses to flooding with fresh or saline water in *Jatropha curcas*. Environ Exp Bot 78:47–55

Gonzelez-Meller MA, Taneva L, Trueman RJ (2004) Plant respiration and elevated atmospheric CO_2 concentration: cellular responses and global significance. Ann Bot 94:647–656

Griffin KL (1994) Calorimetric estimates of construction cost and their use in ecological studies. Funct Ecol 8:551–562

Hodge A (2004) The plastic plant: root responses to heterogeneous supplies of nutrients. New Phytol 162:9–24

IPCC (2007) Climate change 2007: the physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

Jain S, Sharma MP (2009) Biodiesel production from *Jatropha curcas* oil. Renew Sust Energ Rev 14:3140–3147

Korner C (2006) Plant CO_2 responses: an issue of definition, time and resource supply. New Phytol 172:393–411

Kramer PJ, Boyer JS (1995) Water relations of plants and soils. Academic Press, San Diego

Kumar A, Sharma S (2008) An evaluation of multipurpose oil seed crop for industrial uses (*Jatropha curcas* L.): a review. Ind Crop Prod 28:1–10

Langley JA, Megonigal JP (2010) Ecosystem response to elevated CO_2 levels limited by nitrogen-induced plant species shift. Nature 406:96–99

Lei YB, Yin CY, Li CY (2006) Differences in some morphological, physiological, and biochemical responses to drought stress in two contrasting populations of *Populus przewalskii*. Physiol Plant 127:182–191

Lei YB, Wang WB, Feng YL, Zheng YL, Gong HD (2012) Synergistic interactions of CO_2 enrichment and nitrogen deposition promote growth and ecophysiological advantages of invading *Eupatorium adenophorum* in Southwest China. Planta 236:1205–1213

Lilley RM, Ralph PJ, Larkum AD (2010) The determination of activity of the enzyme Rubisco in cell extracts of the dinoflagellate alga *Symbiodinium* sp. by manganese chemiluminescence and its response to short-term thermal stress of the alga. Plant Cell Environ 33:995–1004

Long SP, Ainsworth EA, Rogers A, Ort DR (2004) Rising atmospheric carbon dioxide: plants face the future. Annu Rev Plant Biol 55:591–628

Luo Y, Su B, Currie WS, Dukes JS, Finzi A, Hartwig U, Hungate B, McMurtrie RE, Oren R, Parton WJ, Pataki DE, Shaw MR, Zak DR, Field CB (2004) Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. Bioscience 54:731–739

Maes WH, Achten WMJ, Reubens B, Raes D, Samson R, Muys B (2009) Plant-water relationships and growth strategies of *Jatropha curcas* L. seedlings under different levels of drought stress. J Arid Environ 73:877–884

McGuire AD, Melillo JM, Kicklighter DW, Joyce LA (1995) Equilibrium responses of soil carbon to climate change: empirical and process-based estimates. J Biogeogr 22:785–796

Medlyn BE, Badeck FW, de Pury DGG, Barton CVM, Broadmeadow M, Ceulemans R, De Angelis P, Forstreuter M, Jach ME, Kellomaki S, Laitat E, Marek M, Philippot S, Rye A, Strassemeyer J, Laitinen K, Liozon R, Portier B, Roberntz P, Wang K, Jarvis PG (1999) Effects of elevated [CO_2] on photosynthesis in European forest species: a meta-analysis of model parameters. Plant Cell Environ 22:1475–1495

Mooney HA, Canadell J, Chapin FS, Ehleringer JR, Körner C, McMurtrie RE, Parton WJ, Pitelka LF, Schulze ED (1999) Ecosystem physiology responses to global change. In: Walker BH, Steffen WL, Canadell J, Ingram JSI (eds) The terrestrial biosphere and global change. Cambridge University Press, Cambridge, pp 141–189

Nagel JM, Huxman TE, Griffin KL, Smith SD (2004) CO_2 enrichment reduces the energetic cost of biomass construction in an invasive desert grass. Ecology 85:100–106

Norby RJ, Warren JM, Iversen CM, Garten CT, Medlyn BE, McMurtrie RE (2010) CO_2 enhancement of forest productivity constrained by limited nitrogen availability. Proc Natl Acad Sci USA 107:19368–19373

Poorter H, Navas ML (2003) Plant growth and competition at elevated CO_2: on winners, losers and functional groups. New Phytol 157:175–198

Poorter H, Pepin S, Rijkers T, De Jong Y, Evans JR, Körner C (2006) Construction costs, chemical composition and payback time of high- and low-irradiance leaves. J Exp Bot 57:355–371

Pregitzer KS (2002) Fine roots of trees—a new perspective. New Phytol 154:267–273

Reich PB, Hungate BA, Luo YQ (2006) Carbon-nitrogen interactions in terrestrial ecosystems in response to rising atmospheric carbon dioxide. Annu Rev Ecol Evol Syst 37:611–636

Sage RF, Way DA, Kubien DS (2008) Rubisco, rubisco activase, and global climate change. J Exp Bot 59:1581–1595

Wang D, Heckathorn SA, Wang XZ, Philpott S (2012) A meta-analysis of plant physiological and growth responses to temperature and elevated CO_2. Oecologia 169:1–13

Warren JM, Norby RJ, Wullschleger SD (2011) Elevated CO_2 enhances leaf senescence during extreme drought in a temperate forest. Tree Physiol 31:117–130

Williams K, Field CB, Mooney HA (1989) Relationships among leaf construction costs, leaf longevity, and light environment in rainforest plants of the genus Piper. Am Nat 133:198–211

Xiao CW, Sun OJ, Zhou GS, Zhao JZ, Wu G (2005) Interactive effects of elevated CO_2 and drought stress on leaf water potential and growth in *Caragana intermedia*. Trees 19:711–720

Xu ZZ, Zhou GS, Wang YH (2007) Combined effects of elevated CO_2 and soil drought on carbon and nitrogen allocation of the desert shrub *Caragana intermedia*. Plant Soil 301:87–97

Ye LF, Fu X, Ge F (2011) Enhanced sensitivity to higher ozone in a pathogen-resistant tobacco cultivar. J Exp Bot 63:1341–1347

Zhang YB, Duan BL, Qiao YZ, Wang KY, Korpelainen H, Li CY (2008) Leaf photosynthesis of *Betula albosinensis* seedlings as affected by elevated CO_2 and planting density. For Ecol Manage 255:1937–1944