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Photosynthesis in plants of four tropical species growing under elevated CO_2

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Abstract

We studied the responses of leaf gas exchange and growth to an increase in atmospheric CO_2 concentration in four tropical deciduous species differing in carbon fixation metabolism: *Alternanthera crucis*, C3-C4; *Ipomoea carnea*, C3; *Jatropha gossypifolia*, C3; and *Talinum triangulare*, inducible-CAM. In the first stage, plants were grown in one open-top chamber at a CO_2 concentration of 560±40 µmol mol^{-1} (EC), one ambient CO_2 concentration chamber (AC), and one unenclosed plot (U). In the second stage, plants were grown in five EC chambers (CO_2 concentration = 680±30 µmol mol^{-1}), five AC chambers, and five unenclosed plots. During the first weeks under EC in the first stage, plants of all the species had a very marked increase in their maximal net photosynthetic rates (P_{max}) of 3.5 times on average; this stimulatory effect was maintained for 11-15 weeks, rates dampening afterward to values still higher than controls for 37 weeks. After a suspension of CO_2 enrichment for 6 weeks, an increase in P_{max} of EC plants over the controls was found in plants of all the species until week 82 of the experiment. Stomatal conductance (g) showed no response to EC. Carboxylation efficiency decreased in all the species under EC

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Abbreviations: C_a = external CO_2 concentration; C_i = intercellular CO_2 concentration; EC = elevated CO_2; AC = ambient (control) CO_2; U = unenclosed plot; g = leaf conductance; PPFD = photosynthetic photon flux density; P_N = net photosynthetic rate; P_{max} = maximum photosynthetic rate; RuBPCO = ribulose-1,5-bisphosphate carboxylase/oxygenase; T = temperature.

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and this was correlated with a decrease in ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) content in all the species except for *T. triangulare*. During drought P_{max} was higher in all species, except for *T. triangulare*, grown under EC relative to controls. Ecosystem photosynthetic rates at EC were higher than in the controls during the second stage under irrigation as well as after 30 d of drought.

Additional key words: *Alternanthera*; C3, C3-C4, and CAM plants; *Ipomoea*; *Jatropha*; ribulose-1,5-bisphosphate carboxylase/oxygenase; stomatal conductance; *Talinum*.

Introduction

The increase in atmospheric CO_2 concentration is one of a series of global climatic changes that affect the functioning of natural and managed ecosystems. Photosynthesis and productivity in C3 species are predicted to increase with atmospheric CO_2 concentration (*e.g.*, Kimball 1983, Poorter 1993).

Growth enhancement has been found in open-top chamber systems in the C3 sedge *Scirpus olneyi* grown under 680 μmol mol^{-1} CO_2 (Arp and Drake 1991) and in C3 tree seedlings grown under 650 μmol mol^{-1} CO_2 (Gunderson *et al.* 1993). The duration of the increase in net photosynthetic rate (P_N) with elevated CO_2 concentration (EC) can vary from a few days (Besford *et al.* 1990) to weeks or months (Wulff and Strain 1982, Jackson *et al.* 1994) and years (Arp and Drake 1991), depending on species and/or growth conditions. Nevertheless, P_N often decreases after this stimulation by CO_2 to values similar to or even lower than those of plants under ambient CO_2 concentration (AC) (Wulff and Strain 1982). In the latter case, this inhibition of P_N can be reversed by the utilization of stores of saccharides during periods of rapid growth (Woodward *et al.* 1991).

Water-use efficiency (WUE) increases in some plants grown under EC, due to an increase in P_N and a reduction in leaf conductance (g) (Jackson *et al.* 1994); however, no effect of EC on WUE was found in *Lolium perenne* (Nijs *et al.* 1988). Information on the response of tropical wild plants to EC is very limited, existing reports deal mainly with watered plants of species which do not experience water deficit frequently in their natural habitat (Hogan *et al.* 1991, Körner and Arnone 1992).

Based on the variability of responses to EC found among temperate species, it is necessary to study the effects of a long-term exposure to EC in tropical wild species. The present study aims to determine the response of gas exchange and related parameters to long-term exposure to EC in four species differing in photosynthetic metabolism, habit (shrub and herb), size, and drought resistance mechanisms, that grow in the semiarid zones of northwestern Venezuela. These plants restrict their annual growth cycle to short and unpredictable rainy periods, which makes them interesting study subjects, particularly in view of the predicted increase in aridity in the tropics that will accompany the continued increase in CO_2 concentration (Erikson 1992). The species studied were chosen because they are important components of their natural ecosystem. Plants were grown in open-top chambers at

EC; control chambers and unenclosed plots were included. Effects of drought on gas exchange under these conditions are also reported.

Materials and methods

Experimental sites: The study was carried out in two stages. In each stage, CO_2 concentration in EC chamber was approximately 200 µmol mol^{-1} higher than ambient (AC). In the first stage, one EC open-top chamber (C_a = 560±40 µmol mol^{-1}), one AC open-top chamber (C_a = 350±40 µmol mol^{-1}), and one unenclosed plot (U) were placed on an area of approximately 100 m^2 in the grounds of the Instituto de Biología Experimental (Caracas) at 900 m. Mean annual maximum temperature is 25±3 °C and rainfall is distributed from May to December for a yearly total of 1 100 mm. The second stage was carried out at the experimental site of the Centro de Investigaciones Agrícolas y Pecuarias (CENIAP) in the town of Maracay, Estado Aragua, Venezuela, where the rainy season spans the same months as in Caracas with a yearly total rainfall of 1200 mm and a mean annual temperature of 30±2 °C. Five EC chambers (C_a = 680±30 µmol mol^{-1}), five AC chambers (C_a = 480±20 µmol mol^{-1}), and five U plots were established on an area of approximately 800 m^2. The field was divided in 20 square portions of 6×6 m, and chambers and plots allotted in such a way that chambers were as far apart from each other as possible.

Open-top chambers: The open-top chambers were built after modifying a design by Ashenden *et al.* (1992). Each chamber consisted of a cylinder 1.55 m high and 2.00 m wide made of aluminum-tubing covered with a 2 mm thick polymethylacrylate sheet transparent to all visible and UV wavelengths. The cylinder was topped with a fustrum of the same material set at an angle of 60°. Ventilation was provided by a system consisting of a 7.6 cm diameter, 1.5 m long PVC pipe in whose distal end a fan was installed. The pipe was connected with a PVC T-piece to a 7.6 cm diameter flexible perforated aluminum pipe placed 15 cm above the ground and encircling the inside of the chamber wall. The CO_2 was injected when gas from a 25 kg cylinder (*AGA de Venezuela*, Caracas, Venezuela) was released through a flux regulator into the PVC ventilation pipe. The CO_2 concentration in EC was controlled by a *WMA-2* IRGA (*PP Systems*, Hitchin, U.K.), which sampled air from the chamber through a pipe placed 25 cm above the ground. Frequency and width of the square pulse used by the IRGA were set empirically so that CO_2 was injected when concentration was lower than that set.

Plants: Perennial deciduous *Ipomoea carnea* Jacq. (*Convolvulaceae*), a C3 shrub, *Jatropha gossypifolia* L. (*Euphorbiaceae*), a C3 shrub; *Alternanthera crucis* (Moq.) Bondingh (*Amaranthaceae*), a C3-C4 herb; and *Talinum triangulare* (Jacq.) Willd. (*Portulacaceae*), an inducible-CAM herb, were used. The mechanisms of drought resistance in these species have been studied in natural as well as greenhouse conditions by Herrera *et al.* (1991, 1994) and Tezara *et al.* (1998): *I. carnea, J. gossypifolia,* and *T. triangulare* were classified as drought evaders, and *A. crucis* as drought-tolerant. The inducible-CAM metabolism in *T. triangulare* was characterized by Herrera *et al.* (1991). *A. crucis,* previously reported as a C4 plant on the basis of its Kranz anatomy and low saturating CO_2 concentration for photosynthesis, was afterward characterized as an intermediate, C3-C4, species due to the occurrence of oxygen or Warburg effect on photosynthesis; P_N increased 1.5 times when measured at an O_2 concentration of 2 mmol mol^{-1} relative to the value obtained at an O_2 concentration of 210 mmol mol^{-1} (W. Tezara, unpublished).

Plants were grown from seed or cuttings obtained in thorn scrubs of northwestern Venezuela. In the first stage (Caracas) three young individuals of similar size (approximately 30-cm high) per species were planted in each treatment. Since soil in the experimental site was rocky and apparently poor, chambers and unenclosed plot were set over previously excavated circular holes 50-cm deep with an area of 3.14 m^2 filled with 1.6 m^3 each of commercial organic soil. Plants were fertilized with commercial N:P:K (15:15:15) twice a month and watered frequently. On week 46, CO_2 enrichment was stopped for 6 weeks due to technical failure and resumed afterwards; plants were pruned shortly before in order to examine the effects of EC on new foliage. When the dry season began, watering was suspended (week 82), to be resumed on week 86.

In the second stage (Maracay) young individuals of similar size were planted one per species per treatment. The soil was 75 % sand, 15 % silt, and 10 % clay with a pH of 5.9. Plants of *I. carnea* were excluded from this stage because, being much taller, they shaded the other plants; also, the C3 metabolism was already represented by *J. gossypifolia.* Plants were frequently watered and fertilized as above. Since a significant recruitment took place in the chambers and plots during 60 weeks, the larger individuals were removed on week 60 in order to return planting density to values similar to those at the beginning of the experiment. When the dry season began, watering was suspended (week 84).

Microclimatic variables: Air T was measured with thermistors (*Yellow Springs Instruments*, Yellow Springs, Ohio, USA) and relative humidity with a shielded hair hygrometer (*Abbeon CAL*, Santa Barbara, CA, USA). Soil water content was determined gravimetrically in samples taken at a depth of 5.0 cm with a 1.5-cm diameter cork borer.

Gas exchange: P_N and g were measured in the first stage using portable infrared gas analyzers (*LCA2, Analytical Development Co.*, Hoddesdon, UK, and *CIRAS 1, PP Systems*, Hitchin, UK) connected to *PLC(B)* assimilation chambers (same manufacturers as above). Air supply at the desired CO_2 concentration was provided by a cylinder in the case of the *LCA2* IRGA and by pure CO_2 cartridges in the case of the *CIRAS 1* IRGA. Measurements were made approximately every 1.5 h between 09:00 and 16:00; outside of this part of the light period plants showed respiration. During measurements, PPFD ranged from 800 to 1500 $\mu mol\ m^{-2}\ s^{-1}$. The response of P_N to C_i was assessed. Carboxylation efficiency was calculated as dP_N/dC_i on the linear portion of the response curve and operational C_i determined at C_a = 560 $\mu mol\ mol^{-1}$ for EC plants and C_a = 350 $\mu mol\ mol^{-1}$ for AC and U plants. During measurements, leaf T and PPFD were 29±1 °C and 1400±100 $\mu mol\ m^{-2}\ s^{-1}$, respectively.

Ecosystem photosynthetic rate was measured in the second stage by turning off the CO_2 supply to the open-top chamber, covering the chamber with a colourless polyethylene sheet, and recording the decrease in C_a during 5 min using the *LCA2* IRGA. A similar procedure was followed in the AC and U ecosystems. Ecosystem photosynthetic rate was calculated from the slope of the curve CO_2 concentration *vs.* time on the basis of ground area inside the chamber or covered by the unenclosed plot.

Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) content was determined in buffered extracts of leaf discs by ELISA after Metodiev and Demirevska-Kępova (1992) using a rabbit serum antibody prepared against purified RuBPCO (*Sigma Chemical Company*, Missouri, U.S.A.).

Data analysis: All measurements were made in triplicate. Significance at $p<0.05$ was assessed through the Mann-Whitney U-test using the *Statistica 4.0* package. AC chamber effect was assumed when EC was not statistically different from AC. Effects of CO_2 were assumed when EC was statistically different from AC.

Results

Values of most microclimatic variables for clear days of the experiment were similar between the first and the second stages. Mean values of variables during the first/second stage were 32.2/34.8 °C (maximum air T), 20.0/21.2 °C (minimum air T), 92.3/100 % (maximum RH), 41.0/59.6 % (minimum RH), and 19.8/11.7 % DW

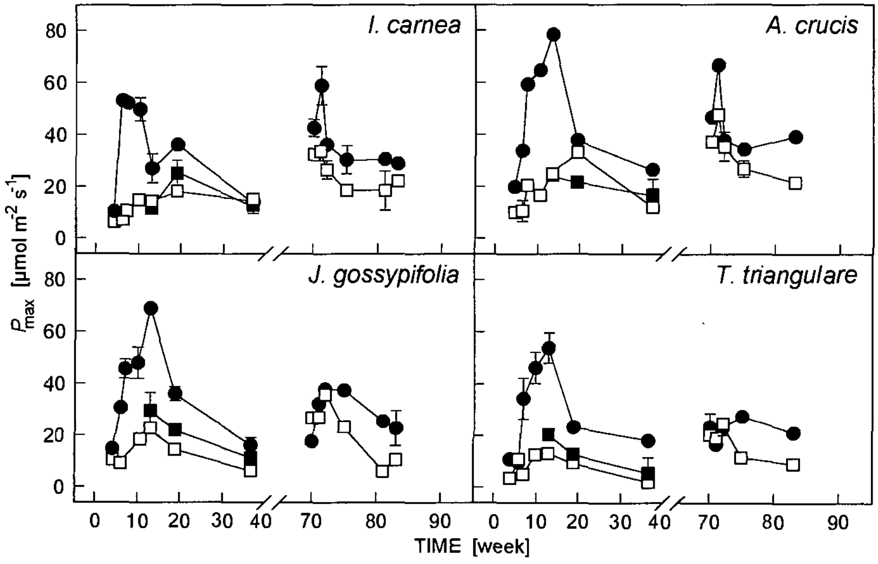

Fig. 1. Time-course of changes in maximum photosynthetic rates, P_{max}, of watered plants grown in the unenclosed plot (□), in the ambient CO_2 chamber (■), or under elevated CO_2 and measured at C_a = 560 µmol mol^{-1} (●) during the first stage of the experiment. Values are means±SE (n = 3). Note the change of scale on the abscissa after the break.

Table 1. Carboxylation efficiency and RuBPCO content measured during the first stage of the study in plants grown for 75 weeks in the open-top chamber at C_a = 560 mmol mol^{-1} (EC), in the control chamber (AC), and in the unenclosed plot (U). Values are means (n = 3). An asterisk denotes significant differences (p<0.05) due to CO_2.

Species	dP_N/dC_i [mol m^{-2} s^{-1}]			RuBPCO content [g m^{-2}]		
	U	AC	EC	U	AC	EC
I. carnea	0.3021	0.2328	0.1647*	6.5	3.2	2.9*
J. gossypifolia	0.1685	0.1697	0.0718*	6.9	6.1	4.4*
A. crucis	0.3556	0.2427	0.0973*	7.5	8.3	5.9*
T. triangulare	0.1185	0.0837	0.0529*	5.1	5.2	5.5

(soil water content), respectively. Open-top chambers were on average 3 °C warmer and 3 % more humid during the light hours than the unenclosed plots (p<0.05).

Gas exchange-first stage (Caracas): Plants of all species showed a significant increase in P_{max} when grown and measured at high CO_2 concentration relative to controls (EC plants measured at C_a = 350 µmol mol^{-1} and U plants; Fig. 1); no significant differences were found among controls (results for AC not shown for clarity). A very large photosynthetic stimulation of 3.5 times on average took place by week 5 and persisted until weeks 11-13. On week 19, rates dampened significantly but were still higher than in the controls. When CO_2 enrichment was resumed after a suspension of six weeks, measurements made on week 71 in plants of *I. carnea* and *A. crucis* showed again a marked increase of 1.4 times in P_{max}; the increase in P_{max} in the other species occurred later in the experiment, rates being higher for EC plants than for AC and U plants of all species until week 81.

Daily carbon balance, the result of integrating diurnal courses of P_N, also increased with EC (Fig. 2). Carbon gain integrated for 37 weeks showed an average

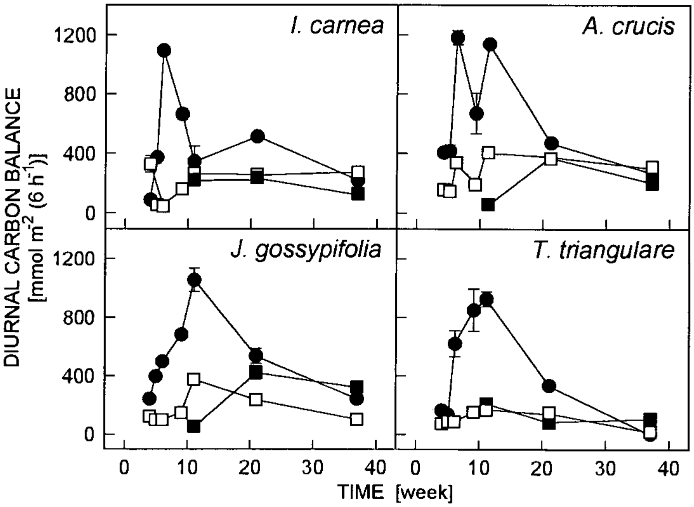

Fig. 2. Time-course of changes in daily carbon balance during the light hours of watered plants grown in the control chamber (■) and the unenclosed plot (□) and grown under elevated CO_2 and measured at C_a = 560 µmol mol^{-1} (●) during the first stage. Values are means±SE (n = 3).

ratio of EC/U = 2.3 for all species.

In P_N/C_i curves done on week 37 (Fig. 3), values of P_N at the same C_i of plants grown in EC compared to U grown plants were lower in *I. carnea* and higher in *A. crucis* and *T. triangulare*, no differences being observed for *J. gossypifolia*. Photosynthetic rates measured at the operational C_i were higher in all species grown under EC, except for *I. carnea*. On week 75, when P_{max} was higher in EC than in AC and U plants, elevated CO_2 concentration decreased carboxylation efficiency in all species and RuBPCO content in *I. carnea, J. gossypifolia,* and *A. crucis* (Table 1).

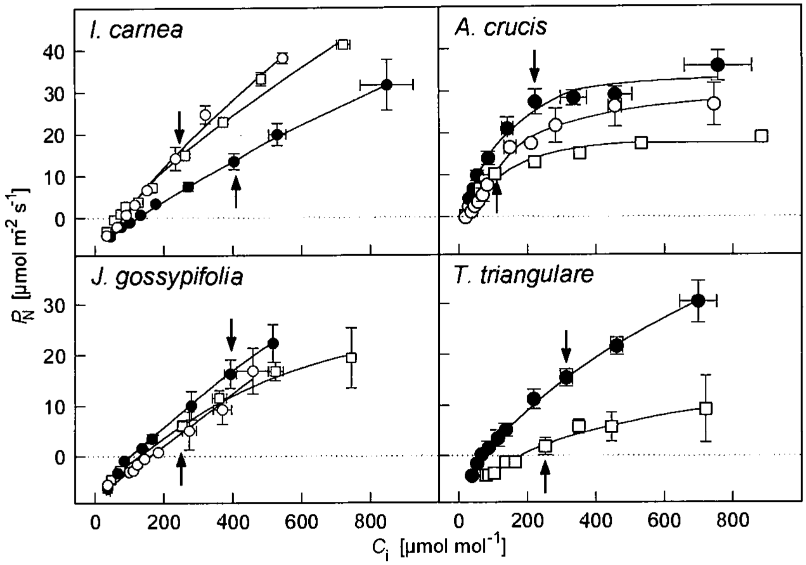

Fig. 3. Response curves of net photosynthetic rate, P_N, to intercellular CO_2 concentration, C_i, of watered plants grown in the open-top chamber under elevated CO_2 (●), in the control chamber (○), and in the unenclosed plot (□), measured on week 37 during the first stage. Values are means±SE (n = 3). Arrows indicate the mean value of operational C_i at the corresponding growth C_a.

After four weeks of drought, P_{max} was significantly higher in EC treatment than in AC and U grown plants of *I. carnea* and *A. crucis*; re-watering led to higher values of P_{max} in EC than in AC and U grown plants of all the species, except for *A. crucis* (Table 2). Soil water content decreased with drought by 44 % in EC, 65 % in AC, and 57 % in U ($p<0.05$).

Leaf conductance of watered plants on week 82 was not diminished by EC in any of the species except for *J. gossypifolia*; in *I. carnea* and *T. triangulare*, *g* was even higher under EC than in the controls; a significant chamber effect of reduction of P_{max} in *A. crucis* was reverted by EC (Table 3). In droughted plants, which had significantly lower values of *g* than watered plants, a significant effect (increase) of EC was detected in *I. carnea* and *A. crucis*.

Table 2. Photosynthetic rates of watered plants measured after 82 weeks of treatment and droughted plants measured after 4 weeks of drought during the first stage of the study. Plants were under drought from week 82 to 86 and then re-watered; measurements in re-watered plants were made on week 89. Values are means ($n = 3$). Measurements in the U plot and in the AC chamber were made at $C_a = 350$ µmol mol^{-1}, and in the EC chamber at $C_a = 560$ µmol mol^{-1}. An asterisk denotes significant differences ($p<0.05$) between EC and controls due to CO_2.

Species	Treatment	P_{max} [µmol m^{-2} s^{-1}] U	AC	EC
I. carnea	watered	21.9	20.0	28.7*
	droughted	-1.0	-1.0	9.5*
	re-watered	14.6	12.4	33.3*
J. gossypifolia	watered	10.7	14.9	22.5*
	droughted	1.2	0.2	1.1
	re-watered	11.5	16.9	22.8*
A. crucis	watered	22.5	24.6	40.2*
	droughted	10.7	7.3	24.7*
	re-watered	22.2	19.9	29.2
T. triangulare	watered	10.2	6.4	22.4*
	droughted	-0.2	-0.9	-0.2
	re-watered	9.1	10.6	27.1*

Gas exchange-second stage (Maracay): After 36 weeks of treatment, P_N at saturating PPFD was higher in plants of *J. gossypifolia* and *A. crucis* of all growth conditions when measured at $C_a = 700$ µmol mol^{-1} than when measured at $C_a = 350$ µmol mol^{-1} (Fig. 4*A,B*). Similar results in *T. triangulare* are not reported because plants grew very poorly, due apparently to competition with the faster-growing plants of *J. gossypifolia* and *A. crucis*.

Table 3. Leaf conductance (at P_{max}) of watered and droughted plants in the first stage. Plants were under drought from weeks 82 to 86. Values are means ($n = 3$). Measurements in the U plot and in the AC chamber were made at $C_a = 350$ µmol mol^{-1}, and in the EC chamber at $C_a = 560$ µmol mol^{-1}. An asterisk denotes significant differences ($p<0.05$) between EC and controls due to CO_2.

Species	Treatment	*g* [mmol m^{-2} s^{-1}] U	AC	EC
I. carnea	water	324	425	659*
	drought	59	71	154*
J. gossypifolia	water	867	513	317*
	drought	33	16	17
A. crucis	water	483	340	465*
	drought	137	70	153*
T. triangulare	water	281	212	475*
	drought	1	13	12

Leaf conductance corresponding to these values of P_N increased in *J. gossypifolia* with measurement C_a, showing a strong decrease in EC plants relative to controls, and decreased with measurement C_a in *A. crucis* (Fig. 4*C,D*). In the latter, plants grown under EC and measured at $C_a = 350$ µmol mol^{-1} showed a strong reduction in *g* relative to AC and U grown plants but in plants measured at $C_a = 700$ µmol mol^{-1} no statistically significant differences in *g* were found among growth conditions.

Ecosystem photosynthesis was higher in EC chambers than in the controls during 88 weeks of growth, with the exception of the period between weeks 15 and 39, when an interruption of the CO_2 supply due to technical failure returned values of EC to those of the controls (Fig. 5). After four weeks under drought, between weeks 84 and 88, values in EC were still significantly higher than in the controls. On a leaf area basis, rates on week 60 (when plants were pruned) were 6, 5, and 4 times higher in EC, AC, and U, respectively, than on a chamber area basis, thus resembling more closely P_N of individual leaves.

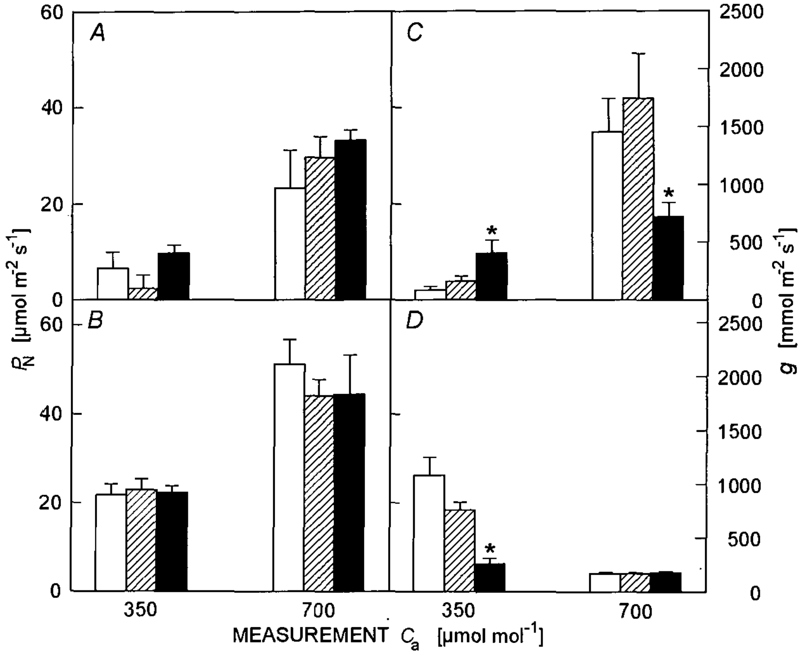

Fig. 4. Effects of growth conditions and measurement C_a on leaf net photosynthetic rate, P_N, in plants of *Jatropha gossypifolia* and *Alternanthera crucis* grown during the second stage in unenclosed plots (*empty bars*), chamber at ambient CO_2 (*stippled bars*) and chamber at elevated CO_2 (*filled bars*). *A*, *B*: photosynthetic rate in *J. gossypifolia* and *A. crucis*, respectively; *C*, *D*: leaf conductance in *J. gossypifolia* and *A. crucis*, respectively. Measurements were made at saturating PPFD (1500-2000 µmol m^{-2} s^{-1}). Values are means±SE ($n = 3$). An asterisk indicates significant differences ($p<0.05$) due to growth under elevated CO_2 for any given species.

Discussion

Growth under EC caused a significant increase in P_{max} and daily carbon balance in all the species studied, independently of carbon fixation metabolism and habit. The highest P_{max} values measured in *I. carnea, J. gossypifolia,* and *A. crucis* were 3.1, 3.8, and 1.6 times higher, respectively, than the highest values measured in the same species in their natural habitat (Tezara *et al.* 1998). Daily carbon balance followed approximately the same trend as P_{max}, similarly to previous findings in the field, where the correlation coefficient was $r^2 = 0.86$ (Tezara *et al.* 1998). Increases of 0.67-2.42 times in P_{max} in C3 woody plants have been reported (Gunderson and Wullschleger 1994). Photosynthetic rate of the C3-C4 plant, *Mollugo lotoides,* increased 50 % when C_a increased from 350 to 500 µmol mol^{-1} (Kennedy *et al.* 1980). In plants of the C4 species, *Amaranthus retroflexus,* P_N increased 30 % under EC relative to AC (Ziska and Bunce 1997).

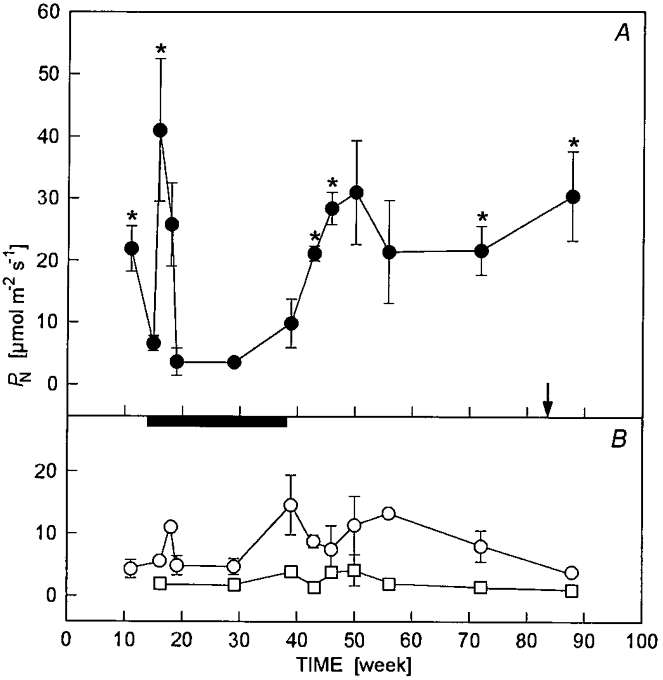

Fig. 5. Time-course of changes in ecosystem net photosynthetic rate, P_N. Measurements were made in the unenclosed plots (□) and in the chambers at ambient CO_2 (○) and in the elevated CO_2 (EC) chambers at elevated CO_2 (●). Values are means ±SE ($n = 3$). An asterisk indicates significant differences ($p<0.05$) between EC and the controls at any given time. The *filled bar* on the abscissa indicates the length of time during which CO_2 was not supplied. The *arrowhead* on the abscissa indicates the beginning of drought.

The dampening in P_N observed after the initial large stimulation during the first weeks of growth was apparently not due to either PPFD, since all measurements were made at PPFD = 1200±300 µmol m^{-2} s^{-1}, nor T, which was on average 33 °C throughout the period encompassing the beginning of determinations, the peak of P_{max}, and the dampening of rate. Results from P_N/C_i curves done in the field (Tezara *et al.* 1998) suggest that temperatures higher than 37 °C are optimum for photosynthesis in these species. The decrease in the daily carbon balance of all species but *A. crucis* to values as low as the controls may not be real because measurements were made in January, the coolest part of the year, when mean maximum T was 28 °C, which is sub-optimum for photosynthesis in these species.

Reasons for the decrease in P_{max} and daily carbon balance may have been developmental, since plants increased markedly in size. A different pattern of assimilate allocation probably ensued after the initial large carbon acquisition, modulating but not inhibiting (down-regulating) P_N. This may have occurred during the first stage because plants were initially small and also because they were pruned on week 46 and new shoots may have responded as small plants.

Also, the decrease in the large stimulation of P_{max} may have occurred as a result of long-term exposure under watered conditions which did not mimic the precipitation of the natural habitat of these species, which is approximately 400 mm y^{-1} in a highly unpredictable pattern (Herrera *et al.* 1994). Frequent watering may have promoted plant and, particularly, root growth above the rate that may be found in the natural habitat, preventing a regulation of the root/shoot ratio and causing substrate limitation. Recurrent periods of water deficit, which may alter source-sink relationships, were invoked as a possible reason for the lack of inhibition of photosynthesis in *Quercus ilex* (Scarascia-Mugnozza *et al.* 1996).

Ecosystem P_N was higher under EC than under AC, similarly to a previous report for greenhouse "tropical ecosystems" (Körner and Arnone 1992), and comparable to mean photosynthetic rates of individual leaves. The finding of higher ecosystem P_N under EC during the second experimental stage confirms the observation of increased P_{max} under EC during the first stage.

Carboxylation efficiency measured in the first stage decreased in EC grown plants, similar to plants of *Acacia mangium* after three months of growth under EC (Ziska *et al.* 1991). A decrease in RuBPCO content was also found in EC plants of all species, except for *T. triangulare*. A decrease in carboxylation efficiency and/or RuBPCO content does not necessarily imply a decrease in P_{max}. In plants of *S. olneyi* measured in open-top chambers, a decrease in RuBPCO content was observed together with maintenance of higher P_N under EC relative to controls (Jacob *et al.* 1995). In contrast, in plants of *Dactylis glomerata* a downward acclimation of P_N to EC was accompanied by a decrease in carboxylation efficiency (Tuba *et al.* 1996).

The decrease in carboxylation efficiency was related to inhibition of photosynthesis only in *I. carnea*. In the other three species a pattern of resource allocation may have favoured, in the presence of a higher supply of the substrate for photosynthesis, other sinks at the expense of enzyme construction, since less RuBPCO is required (Drake *et al.* 1997).

Evidence of acclimation, *i.e.*, biochemical or physiological changes that result from growth under EC (Gunderson and Wullschleger 1994), was provided by the decrease in carboxylation efficiency and RuBPCO content, and the differences detected in P_N at the operational C_i. An increase in P_N at operational C_i was found in five tropical species after three months of growth under EC (Ziska *et al.* 1991).

During drought, the promoting effect of CO_2 on P_{max} observed in watered plants persisted in *I. carnea* and *A. crucis* (first stage), apparently coupled to a smaller decrease in soil water content in the EC compared to the AC and U treatments; the effect was also found in the ecosystem. Higher P_N in plants growing under EC than at AC during drought have been reported in seedlings of tree species (Tschaplinski *et al.* 1995) and in *Quercus ilex* (Scarascia-Mugnozza *et al.* 1996). The reduction of P_{max} due to drought in EC grown plants was slower in *I. carnea* and *A. crucis*, the latter known to affect osmotic adjustment (Rengifo 1997). The fastest reduction of P_{max} was observed in *J. gossypifolia*, a drought evader, and in *T. triangulare*, in which carbon gain occurs mostly by dark fixation once CAM is induced after approximately ten days of drought (Herrera *et al.* 1991).

Stomatal closure has been reported as one of the direct effects of EC (Hogan *et al.* 1991) but in the present study g was actually higher under EC. Intrinsic WUE (P_N/g, results not shown) was significantly increased by CO_2 in watered plants of *J. gossypifolia* and *T. triangulare* and in droughted plants of *J. gossypifolia* and *A. crucis* due to an increase in P_{max} in watered as well as droughted plants, rather than to a reduction in g.

Plants of the four species responded with gas exchange and other physiological and biochemical characteristics to EC as expected, since all but *A. crucis* operated basically through the C3 metabolism when watered (the proportion of CO_2 fixed directly through the PCR cycle in *A. crucis* remains to be determined). Photosynthesis responded to EC through direct and indirect effects of CO_2 on the photosynthetic apparatus. In a future atmosphere with a higher CO_2 concentration, these species should be able to show a higher productivity than today during periods of sufficient water supply and to lengthen the period of carbon acquisition during drought.

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