Positive effects of elevated CO2 and its interaction with nitrogen on safflower physiology and growth

Shiren Mohamed, Anita Jellings, Michael Fuller

Agron. Sustain. Dev. (2013) 33:497–505
DOI 10.1007/s13593-013-0137-x

RESEARCH ARTICLE

Positive effects of elevated CO_2 and its interaction with nitrogen on safflower physiology and growth

Shiren J. Mohamed · Anita J. Jellings · Michael P. Fuller

Accepted: 11 February 2013 / Published online: 15 March 2013

Abstract Over the last two decades, the impact of elevated CO_2 on crops has become a major issue in the context of climate change. Increasing CO_2 levels should modify the plant demand for nutrients, but precise effects on plant physiology are poorly known. Here, we studied the effect of ambient CO_2 at 400 μmol mol^{-1} and high CO_2 at 1,000 μmol mol^{-1} on safflower (*Carthamus tinctorius* L.) at N levels from 25 to 175 kg ha^{-1}. Growth and physiology of safflower were assessed in pots in controlled enclosure chambers in a glasshouse. Overall results show that high CO_2 increased assimilation rate by +27 %, leaf area index (LAI) by +28 %, total above-ground dry weight by +51 %, and total above-ground dry weight by +43 % at harvest. High CO_2 reduced stomatal conductance by −29 % and transpiration rate by −18 %. At anthesis, results show that high CO_2 increases assimilation rate by +13 %, LAI by +2 %, and above-ground dry weight by +34 %. At anthesis, results also show that high CO_2 decreases leaf N and chlorophyll content.

Keywords Elevated CO_2 · Safflower · Gas exchange · Assimilation rate · LAI · Biomass

1 Introduction

Plant growth and productivity are affected by climate primarily due to changes in photosynthetic rate (Reddy et al. 2010). It is frequently reported (Ainsworth and Long 2005; Ainsworth and Rogers 2007) that the rate of photosynthesis of C_3 plants grown at elevated CO_2 activates the carboxylation binding site of Rubisco, decreases the oxidation activity and hence inhibits photorespiration (Taiz and Fizeiger 2002). In addition, the higher CO_2 reduces the stomatal conductance (Ainsworth and Rogers 2007) leading to enhanced water use efficiency (WUE) (Hsiao et al. 1999). As a result, enhancement of plant growth is expected, but the magnitude of the response differs between different crops (Ainsworth and Long 2005). Productivity is also strongly related to vegetative growth in terms of leaf area index (LAI) and directly associated with biomass since LAI determines the amount of light intercepted and ultimately the net photosynthetic rate (Gastal and Lemaire 2002). LAI is widely reported to be increased at elevated CO_2 (Campbell et al. 2001; Manderscheid et al. 2003).

In general, increased growth at elevated CO_2 has been reported to increase demand for mineral nutrients (Reddy et al. 2004), and under low nitrogen supply, a reduction in photosynthetic capacity has often been suggested (Del Pozo et al. 2007; Drake et al. 1997). Primarily, because the enzymes of the Calvin cycle and thylakoids equate to the majority of the plant's protein content (Taiz and Fizeiger 2002) indirectly, nitrogen deficiency leads to a limit of sink development to utilize additional photoassimilate from the enhanced photosynthesis (Ainsworth et al. 2003). However, elevated CO_2 can also increase photosynthetic nitrogen use efficiency (NUE) (Zerihun et al. 2000), and as a result, the negative effect of nitrogen deficiency may be ameliorated by elevated CO_2 (Radoglou et al. 1992), and plant growth and biomass are sometimes not significantly different between low and high-nitrogen treatments at elevated CO_2 (Larigauderie et al. 1988). To date, there are no reports in the literature concerning the effect of elevated CO_2 on safflower physiology and growth in spite of the medical,

S. J. Mohamed · A. J. Jellings · M. P. Fuller (✉)
School of Biological and Biomedical Sciences,
Plymouth University, Plymouth PL4 8AA, UK
e-mail: mfuller@plymouth.ac.uk

S. J. Mohamed
Field Crops Department, College of Agriculture,
University of Sulaimani, Sulaimani, Kurdistan, Iraq

pharmaceutical and economic importance that have been demonstrated for safflower (Dajue and Mundle 1996). Safflower is typically grown in the arid or semi-arid regions of the world (Johnston et al. 2002), many of which are facing potentially favourable climate change (Shaw et al. 2005), but many of these ecosystems are currently facing net loss of nutrient due to exploitative non-sustainable land use (Evans and Belnap 1999). It is important to evaluate the response of semi-arid crop species so that sustainable agronomic management plans can be developed to cope with future climate change scenarios.

The results reported here are the initial investigations of the effect of CO_2 enrichment on physiology and growth of this crop grown in enclosed chambers using a perlite-based hydroponic system.

2 Materials and methods

Two experiments were carried out during the years 2011 and 2012 at Plymouth University, UK, and each experiment lasted approximately 6 months. Seeds of "Richters" Lemon Yellow (Richter Seeds Ltd, UK) were pre-germinated in an incubator for 3 days using a fluctuating 12-h/12-h temperature of 12 and 20–24 °C. These pre-germinated seeds were sown in pots constructed from a cylindrical polypropylene pipe 30 cm high×10 cm in diameter filled with standard grand perlite (William Sinclair Horticulture Ltd, UK). The base of each pot was a loosely fitting transparent graduated 500-mL plastic beaker so that the level of the drainage fluid could be monitored after watering. Pots were placed in eight tightly sealed ventilated chambers (60×60×140 cm) located in a semi-controlled glasshouse with 16 pots per chamber (Fig. 1). Four chambers were supplied with elevated CO_2 (approx. 1,000 μmol mol^{-1}) and four with ambient air (400 μmol mol^{-1}). The experimental design was a randomized block with four replicates, with two ambient and two elevated chambers at each side of the glasshouse, and treatments were randomly assigned to the chambers. Pots were watered with 10–30 mL of a standard hydroponics growth solution (VitaLink Max Grow (soft water) A and B) every 3–5 days until the plants reached the beginning of stem elongation (plants completely established). Thereafter, the amount of solution was increased to 50–200 mL and supplied every week to leave drainage fluid in the drainage beaker up to the 200-mL graduation. Water was applied thereafter according to demand.

Fig. 1 Safflower plants growing in an enclosed elevated CO_2 chamber

In the second experiment, the study layout was a split-plot design with CO_2 as the main plot and nitrogen as sub-plots. Four chambers were supplied with elevated CO_2 and four with ambient air, and nitrogen fertiliser equivalent to 25, 70, 125 and 175 kg N ha^{-1}. Each chamber contained 16 pots (four pots per level of nitrogen). Pots were watered with 10–30 mL of standard hydroponics growth solution A and B every 3–5 days for 1 month. Thereafter, plants were irrigated with 50–200 mL complete Hoagland's solution minus nitrogen (for the recipe, see Hershey 1995) every 5–7 days. Different nitrogen rates were achieved by supplementing the Hoagland's at watering time. Four levels of nitrogen solution were prepared by dissolving 0.7, 1.9, 3.14 and 4.22 g ammonium nitrate in 6.4 L of Hoagland solution to give the equivalent of 25, 75, 125 and 175 kg N ha^{-1} split into four doses at monthly intervals using 200 mL of Hoagland's solution per pot. Between fertigations, appropriate amounts of water were applied according to the demand as in expt 1.

Carbon dioxide was supplied using cylinders of compressed bottled CO_2 (BOC Ltd, UK) coupled to an infrared gas analyser controller (Eurotherm Ltd, UK) which continuously ventilated air in the chambers and pulsed CO_2 accordingly. Natural daylight in the chambers was supplemented using SonT sodium vapour lamps (Phillip Ltd, Holland) during the winter months to maintain a 12-h photoperiod. Monitors (Telaire Ltd, UK) were used to measure CO_2, temperature and humidity at 6-min intervals in each chamber, and the data recorded to dataloggers (Hobo Inc., USA). In the first experiment, the overall mean temperature was 24 °C, and the mean humidity was 85.4 %. In the second experiment, the mean temperature was 25.2 °C, and the mean humidity was 86.3 %. Ranges in temperature were within that normally experienced in the field for this crop.

At 50 % anthesis, the following physiological parameters were measured: assimilation rate (A), stomatal conductance (g_s), transpiration rate (E) and the intercellular CO_2 concentration (Ci) on the three youngest top expanded leaves of all plants using a LCi-SD Portable photosynthesis system (ADC Ltd, UK) equipped with a 6-cm^2 chamber, and the lamp was operated at 11 V DC. In both experiments, the same three youngest leaves used for physiological parameters were used to determine nitrogen and chlorophyll content 1 day after the measurement of physiological parameters. Half of the plants in each chamber in the first experiment and all plants of the six

chambers (three from each ambient and elevated) were harvested, and two chambers, one from each treatment, were left to mature until harvest. Leaf area was measured for all plants at anthesis using an image analysis system (DIAS™ Delta-T Instruments Ltd, UK), and the LAI was calculated by dividing total leaf area by pot surface area. From the harvested plants, three plants from each chamber were sub-sampled, and three sub-samples were used to determine chlorophyll content and three other samples used to determine leaf nitrogen content in the first experiment. In the second experiment, two sub-samples were taken from two plants from each nitrogen treatment in each chamber for chlorophyll and nitrogen content determination; other plants were separated into above-ground fractions (stem, branch, leaf and capitula) and dried at 80 °C for 8 h in a forced air drying oven (Gallenkamp Ltd, UK), and total above-ground dry weight was recorded. Leaf nitrogen concentration was determined using Kjeldahl analysis according to Rudmann et al. (2001), and chlorophyll content on a fresh weight basis was determined using a spectrophotometer set at 645 and 663 nm against an 80 % acetone solvent blank (Taiz and Fizeiger 2002). The amount of chlorophyll present in the extract was calculated on the basis of milligrammes of chlorophyll per gramme of leaf tissue according to the following equations:

$$\text{mg chlorophyll a/g tissue} = [12.7(\text{D}\,663) - 2.69(\text{D}\,645)] \times \text{V}/1,000 \times \text{W}$$

$$\text{mg chlorophyll b/g tissue} = [22.9(D\,645) - 4.68(D\,663)] \times V/1{,}000 \times W$$

$$\text{mg total chlorophyll/g tissue} = [20.2(\text{D}\,645) + 8.2(\text{D}\,663)] \times \text{V}/1,000 \times \text{W}$$

$$\text{mg total chlorophyll/g tissue} = \text{D}\,652 \times 1,000/34.5 \times \text{V}/1,000 \times \text{W}$$

- D The optical density reading of the chlorophyll extract at the specific indicated wavelength
- V The final volume of the 80 % acetone–chlorophyll extract
- W The fresh weight in grammes of the leaf extracted

Statistical analyses of data were performed using Minitab V. 15 using two-way ANOVA in the first experiment and using Minitab V. 15 using nested ANOVA for the second experiment. Least significant difference ($LSD_{0.05}$) was calculated to indicate differences between treatments. Response curves were fitted to data using sequential polynomial fitting to maximise the coefficients of determination, and all curves presented are second-order polynomials with R^2 values between 0.80 and 0.99.

3 Results and discussion

3.1 Gas exchange and assimilation rate

This research reports the results of an investigation of the physiological response of the semi-arid oil-seed crop safflower to elevated CO_2. Elevated CO_2 significantly ($p<0.001$) increased the mean assimilation rate (A) by 27 % compared to ambient at anthesis (Table 1). This was interpreted as a result of increased CO_2 at a carboxylation site of Rubisco and inhibited photorespiration. Such responses are commonly reported (Ainsworth and Long 2005; Ainsworth and Rogers 2007). In fact, it has been reported that elevated CO_2 enhanced assimilation rate in C_3 plants by 31 %, but the magnitude of the increment varied among different species and environments (Ainsworth and Rogers 2007). In general, some modelling studies have reported that assimilation rates were sustained over the time at short-term exposure to rising CO_2, but were down regulated after long-term exposure to CO_2 enrichment (Ainsworth et al. 2002). From his review, Ainsworth et al. (2002) revealed that down regulation in photosynthetic capacity (acclimation) after long-term exposure to elevated CO_2 was related to the size of the pot in which plants were grown because the volume of the pots impacted on the sink size by restricting root growth. Elevated CO_2 significantly ($p<0.001$) reduced stomatal conductance (g_s) by an average of 29 % (Table 1), and as a consequence, the transpiration rate (E) was significantly ($p<0.001$) reduced by 18 % under elevated CO_2 (Table 1). These reductions may be attributed to improvements in WUE and enhanced the assimilation rate as reported elsewhere (Hsiao et al. 1999). However, the reduction in transpiration rate that resulted from increased CO_2 is altered somewhat by larger leaf area induced by doubled CO_2 so that the effect of elevated CO_2 on plant growth is offset (Allen 1999).

It has been frequently reported that increases in both atmospheric CO_2 concentration and nitrogen supply result in large and sustained increases in light-saturated assimilation rate (Johnson et al. 1995; Radoglou et al. 1992; Sanz-Sáez et al. 2010). For example, CO_2 elevated to 700 μmol mol^{-1} resulted in an increase of 30 and 40 % in assimilation rate under high nitrogen and low nitrogen (without nitrogen supplied), respectively, as compared to ambient CO_2 (350 μmol mol^{-1}) in fully expanded primarily leaves of *Phaseolus vulgaris* (Radoglou et al. 1992).

Table 1 Leaf physiological characteristics at anthesis of safflower grown under ambient and elevated CO_2

n=16	Ambient CO_2 mean ($SE_{0.05}$)	Elevated CO_2 mean ($SE_{0.05}$)
Assimilation rate (A), µmol m^{-2} s^{-1}	13.17 (1.22)	927 (0.98)
Stomatal conductance (g_s), mmol m^{-2} s^{-1}	0.221 (0.042)	0.316 (0.019)
Transpiration rate (E), mmol m^{-2} s^{-1}	4.79 (0.417)	5.83 (0.313)
Sub-stomatal CO_2 concentration (Ci), µmol mol^{-1}	504.4 (17.78)	451.1 (44.44)

The results obtained in the second experiment presented here confirmed these findings. At anthesis, all levels of nitrogen supplied under elevated CO_2 (to 1,000 µmol mol^{-1}) increased assimilation rate (A) by 18 % as compared to ambient CO_2 (400 µmol mol^{-1}), and elevated CO_2 resulted in an increase of 15 % in assimilation rate for plants grown with the highest nitrogen supply and 26 % for plants grown under the lowest nitrogen supply (Fig. 2). This increase was a result of increased intercellular CO_2 concentration (Ci) and compensated for limited nitrogen-grown plants under elevated CO_2; as a result, the stomatal conductance (g_s) and, correspondingly, transpiration rate (E) did not significantly (p>0.0) decline under the effect of elevated CO_2. At both ambient and elevated CO_2, stomatal conductance and transpiration rate significantly (p<0.05) increased, respectively, by 26 and 24 % at 125 kg N ha^{-1} as compared to the lowest nitrogen levels (Fig. 2) which was consistent with the finding that stomata have been shown to be sensitive to the intercellular CO_2 concentration (Mott 1988). This can be interpreted as the carboxylation capacity of ribulose-1, 5-bisphosphate carboxylase/oxygenase protein (Rubisco) under elevated CO_2 at high nitrogen (Ainsworth and Long 2005; Ainsworth and Rogers 2007; Jifon and Wolfe 2002). The results presented here also support those previously reported by Radoglou et al. (1992). The results in this study suggest that the effects of CO_2 and nitrogen on assimilation rate are independent of each other. However, Anten et al. (2004) found an interactive effect of nitrogen and CO_2 on canopy carbon gain, indicating that photosynthesis rate is dependent on both, and showed that this interaction resulted from an increase in LAI with increasing nitrogen input, which led to the positive effect of receiving a higher quantum yield of light at elevated CO_2. In fact, the interactive effect between CO_2 and

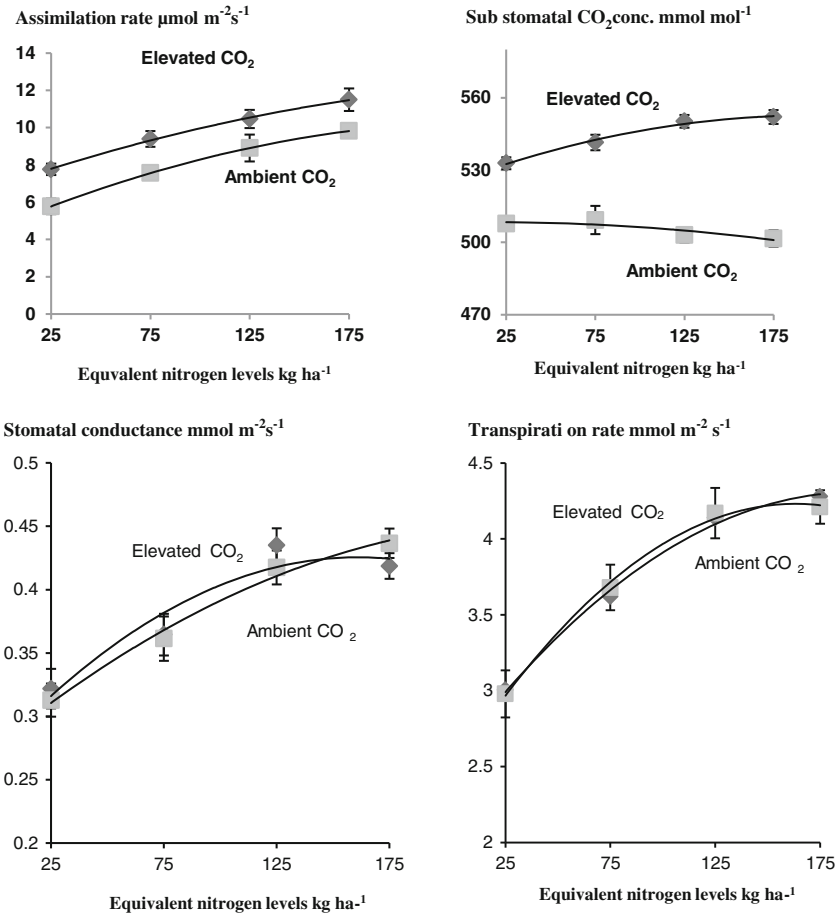

Fig. 2 Trends in aspects of assimilation (assimilation rate, stomatal conductance, sub-stomatal CO_2 and transpiration rate) of safflower grown under ambient and elevated CO_2 at varying levels of nitrogen availability showing how assimilation rate is raised with elevated CO_2 as a result raising internal leaf CO_2 levels. This response is consistently evident across all nitrogen levels, and nitrogen improved the response with each increment. Stomatal conductance and transpiration rate were unaffected by the level of CO_2 but responded to nitrogen incrementally

nitrogen on photosynthetic rate and growth could operate through two mechanisms: nitrogen uptake which decreases photosynthetic acclimation to elevated CO_2, and this has previously been shown to be more obvious when nitrogen is limited (Stitt and Krapp 1999), or long exposure to elevated CO_2 which can lead to photosynthetic acclimation caused by carbohydrate accumulation, which tends to be more pronounced under lower than higher nitrogen availability (Ainsworth and Rogers 2007).

In contrast to these studies, under conditions where nitrogen deficiency decreased leaf area, assimilation and sugar levels and increased allocation of biomass to non-photosynthetic tissue and photosynthetic acclimation to elevated CO_2 were more pronounced in high compared to low nitrogen supply (Jifon and Wolfe 2002). Moreover, Rubisco has not been shown to decrease with nitrogen deficiency under elevated CO_2 (Farage et al. 1998).

3.2 Nitrogen and chlorophyll content

In the first experiment, the youngest fully expanded leaves significantly concentrated less nitrogen at elevated CO_2 compared with ambient CO_2.

Chlorophyll a and b, and total chlorophyll were not significantly affected by elevated CO_2 treatment (Table 2). This trend is consistent with the observation that plants change their nitrogen allocation to optimize energy cost (Jablonski et al. 2002). Moreover, this was interpreted in turn by the increased ability of plants to use nitrogen more efficiently as a result of the marked increase in photosynthetic rate at doubled CO_2 (Cruz et al. 2003). Another explanation is the consequence of a decrease of transpiration rate that resulted from reduced stomatal closure in response to elevated CO_2 which possibly limited plant nitrogen uptake from the root environment, but this possible explanation has received little attention in the literature (Kanemoto et al. 2009).

Safflower leaves concentrated less nitrogen which did not lead to big changes in chlorophyll content at elevated CO_2 (Fig. 3). Previous work on cotton (Delucia et al. 1985) proposed that elevated CO_2 (1000 μl L^{-1}) reduced leaf chlorophyll content, and the ratio of chlorophyll a/b changed with reductions in transpiration rate and nitrogen content. The reduction in leaf chlorophyll content under elevated CO_2 was attributed to mild chlorosis in leaf in response to CO_2 increase.

Table 2 Leaf nitrogen and chlorophyll contents at anthesis of safflower grown under ambient and elevated CO_2

n=3	Ambient CO_2 mean ($SE_{0.05}$)	Elevated CO_2 mean ($SE_{0.05}$)
Leaf nitrogen, g 100 g^{-1} dry wt	2.70 (0.162)	3.57 (0.189)
Chlorophyll a, mg g^{-1} fresh wt	0.52 (0.089)	0.48 (0.054)
Chlorophyll b, mg g^{-1} fresh wt	0.50 (0.051)	0.48 (0.049)
Total chlorophyll, mg g^{-1} fresh wt	1.32 (0.067)	1.28 (0.093)

In the second experiment reported here, at anthesis, elevated CO_2 also significantly ($p<0.05$) reduced nitrogen concentration in expanded leaves with an 8 % reduction compared to ambient CO_2 (Fig. 3), with the greatest reduction under the lowest nitrogen levels. There was no significant CO_2 and nitrogen interaction. It can be concluded that the reduction in plant leaf nitrogen concentration under elevated CO_2 and at all nitrogen treatments did not lead to photosynthetic acclimation, and photosynthetic acclimation has been strongly related to a decrease in leaf nitrogen concentration under low nitrogen supply (Ainsworth and Long 2005). As mentioned earlier, the mean photosynthetic rate (A) for plants grown under the lowest nitrogen level was significantly ($p<0.05$) higher than for the plants grown under the lowest nitrogen level at ambient CO_2. Therefore, this study lends further support to the hypothesis that elevated CO_2 improves the photosynthetic NUE. In agreement with our study, the photosynthetic NUE in sunflower (*Helianthus annuus* L.) increased by an average of 50 % in sunflower at CO_2 enrichment over ambient (Zerihun et al. 2000). Similarly, elevated CO_2 significantly ($p<0.05$) reduced leaf chlorophyll content, and the most reduction was at the lowest nitrogen input (Fig. 3), with a 4, 5 and 8 % reduction in chlorophyll a and b, and total chlorophyll, respectively, under the elevated CO_2 as compared to ambient CO_2, and a reduction of 24, 44 and 31 % in chlorophyll a and b, and total chlorophyll, respectively, at the lowest nitrogen supplied under elevated CO_2 as compared with the highest nitrogen supplied. There was a significant CO_2 × nitrogen interaction for total chlorophyll (Fig. 3). One reason for chlorophyll reduction at elevated CO_2 may be related to a reduction in nitrogen uptake (Nakano et al. 1997) resulting in a decrease in leaf chlorophyll content which resulted in chlorosis as previously reported (Radoglou et al. 1992). In a 2-year study on spring wheat (*Triticum aestivum* L. cv. Alcala), in one of the years, leaf chlorophyll content was not significantly ($p>0.05$) changed by CO_2, but it was significantly reduced under nitrogen deficiency. However, in the other year, the acclimation of leaf photosynthesis rate (A) and stomatal conductance (g_s) in elevated CO_2 over the ambient CO_2 were associated with lower total leaf chlorophyll content (Del Pozo et al. 2005) indicating that other factors are also interacting with those measured. There was no significant ($p>0.05$) interaction effect of elevated CO_2 and nitrogen reported here on leaf nitrogen concentration and all types of chlorophyll content as all increased with each increase in nitrogen levels at both ambient and elevated CO_2. In contrast, Jifon and Wolfe

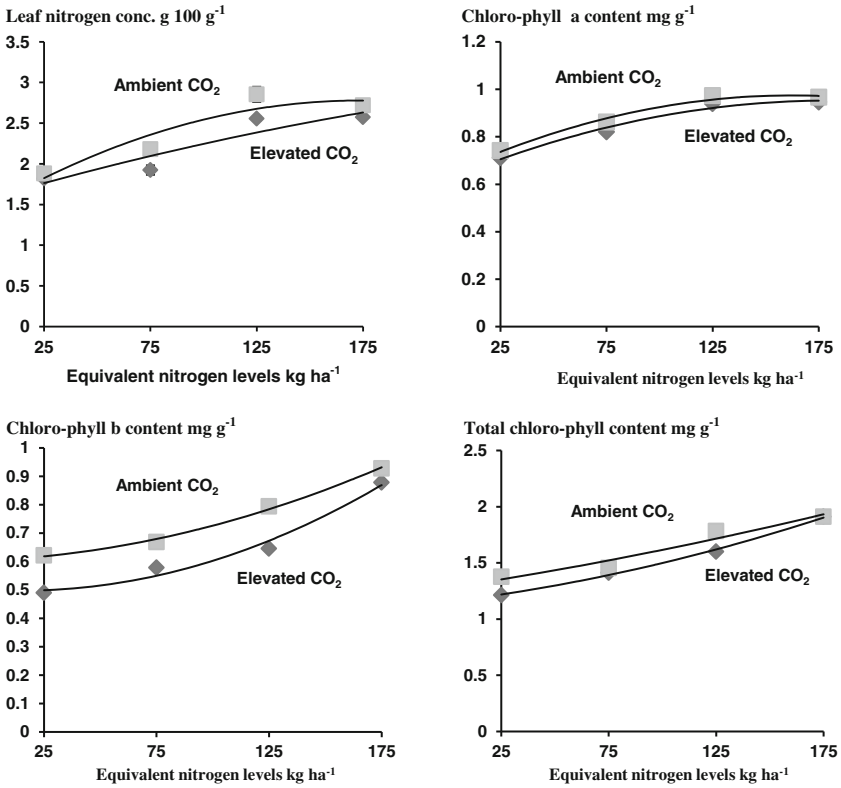

Fig. 3 Trends in aspects of leaf composition (nitrogen and chlorophyll content) of safflower grown under ambient and elevated CO_2 at varying levels of nitrogen availability showing elevated CO_2 has a small but consistent effect of lowering nitrogen and chlorophyll content. Increased nitrogen application incrementally increased both nitrogen and chlorophyll content

(2002) stated that leaf nitrogen and chlorophyll concentration were not significantly affected by elevated CO_2, and the leaf nitrogen concentration was higher under lower nitrogen than high nitrogen, while the chlorophyll content was significantly higher in high-nitrogen than low-nitrogen plants in all CO_2 levels.

3.3 Leaf area index and biomass

In the first experiment, elevated CO_2 significantly increased LAI by 28 % compared to ambient at anthesis (Table 3). The total above-ground dry weight subjected to a higher level of CO_2 followed the same pattern as that for LAI at both anthesis and harvest. LAI increased due to an increase in assimilation rate and growth as previously reported by Manderscheid et al. (2003) who found that increased LAI did not lead to a noticeable change in canopy light absorption at elevated CO_2. However, some authors predict that LAI would increase in upper canopies under doubled CO_2 because elevated CO_2 reduces the light compensation point for photosynthesis and thus stimulates leaf production (Campbell et al. 2001). Others showed no significant effect of elevated CO_2 on LAI (Sims et al. 1999) or found that elevated CO_2 failed to increase the leaf area and decreased specific leaf area (Ainsworth and Long 2005). There was a strong positive response of above-ground biomass to elevated CO_2 observed here with increases of 51 % at anthesis and 43 % at harvest compared to ambient. This increase in biomass could be due to a contribution of photosynthetic capacity improvement due to increased LAI and radiation use efficiency. This result agreed with Manderscheid et al. (2003). In contrast, Yuan et al. (2009) stated that higher resource use efficiency occurred per unit of leaf area rather than increasing the leaf area at elevated CO_2, and they found that total plant dry weight increased at high levels of CO_2 (600 and 800 μmol mol^{-1}) but was not correlated with LAI. However, in the experiment reported here, the increased assimilation rate and LAI did not result in a significant increase in seed number.

In the second experiment, the greatest LAI increase was 6 % at 125 kg N ha^{-1} as compared to 25 kg N ha^{-1}, and this parameter peaked at 125 kg N ha^{-1} at both CO_2 levels (Fig. 4). Such a proportional increase in leaf area and LAI with

Table 3 Leaf area index and biomass of safflower grown under ambient and elevated CO_2

	Ambient CO_2 mean ($SE_{0.05}$)	Elevated CO_2 mean ($SE_{0.05}$)
Leaf area index (LAI) at anthesis	4.60 (0.02)	4.36 (0.03)
Above-ground biomass dry weight at anthesis, g $plant^{-1}$	5.48 (0.522)	3.13 (0.444)
Above-ground biomass dry weight at harvest, g $plant^{-1}$	10.00 (0.522)	4.01 (0.720)

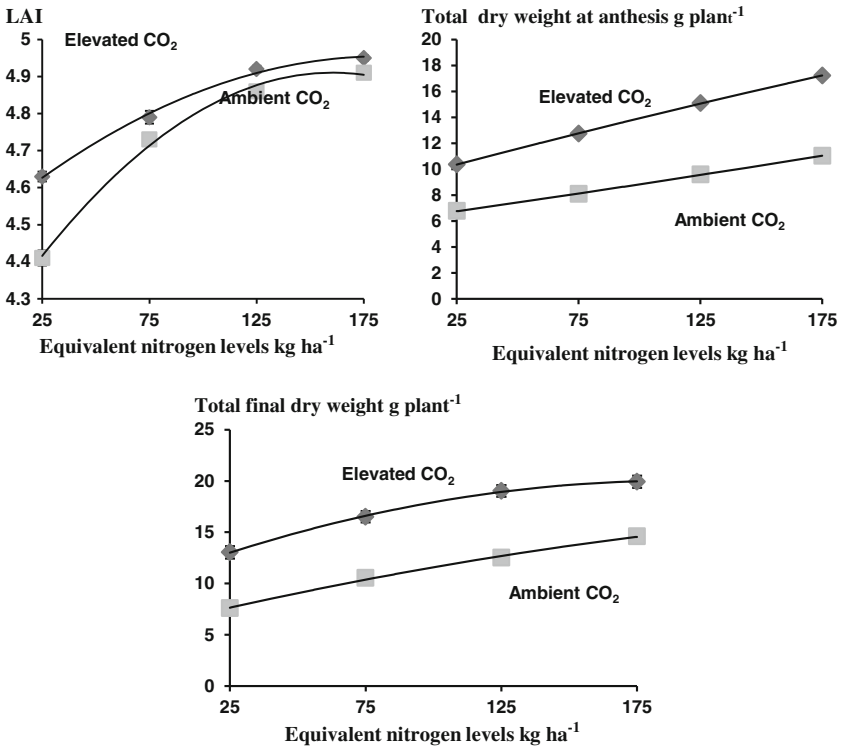

Fig. 4 Mean of LAI at anthesis (LSD CO_2=0.04, N=0.05 and CO_2 × N=0.07), total above-ground biomass at anthesis (LSD CO_2=1.42, N=2.01 and CO_2 × N=n.s) and total above-ground biomass at harvest (LSD CO_2= 0.68, N=0.96 and CO_2 × N=n.s) at elevated CO_2 and different levels of nitrogen fertiliser. *Vertical bars* are standard error of the mean (n=4) at 0.05 levels

nitrogen input is commonly reported (Li et al. 2004). Other studies have also reported that leaf area increased with nitrogen input, but at different CO_2 levels, growth was similar (Anten et al. 2004) indicating that optimum LAI does not increase at elevated CO_2 when nitrogen is low. Two factors may be responsible for this; one is that plants grown in elevated CO_2 have higher quantum yield which makes the leaves in their canopy more nitrogen limited; the other is a possible increase in dark respiration rate under elevated CO_2 which leads to a higher light compensation point and lowers LAI (Hirose et al. 1997). Others state that LAI increases at elevated CO_2 when nitrogen uptake is high, but not when nitrogen uptake is low (Kim et al. 2001).

In this experiment, increased assimilation rate and leaf area both attributed to the increase in above-ground biomass (Fig. 4). Above-ground dry weight was increased by 35 and 36 % at anthesis and harvest, respectively, under elevated CO_2, and the higher values under the higher nitrogen input were 35 and 40 % at anthesis and harvest, at 125 kg N ha^{-1} as compared with the lowest nitrogen level, respectively. The above-ground dry weight for plants under elevated CO_2 and lowest nitrogen rate was 42 % higher than the ambient CO_2 and lowest nitrogen rate-grown plants. This suggests that elevated CO_2 compensates for the negative effect of nitrogen deficiency. At the same time, nitrogen supply is preventing photosynthesis from CO_2 acclimation as reported by Kim et al. (2003). It can be concluded, from the short-term exposure to elevated (700 μmol mol^{-1}) and ambient CO_2 of alfalfa and three levels of nitrogen fertiliser, that the plants grown under elevated CO_2 and zero nitrogen significantly increased photosynthetic activity due to increased nitrogen use efficiency, but there were no significant differences across all CO_2 and nitrogen levels, and photosynthetic down regulation had occurred. However, the long-term exposure to elevated CO_2 markedly enhanced leaf area and plant biomass over the ambient CO_2 when the plant was irrigated with nitrogen and photosynthesis activity was maintained (Sanz-Sáez et al. 2010). This finding clearly supports the hypothesis that growth in limited nitrogen restricted the sink development capacity to utilize the photo assimilate and led to an accumulation of non-structural carbohydrate which increased the C/N ratio and exacerbated the acclimation of photosynthesis (Ainsworth et al. 2003; Ainsworth and Rogers 2007). At all nitrogen treatments, elevated CO_2 significantly (p<0.05) increased the branch number and corresponding capitula number (data not shown), with the highest values occurring at 125 kg N ha^{-1}. In the present study, there was a significant (p<0.05) interaction effect of CO_2 and nitrogen on LAI at anthesis and branch and capitula number at harvest. It can therefore be predicted that the response of branch and capitula number of safflower here is dependent on CO_2 concentration, and the data suggest that 125 kg N ha^{-1} is sufficient for safflower to produce greater LAI, above-ground biomass and the most branches and capitula; this is dependent on LAI and leaf and shoot dry matter accumulation. In support of the results reported here for safflower, cotton showed a significant interaction effect of CO_2 and nitrogen on branch and boll number, but no significant interaction effect on plant height and total above biomass (Reddy et al. 2004). The

explanation for this interactive effect was the increased assimilation rate (A) and growth under elevated CO_2; the demand for nitrogen input increases, and a greater sink capacity can be provided and photosynthetic rate sustained.

4 Conclusions

The results presented here indicate that CO_2 levels, elevated to approximately twice the levels of ambient, and optimal water and nutrient supply increased the assimilation rate in safflower. This increase can be linked to reduction in stomatal conductance and transpiration rate. The above-ground biomass markedly increased and was associated with a noticeable increase in leaf area index and assimilation rate at elevated CO_2. It was also shown that the positive CO_2 response was maintained at each increase in nitrogen nutrition input. Since the assimilation rate still significantly increased under the lowest nitrogen in response to elevated CO_2 compared to ambient CO_2, it can also be concluded that the negative impact of nitrogen deficiency on photosynthesis can be ameliorated by elevated CO_2. A nitrogen level of 125 kg N ha^{-1} was sufficient for optimum growth and biomass production in response to elevated CO_2. From the interactive effect of CO_2 and nitrogen on growth, it can be also concluded that the growth at increased CO_2 needed 125 kg N ha^{-1} to avoid photosynthetic down regulation. Safflower is normally grown in semi-arid soils with low nitrogen content, and the results here show that in the near future, as atmospheric CO_2 levels rise, production levels will rise with carbon fertilisation, but extra production will be achieved if nitrogen application levels are raised up to 125 to 175 kg N ha^{-1}.

Acknowledgments The Iraqi Ministry of Higher Education is gratefully acknowledged for a PhD scholarship for Shiren Mohamed.

References

Ainsworth AE, Davey AP, Bernacchi JC, Dermody CO, Heaton AE, Moore AD, Nargan BP, Naidu LS, Shimyoora H, Guangzhu X, Curtis P, Long PS (2002) A meta-analysis of elevated CO_2 on soybean (*Glycine max*) physiology, growth and yield. Global Change Bio 8:695–709. doi:10.1046/j.1365-2486.2002.00498.x

Ainsworth E, Davey P, Hymus G, Osborne C, Rogers A, Blum H, Nösberger J, Long S (2003) Is stimulation of leaf photosynthesis by elevated carbon dioxide concentration maintained in the long term? A test with *Lolium perenne* grown for 10 years at two nitrogen fertilization levels under Free Air CO_2 Enrichment (FACE). Plant Cell Environ 26:705–714. doi:10.1046/j.1365-3040.2003.01007.x

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 165:351–372. doi:10.1111/j.1469-8137.2004.01224.x

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. doi:10.1111/j.1365-3040.2007.01641.x

Allen L Jr (1999) Evapotranspiration responses of plants and crops to carbon dioxide and temperature. In: Kirkham MB (ed) Water use in crop production. Food Products Press Inc, Binghampton, pp 37–70

Anten N, Hirose T, Onoda Y, Kinugasa T, Kim H, Okada M, Kobayashi K (2004) Elevated CO_2 and nitrogen availability have interactive effects on canopy carbon gain in rice. New Phytol 161:459–471. doi:10.1046/j.1469-8137.2003.00943.x

Campbell CS, Heilman JL, McInnes KJ, Wilson LT, Medley JC, Wu G, Cobos DR (2001) Seasonal variation in radiation use efficiency of irrigated rice. Agr For Meteoro 110:45–54. doi:10.1016/S0168-1923(01)00277-5

Cruz C, Lips H, Martins-Loução MA (2003) Nitrogen use efficiency by a slow-growing species as affected by CO_2 levels, root temperature, N source and availability. J Plant Phys 160:1421–1428. doi:10.1078/0176-1617-00998

Dajue LI, Mundle HH (eds) (1996) Safflower. *Carthamus tinctorius*, International Plant Genetic Resources Institute, Rome

Del Pozo A, Pérez P, Morcuende R, Alonso A, Martínez-Carrasco R (2005) Acclimatory responses of stomatal conductance and photosynthesis to elevated CO_2 and temperature in wheat crops grown at varying levels of N supply in a Mediterranean environment. Plant Sci 169:908–916. doi:10.1016/j.plantsci.2005.06.009

Del Pozo A, Pérez P, Gutiérrez D, Alonso A, Morcuende R, Martinez-Carrasco R (2007) Gas exchange acclimation to elevated CO_2 in upper-sunlit and lower-shaded canopy leaves in relation to nitrogen acquisition and partitioning in wheat grown in field chambers. Environ Exp Bot 59:371–380. doi:10.1016/j.envexpbot.2006.04.009

Delucia EH, Sasek TW, Strain BR (1985) Photosynthetic inhibition after long-term exposure to elevated levels of atmospheric carbon dioxide. Photosynth Res 7:175–184. doi:10.1007/BF00037008

Drake BG, Gonzàlez-Meler MA, Long SP (1997) More efficient plants: a consequence of rising atmospheric CO_2? Annu Rev Plant Bio 48:609–639. doi:10.1146/annurev.arplant.48.1.609

Evans R, Belnap J (1999) Long-term consequences of disturbance on nitrogen dynamics in an arid ecosystem. Ecology 80:150–160. doi:10.1890/0012-9658(1999)080[0150:LTCODO]2.0.CO;2

Farage PK, McKee IF, Long SP (1998) Does a low nitrogen supply necessarily lead to acclimation of photosynthesis to elevated CO_2? Plant Phys 118:573–580. doi:10.1104/pp.118.2.573

Gastal F, Lemaire G (2002) N uptake and distribution in crops: an agronomical and ecophysiological perspective. J Exp Bot 53:789–799. doi:10.1093/jexbot/53.370.789

Hershey DR (1995) Plant biology science project. Wiley, New York

Hirose T, Ackerly D, Traw M, Ramseier D, Bazzaz F (1997) CO_2 elevation, canopy photosynthesis, and optimal leaf area index. Ecology 78:2339–2350. doi:10.1890/0012-9658(1997)078[2339:CECPAL]2.0.CO;2

Hsiao TC, Jackson RB, Yiqi L, Harold AM (1999) Interactive effects of water stress and elevated CO2 on growth, photosynthesis, and water use efficiency. In: Luo Y, Mooney H (eds) Carbon dioxide and environmental stress. Academic, San Diego, pp 3–31

Jablonski LM, Wang X, Curtis PS (2002) Plant reproduction under elevated CO_2 conditions: a meta–analysis of reports on 79 crop and wild species. New Phytol 156:9–26. doi:10.1046/j.1469-8137.2002.00494.x

Jifon JL, Wolfe DW (2002) Photosynthetic acclimation to elevated CO_2 in *Phaseolus vulgaris* L. is altered by growth response to nitrogen supply. Global Change Bio 8:1018–1027. doi:10.1046/j.1365-2486.2002.00531

Johnson DW, Ball T, Walker RF (1995) Effects of elevated CO_2 and nitrogen on nutrient uptake in ponderosa pine seedlings. Plant Soil 168:535–545. doi:10.1007/BF00029366

Johnston AM, Tanaka DL, Miller PR, Brandt SA, Nielsen DC, Lafond GP, Riveland NR (2002) Oilseed crops for semiarid cropping systems in the northern Great Plains. Agron J 94:231–240. doi:10.2134/agronj2002.2310

Kanemoto K, Yamashita Y, Ozawa T, Imanishi N, Nguyen NT, Suwa R, Mohapatra PK, Kanai S, Moghaieb RE, Ito J (2009) Photosynthetic acclimation to elevated CO_2 is dependent on N partitioning and transpiration in soybean. Plant Sci 177:398–403

Kim YH, Liffering M, Miura S, Kobayashi K, Okada M (2001) Growth and nitrogen uptake of CO_2 enrichment rice under field condition. New Phytol 150:223–229. doi:10.1046/j.1469-8137.2001.00111.x

Kim YH, Lieffering M, Kobayashi K, Okada M, Mitchell WM, Gumpertz M (2003) Effect of free air CO_2 enrichment and nitrogen supply on the yield of temperate paddy rice crops. Field Crop Res 83:261–270. doi:10.1016/S0378-4290(03)00076-5

Larigauderie A, Hilbert DW, Oechel WC (1988) Effect of CO_2 enrichment and nitrogen availability on resource acquisition and resource allocation in a grass, *Bromus mollis*. Oecologia 77:544–549. doi:10.1007/BF00377272

Li F, Kang S, Zhang J (2004) Interactive effects of elevated CO_2, nitrogen and drought on leaf area, stomatal conductance, and evapotranspiration of wheat. Agr Water Manag 67:221–233. doi:10.1016/j.agwat.2004.01.005

Manderscheid R, Burkart S, Bramm A, Weigel HJ (2003) Effect of CO_2 enrichment on growth and daily radiation use efficiency of wheat in relation to temperature and growth stage. Eur J Agron 19:411–425. doi:10.1016/S1161-0301(02)00133-8

Mott KA (1988) Do stomata respond to CO_2 concentrations other than intercellular? Plant Phys 86:200–203. doi:10.1104/pp.86.1.200

Nakano H, Makino A, Mae T (1997) The effect of elevated partial pressures of CO_2 on the relationship between photosynthetic capacity and N content in rice leaves. Plant Phys 115:191–198. doi:10.1104/pp.115.1.191

Radoglou K, Aphalo P, Jarvis P (1992) Response of photosynthesis, stomatal conductance and water use efficiency to elevated CO_2 and nutrient supply in acclimated seedlings of *Phaseolus vulgaris* L. Ann Bot 70:257–264, 0305-7364/92/090245+ 12 S08 00/0

Reddy AR, Rasineni GK, Raghavendra AS (2010) The impact of global elevated CO_2 concentration on photosynthesis and plant productivity. Current Sci 99:46–54

Reddy KR, Koti S, Davidonis GH, Reddy VR (2004) Interactive effects of carbon dioxide and nitrogen nutrition on cotton growth, development, yield, and fiber quality. Agron J 96:1148–1157. doi:10.2134/agronj2004.1148

Rudmann SG, Milham PJ, Conroy JP (2001) Influence of high CO_2 partial pressure on nitrogen use efficiency of the C_4 grasses *Panicum coloratum* and *Cenchrus ciliaris*. Ann Bot 88:571–577. doi:10.1006/anbo.2001.1503

Sanz-Sáez Á, Erice G, Aranjuelo I, Nogués S, Irigoyen JJ, Sánchez-Díaz M (2010) Photosynthetic down-regulation under elevated CO_2 exposure can be prevented by nitrogen supply in nodulated alfalfa. J Plant Phys 167:1558–1565. doi:10.1016/j.jplph.2010.06.015

Shaw MR, Huxman TE, Lund CP (2005) Modern and future semi-arid and arid ecosystems. In: Ehleringer JR et al (eds) A history of atmospheric CO_2 and its effects on plants, animals, and ecosystems. Springer Science and Business Media, Inc, New York

Sims DA, Cheng W, Luo Y, Seemann JR (1999) Photosynthetic acclimation to elevated CO_2 in a sunflower canopy. J Exp Bot 50:645–653. doi:10.1093/jxb/50.334.645

Stitt M, Krapp A (1999) The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. Plant Cell Environ 22:583–621. doi:10.1046/j.1365-3040.1999.00386.x

Taiz L, Fizeiger E (eds) (2002) Plant physiology (3rd edition). Sinaur Associates Inc., Sunderland, MA, USA

Yuan JS, Himanen SJ, Holopainen JK, Chen F, Stewart CN Jr (2009) Smelling global climate change: mitigation of function for plant volatile organic compounds. Trends Ecol Evol 24:323–331. doi:10.1016/j.tree.2009.01.012

Zerihun A, Gutschick VP, Bassirirad H (2000) Compensatory roles of nitrogen uptake and photosynthetic N-use efficiency in determining plant growth response to elevated CO_2: evaluation using a functional balance model. Annals Bot 86:723–730. doi:10.1006/anbo.2000.1234