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Diurnal flux in CO_2 associated with *Pongamia pinnata* (L.) Pierre grown under controlled microclimatic conditions

Aparna Sreekumar*, Sashna N.C. and Harilal C.C.

Environmental Sciences Division, Department of Botany, University of Calicut, Tenjipalam, 673 635, Malappuram, Kerala, India

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ABSTRACT

The present study is an attempt to assess the growth and biochemical responses of *Pongamia pinnata* grown under elevated levels of CO_2 supply. Saplings (18 months) maintained in the nursery were brought to experimentation in two growth chambers having controlled supply of air and air- CO_2 mixture, for a period of 15 days. The control and treated chambers were retained at an average CO_2 concentration of 505.28± 17.39 ppm and 1053.85± 24.99 ppm respectively. Day and night fluxes of CO_2 within the chambers were analysed daily, along with a periodic assessment of the growth and biochemical responses associated with the plants. A standardization study, excluding plants, was also attempted to assess the resultant flux of CO_2 associated with the growth chambers. The day flux of CO_2 in the control and treated chambers was different from that of the night flux in both experimentation and standardization studies. Higher CO_2 assimilation by the plants in the CO_2 enriched system during the day time has also resulted in increased plant height, stem thickness, leaf area and also in the assimilation of carbohydrates, sodium and potassium. Increased Carotenoids and phenol are implications of the stress to which the plants are subjected to under elevated levels of CO_2 supply. The present study confirms that, despite certain signs of stress, *Pongamia pinnata* can be an ideal candidate for carbon offset planting.

Key words *: CO_2 controlled chambers, Elevated CO_2, Pongamia pinnata, CO_2 flux, Growth and biochemical responses.*

Introduction

Global warming and climate change, attributed by greenhouse gases (GHGs), are the most serious environmental concerns of the present millennium. Atmospheric CO_2, which accounts for 70 to 80 % of the total emissions (IPCC, 2007) is responsible for the disturbances in atmospheric chemistry. In the 1960s, the global annual increase in atmospheric CO_2 was 0.6 ppm, which in the last decade was close to 2.3 ppm (Dlugokencky *et al.*, 2018). TheEarth's average surface temperature has increased by 0.8°C over the past 100 years and about 0.6°C over the past three decades (IPCC, 2001). The projected concentration of CO_2 in the atmosphere can vary from 540 ppm to 970 ppm in 2100, compared to 280ppm in the pre-industrial era and 416.82 ppm in 2020 (Lindsey, 2020).

Afforestation and reforestation are considered as one among the effective ways to curb atmospheric CO_2 levels. Stimulation of productivity under increasing CO_2 levels in forest ecosystems (CO_2 fertilization effect) has been demonstrated in various field experiments (Norby *et al*., 2005; Karnosy *et al*., 2006). The potential role of forests in mitigating climate change by confiscating CO_2 from the atmosphere has

been demonstrated in integrated assessment models (IPCC, 2018). In all these attempts, CO_2 assimilation potentials of tree species are significant.

Responses of tree species to elevated CO_2 levels have been attempted by many. Trees are characterized by their potential for acclimatization, adaptability and longevity (Caulemans and Mousseau, 1994). Study by Rey and Jarvis (1997) reported that elevated CO_2 has resulted in 58% increase in biomass in *Betula pendula*. FACE experimental studies on *Acer saccharum*, *Betula papyrifera*, and *Populus tremuloid s*revealed that the biomass production of these trees increased under elevated levels of CO_2 (Kallarackal and Roby, 2012*).* Pine trees grown under elevated CO_2 accumulated 55% more dry mass than trees under ambient CO_2 (Jach and Ceulemans 2000). According to Bohre *et al*. (2014), the net biomass production and carbon sequestration of *Pongamia pinnata* increased with the age of plantations. The changes in biomass production were associated with increased photosynthetic rate, which was reported in *Prunus avium* (Centritto *et al*., 1999), *Fragaria ananassa* (Bunce, 2001), Poplars (Bernacchi *et al*., 2003), *Fagus sylvatica* (Lotfiomran *et al*., 2016), and Spinach (Jain *et al*., 2007). Study by Superales (2016) revealed that saplings of *Swietenia mahagoni* captured more CO_2 in the wood, compared to leaves and bark. Lamani *et al*. (2016) reported a positive response in *Santalum album* with increase in seedling height due to increased use of CO_2 for carbon assimilation and reduced photorespiration. Carbon sequestration potential of *Shorea robusta*, *Eucalyptus tereticornis*, *Populus deltoides*, *Tectona grandis* were studied by Kaul *et al*. (2010) using dynamic growth model CO2FIX. In a study conducted by Maji *et al.* (2017), 10 tree species were compared and found that *Ficus religiosa* was with highest sequestration efficiency and *Melia dubai* with lowest. CO_2 enrichment studies were conducted in tomato (Ho, 1977), strawberry (Campbell and Young, 1986), mungbean (Sharma and Sengupta, 1990) and reported increase in carbon exchange rate in all the experiments. Meta-analysis revealed that the fast-growing species have a greater growth response to elevated CO_2, compared to slow growing ones (Zhang *et al*., 2008).

Carbon offset planting envisions the selection of ideal species with higher sequestration efficiencies and their strategic planting in ideal locations. Only a limited number of tree species have been subjected to efficiency studies and a sizable number are remaining. Assessment of the effects of elevated CO_2 on plant growth is difficult under field conditions and efficient CO_2 fumigation requires shielding the plants from environment using constructed structures like Open Top Chambers (OTC), Controlled Growth Chambers, Free Air CO_2 Enrichment (FACE) systems and Screen Aided CO_2 Control (SACC) systems (Kimball, 2010). With this background, the present study was attempted to assess the responses of *P. pinnata* to elevated levels of CO_2, under controlled growth conditions.

In the present study, changes in the growth and biochemical responses in *Pongamia pinnata* saplings under elevated CO_2 supply in a controlled Chamber was assessed. *Pongamia pinnata* belongs to the fabaceae family, and is used widely in oil industries. *P. pinnnata* is a deciduous tree and one of the widely employed species in afforestation programmes. It is a fast-growing leguminous tree species and hence can be employed in carbon mitigation programmes (Scott *et al*., 2008). Despite the considerable importance of this species in various sectors, especially bio-energy and oil content, the information on carbon content and carbon sequestration efficiency is scanty (Bohre *et al*., 2014). This medium-sized tree is indigenous to the Indian subcontinent and southeast Asia, and has been successfully introduced to humid tropical regions of the world as well as parts of Australia, New Zealand, China and the USA. Historically, this plant has been used in India and neighbouring regions as a source of traditional medicines, animal fodder, green manure, timber, fish poison and fuel (Reddy *et al*., 2015).

Materials and Methods

Plantlets of *P. pinnata* were raised from certified seeds procured from the Seed Centre of Kerala Forest Research Institute, Peechi, Thrissur, Kerala, India. They were then raised in grow bags of size 35x20x20 cm having potting mixture (soil, sand and organic manure) in the ratio 2:1:1. They were then maintained in a polyhouse for 18 months, with adequate supply of water and nutrients and periodic monitoring of growth parameters.

Carbon dioxide- controlled chamber

The experiment was conducted in two controlled growth chambers, each with a size of 1.8 m x 1.8 m x 2.4 m of length, breadth and height, respectively. The chambers were made with PVC pipes, covered with polyethylene sheets. The control chamber was

equipped with the facility for the supply of ambient air through an air compressor, whereas the treatment chamber was equipped with a CO_2 cylinder and an air compressor for the supply of CO_2 – air mixture in specific doses. Both the chambers were fitted with the facility for the analysis of CO_2 (ppm), temperature and humidity, together with an exhaust facility for controlling the micro climatic conditions within the chamber, if required. The chambers were also fitted with a semi-automated facility for the irrigation of plantlets during experimentation. The experimental setup is given in Figure 1.

Fig. 1. *Pongamia pinnata* inside control and CO_2 treated chamber

CO_2 supply and monitoring of micro climatic conditions

Two sets of plants (four each), of 18 months old were selected, of which one set was taken to the control chamber having ambient air supply and the other to the chamber having elevated supply of CO_2. The chambers were then sealed to prevent the exchange of air into or out of the growth system. To the treatment chamber, CO_2 mixed with air has been supplied for about 15 minutes, every day in the morning, maintaining a mean CO_2 level of approx. 1000 ppm. Similarly, the control chamber was supplied with ambient air daily, for 15 minutes. The magnitude of temperature, humidity and carbon dioxide concentration associated with both the chambers were monitored twice a day at 9 am and 6 pm. Estimation of CO_2 associated with both the chambers were carried out through an automated CO_2 analyzer (NDIR type Infrared Gas Analyzer, Fuji Electric, Japan). Temperature and humidity in both the chambers were monitored using Billion bag digital wireless electronic Hygro-thermometer. The experimentation was carried out for 15 days.

Measurements of growth parameters

Growth attributes of plants were assessed at two stages of experimentation, one on the initial day (0DoT) and the other on the final day of treatment (15DoT). Morphological parameters assessed include plant height, stem thickness, number, length, breadth and area of leaves. Plant height was assessed from the level of soil to the region of active meristem, using a measuring tape. Stem thickness was measured at the collar level using a screw gauge. Total number of leaves were counted and the length of leaves together with their breadth at the widest portion was measured. Leaf area was calculated from the measurements of length, breadth and number of leaves.

Estimation of biochemical parameters

Biochemical parameters associated with the plants from the control and CO_2 treatment sets were recorded at 4 stages (0DoT, 5DoT, 10DoT and 15DoT). Parameters analysed include leaf pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoids), carbohydrates, phenol and protein content. Pigments were estimated according to Shoaf and Lium (1976). Carbohydratewas estimated following Dubois *et al*. (1956). Phenol and protein contents were estimated following Malick& Singh (1980) and Lowry *et al*. (1951), respectively. Biochemical parameters were assessed using 1^{st}, 3^{rd}, 5^{th}, 7^{th}, 9^{th} and 11^{th} leaves of different ages.

Estimation of minerals

Estimation of the mineral contents of plants was undertaken at four stages of growth (0DoT, 5DoT, 10DoT and 15DoT). For the estimation of minerals, plants were dried and subjected to perchloric acid - nitric acid - sulphuric acid digestion. Titration method was followed to determine the extent of calcium and magnesium. Elements such as sodium and potassium were estimated using a flame photometer (Systronics, 128).

Standardization studies of growth chambers

A standardisation study was undertaken to assess the retention percentage and daily flux of gases associated with both the empty chambers. For this, the entire experimentation was undertaken in the respective chambers in the absence of plants, with si-

multaneous supply of air / air - CO_2 mixture and subsequent analysis of temperature, humidity, and CO_2 at specific time intervals of the day (9am and 6pm) for 15 days. The data generated were used to validate the retention percentage and daily flux of CO_2 associated with the chambers during experimentation with *P. pinnata*.

Estimation of soil characteristics

The changes in pH, organic carbon content and moisture associated with the soil samples were also assessed at two stages, on the initial day (0DoT) and the final day (15DoT). Soil pH was estimated using a pH meter. Soil organic carbon was estimated using the Walkley and black method (Krishnan *et al*., 2009). Soil moisture was measured gravimetrically.

Statistical analysis

The statistical significance of morphological and biochemical parameters was evaluated by two-way Analysis of Variance (ANOVA) at ($p< 0.05$) and values are expressed in mean ± SD using SPSS version 27.

Results and Discussion

CO_2 flux in the growth chamber

Day flux of CO_2 is estimated as the difference in the extent of CO_2 supplied in the morning with that of the CO_2 retained in the evening. Similarly, night flux is assessed as the extent of CO_2 in the evening with that of its extent in the next morning. The day flux of CO_2 associated with the control and the CO_2 treated chambers of standardisation studies and studies with *P. pinnata* are represented in Table 1. In both standardization studies and experimentation with *P. pinnata*, a progressive reduction in the extent of CO_2 was noticed in the evening, compared to morning. In the case of standardisation study, the percentage decline in day flux in the control chamber was 4.618% and that of the treated chamber was 7.56 %. Also, the day flux in the control chamber of *P. pinnata* was 0.89% and that of its CO_2 treated chamber was 44.36%. Thus a net assimilation (decline) of 36.8% of CO_2 was noticed in the chamber containing *P. pinnata* than that of the standardization study and 43.47% with that of its control.

Day flux are indications of the sequestration efficiencies of plants (Lahive *et al*., 2018). In the present study, plants within the CO_2 treated chamber consumed a higher share of CO_2 compared to control and that of the standardization studies. The higher accumulation and resultant flux in CO_2 within the treated chamber can be attributed to the efficiency of *P. pinnata* plants growing within it.

Table 1. Day flux of CO_2 in control and treated chambers

	Day flux											
	Control						Treated					
	Standardization studies			*Pongamia pinnata*			Standardization studies			*Pongamia pinnata*		
Day	Morning	Evening	Day flux	Morning	Evening	Day flux	Morning	Evening	Day flux	Morning	Evening	Day flux
1	602	617	15	488	484	-4	1025	955	-70	1057	590	-467
2	660	622	-38	516	489	-27	996	938	-58	1044	605	-439
3	685	622	-63	490	498	8	999	942	-57	1075	593	-482
4	650	622	-28	494	493	-1	1020	947	-73	1080	574	-506
5	653	627	-26	518	499	-19	1026	941	-85	1015	581	-434
6	647	625	-22	506	502	-4	1005	929	-76	1050	583	-467
7	658	641	-17	493	522	29	1030	949	-81	1041	604	-437
8	670	637	-33	481	503	22	1001	945	-56	1048	543	-505
9	671	639	-32	503	507	4	1025	929	-96	1073	566	-507
10	687	646	-41	498	499	1	1045	936	-109	1007	638	-369
11	680	644	-36	504	496	-8	1009	941	-68	1053	580	-473
12	673	654	-19	519	510	-9	1030	936	-94	1090	591	-499
13	680	638	-42	515	525	10	1025	930	-95	1085	611	-474
14	694	646	-48	549	484	-65	1022	961	-61	1036	550	-486
Avg	665	634.28	-30.71	505.28	500.78	-4.538	1018.42	941.35	-77.07	1053.85	586.35	-467.53
Sd	23.26	11.56	17.94	17.39	12.3	22.73	14.22	9.52	16.83	24.99	24.59	37.86
% change			-4.62%			-0.89%			-7.56%			-44.36%

Night flux is estimated as the difference in the amount of CO_2 in the evening with that of the next morning. The night flux of CO_2 attributed by *P. pinnata* in the control and treated chambers with that of the standardisation studies are represented in Table 2. The results showed an increase in night flux of CO_2 in both control (4.39%) and treated chambers (17.88%) having *P. pinnata*, and this might be due to the respiration of the plants during night. With an increase in CO_2, night flux is increased in some cases, but decreased in others (Amthor, 1991). According to Wang *et al*. (2001) and Edward *et al*. (2002), plants grown in elevated CO_2 have higher respiration rates than control, which substantiates the findings of the present study. However decreased respiration rates under augmented $CO_2$is supposed to be due to reduction in leaf nitrogen associated with photosynthetic down regulation and lower metabolic demands (Loreto *et al*., 2000; Crows *et al*., 2012; Ayub *et al*., 2014). Regarding standardisation studies the night flux values were 5.7% and 2.39% in control and treatment chambers, respectively.

Microclimatic conditions in the growth chamber

Micro climatic conditions like temperature and humidity experienced within the control and carbon dioxide treated chambers of standardization studies and studies with *P. pinnata* are given in Tables 3 and 4 respectively. The results showed variations in microclimatic conditions over a range and can be attributed to the varying concentrations of CO_2 inside the chambers. In the standardisation studies, morning temperatures in the control and treated chambers (°C) were 40.3 ± 1.9 and 40.78±1.8 and those of evening temperatures were 35.3±1.78 and 35.12± 1.55, respectively. However, the morning and evening temperatures in the control chamber having *P. pinnata* were 38.74±3.16 and 35.68±1.45 and that of the treated chamber was 37.79±2.34 and 35.62±1.19, respectively. Carbon dioxide, as a greenhouse gas, absorbs and emits radiation in the thermal infrared range and can greatly influence temperatures which are in line with the present study, where stimulation in CO_2 showed a slight deduction in temperature. Photosynthetic acclimation of *Medicago sativa* to elevated CO_2 is reported to be temperature dependent (Ziska and Bunce, 1994). According to Cernusak *et al*. (2013), Amthor (1991) higher temperatures increased CO_2 assimilation in plants.

Percentage humidity (table 4) also showed variations, which are attributed to varying concentrations of CO_2. In the standardisation study, morning and

Table 2. Night flux of CO_2 in control and treated chambers

	Night flux											
	Control						Treated					
	Standardization studies			*Pongamia pinnata*			Standardization studies			*Pongamia pinnata*		
Day	Evening	Morning	Night flux	Evening	Morning	Night flux	Evening	Morning	Night flux	Evening	Morning	Night flux
1	617	656	39	484	530	46	955	973	18	590	850	260
2	622	680	58	489	496	7	938	970	32	605	646	41
3	622	649	27	498	506	8	942	978	36	593	647	54
4	622	660	38	493	490	-3	947	949	2	574	620	46
5	627	649	22	499	521	22	941	960	19	581	646	65
6	625	671	46	502	498	-4	929	940	11	583	573	-10
7	641	670	29	522	495	-27	949	962	13	604	583	-21
8	637	676	39	503	516	13	945	973	28	543	673	130
9	639	687	48	507	538	31	929	959	30	566	670	104
10	646	687	41	499	525	26	936	955	19	638	705	67
11	644	672	28	496	536	40	941	963	22	580	737	157
12	654	674	20	510	534	24	936	953	17	591	726	135
13	638	690	52	525	570	45	930	976	46	611	754	143
14	646	671	25	484	565	81	961	984	23	550	847	297
Avg	634.29	670.85	36.57	500.78	522.85	22.07	941.35	963.92	22.57	586.35	691.21	104.85
Sd	11.55	13.31	11.54	12.3	24.98	26.62	9.52	12.36	11.2	24.59	85.26	91.52
% Change			5.70%			4.39%			2.39%			17.88%

evening humidity in the control chamber was 51.08±3.65 and 57.57± 6.02, whereas it was 54± 3.8 and 68.64± 6.01 respectively in the treated chamber. The humidity in the control chamber having *P. pinnata* was 90.5±10.9 (morning) and 93.2±5.14 (evening), whereas in the treated chamber, average morning humidity was 95.64±10.47 and that of evening was 98.43±1.60. Present study showed slight increase in humidity under stimulated CO_2 which is consistent with the results of Elvira and Vasenev (2020). It is postulated that the metabolic status and resultant evapo-transpiration by the aerial and belowground biomass of plants, evaporation from soil, activity of soil microorganisms etc. can attribute humidity to external environmental conditions.

Growth responses of *Pongamia pinnata* under CO_2treatment

Data regarding the growth attributes of *P. pinnata,* subjected to experimental conditions are presented in Table 5. Mean values of growth parameters such as plant height, stem thickness, length, breadth, number and leaf area increased with elevated CO_2. After 15 days of experimentation, an increase of 6.05% in height was noticed in plants under treatment with CO_2. Ainsworth *et al*. (2002) reported a 14% increase in plant height after prolonged exposure to a higher concentration of CO_2 in *Glycine max*. In a study conducted in cassava plants, Ruiz-Vera *et al*. (2021) noticed an increase in plant height under elevated CO_2. On the contrary, there were no appreciable differences in stem thickness under enhanced CO_2. Pal *et al*. (2004) and Lahive *et al*. (2018) observed an increase in stem thickness in *Trifolium alexandrium* and *Theobroma cacao* exposed to elevated conditions of CO_2. In the present study an increase of 2.8 % in leaf length, 0.17% in leaf breadth, 49.58% in leaf number, 44.4% in leaf area was observed under elevated CO_2. Pal *et al*. (2004) observed an increase in leaf length and breadth in *Trifolium alexandrium* exposed to elevated conditions of CO_2. The present study showed a substantial increase in leaf number, which is in accordance with the study of Kull *et al*. (2003) and Lahive *et al*. (2018), where an increase in number of leaves were noticed in *Betula pendula* and *Theobroma cacao,* respectively. Ainsworth *et al*. (2002) reported an 80% increase in the number of leaves after prolonged exposure to a higher concentration of CO_2in *Glycine max*. 43.75 % increase in leaf number under enhanced CO_2 was reported under elevated CO_2. The increased leaf

Table 3. Range of temperatures (Minimum, Maximum and Mean) noticed within the chambers under varying experimental conditions

Experimental condition	Standardisation studies		*Pongamia pinnata*	
	Morning temperature ^{0}C (Min., Max. and Mean)	Evening temperature ^{0}C (Min., Max. and Mean)	Morning temperature ^{0}C (Min., Max. and Mean)	Evening temperature ^{0}C (Min., Max. and Mean)
Control chambers	37.5 – 44.0	32.3 – 37.3	30.7-42.4	32.9-37.7
	40.3±1.9	35.30±1.78	38.74±3.16	35.68±1.45
Treated chambers	39.3-44.4	33.1-37.1	33.5-41.7	33.1-36.9
	40.78±1.8	35.12±1.55	37.79±2.34	35.62±1.19

Table 4. Range of humidity (Minimum, Maximum and Mean) noticed within the chambers under varying experimental conditions

Experimental condition	Standardisation studies		*Pongamia pinnata*	
	Morning Humidity % (Min., Max. and Mean)	Evening Humidity % (Min., Max. and Mean)	Morning Humidity % (Min., Max. and Mean)	Evening Humidity % (Min., Max. and Mean)
Control chambers	44-56	51-70	60-99	84-99
	51.08± 3.65	57.57 ± 6.02	90.50± 10.98	93.21± 5.14
Treated chambers	47-59	59-79	60-99	93-99
	54±3.8	68.64±6.01	95.64±10.47	98.43±1.60

area in the present study might be due to the stimulation of leaf formation, which is associated with leaf cellular expansion. The stimulation in leaf expansion by elevated CO_2 is mainly due to changes in cell wall loosening or extensibility and not related to changes in cellular water content, in growing leaves (Taylor *et al*., 1994). Leaf area increase under elevated CO_2 may be due to increased nitrogen input, increased cell expansion, increased leaf thickness, increase in the number of palisade cell layers (Campbell *et al*., 2001; Sanz-Saez *et al*., 2010; Mohamed *et al*., 2013; Al-Rawahy *et al*., 2013; Lahive *et al*., 2018; Ruiz-Vera *et al*., 2021).

Variations in pigments and metabolites in *Pongamia pinnata* under CO_2 treatment

Plants respond to changes in its external environment by adjusting biochemically (Janani *et al*., 2016). The changes in pigments and other biochemical components in *Pongamia pinnata* in response to elevated levels of CO_2 has been assessed at four stages of growth. Mean values of pigments such as chlorophyll a, chlorophyll b, total chlorophyll, carotenoids and other biochemical components like carbohydrates, protein and phenol contents in plants from the control and elevated CO_2 supply are estimated on the initial, 5th, 10th and 15th day of experimentation and the results are presented in table 6. It is found that except protein, all other pigments and metabolites increased under enhanced CO_2. In the present study, under elevated CO_2 chlorophyll a, chlorophyll b, total chlorophyll and carotenoids increased by 20.23%, 61.68%, 43.32%, 15.15% respectively. The study conducted by Al-Rawahy *et al*. (2013) in *Medicago sativa* reported increased chlorophyll content under elevated levels of CO_2 due to improved substrate availability for assimilation and reduced water loss due to lower stomatal conductance. Carotenoids and chlorophylls play an important role in mediating oxidative stress in plant tissues and plants must be able to actively regulate carotenoid biosynthesis under elevated levels of CO_2 (Ormrod *et al*., 1999). The increased carotenoid content in the present study might be a stress response.

Carbohydrate content in the present study increased by 38.18%, when plants are exposed to elevated CO_2 for 15 days. Aguera *et al.* (2006), Hendrix *et al*. (1994), Urbonaviciute *et al*. (2006), Keutgen and Chen (2001), Faria *et al*. (1996), Ainsworth *et al*. (2002) reported higher carbohydrate content under elevated CO_2levels in *Solanum lycopersicum, Gossipium hirsutum*, *Raphanus sativus*, *Elaeis guineensis, Citrus limon*, *Quercus suber* and *Glycine max*. A study conducted in *Melia dubia* by Janani *et al*. (2016) showed a reduction in the amount of protein, since the plants lost the ability to take up soil nitrate and convert it to protein at enriched levels of CO_2. The reduction was due to significant leaf N- re allocation to supplemental sinks (Norby *et al*., 1996). Reduced protein under CO_2 enrichment was also reported by Dong *et al*. (2018) and Taub *et al.* (2008) which is in line with the results of the present study, where the protein decreased by 45.11% under enriched CO_2.

An increase of 54.5% in phenol content was observed under treated conditions, compared to the initial. Phenol content in all the plants increased under elevated CO_2. Increased phenol content under elevated CO_2 was reported by Ghasemzadeh *et al*. (2010) and Tognetti *et al*. (1999) in *Zingiber officinale* and *Quercus robur*, respectively. According to CNB hypothesis, if plants increase photosynthesis and carbon gain under enriched CO_2, the excess carbon will be assigned to carbon-based defences, which is evident from the increased phenol content under elevated CO_2 (Bryant *et al.,* 1983).

Table 5. Variations in growth parameters of *Pongamia pinnata* under control and CO_2 treated conditions

Parameters	Control		%	CO_2 treated		%	P
	DoT1	DoT15	Change	DoT1	DoT15	Change	value
Plant height (cm)	174.37±18.98	179.25± 14.9	+2.7	181.75±12.58	192.75±9.91	+6.05	< 0.05
Stem thickness (cm)	1.59±0.054	1.61±0.05	+1.25	1.58±0.07	1.58±0.07	nil	NS
Leaf length (cm)	12.45±0.56	12.67±1.02	+1.76	11.06±1.28	11.37±1.30	+2.8	< 0.05
Leaf breadth (cm)	6.29±3.18	6.29±0.3	nil	5.78±0.54	5.79±0.55	+0.17	NS
Leaf number	105.75±32.98	142.75±33.12	+34.98	149.25±81.29	223.25±35.66	+49.58	< 0.05
Leaf area (m^2)	0.89±0.65	0.96±0.35	+7.86	0.81±0.55	1.17±0.42	+44.4	< 0.05

(DoT - Day of treatment)
(NS- Not significant)

Table 6. Biochemical responses of *Pongamia pinnata* to treatment with elevated levels of CO_2

Experimental condition	Day of Treatment (DoT)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll	Carotenoids (mg/g)	Carbohydrate (mg/g)	Protein (mg/g)	Phenol (mg/g)
Control	DoT 0	3.01±0.88	4.22±2.09	6.77±2.76	0.61±0.12	69.54±7.56	312±29.16	190.42±133.19
	DoT 5	2.08±1.19	2.38±1.83	4.17±2.78	0.52±0.04	40.32±6.54	365.36±74.25	40.6±5.76
	DoT 10	3.4±1.16	7.41±2.86	10.07±3.72	0.62±0.17	41.22±3.68	327.09±49.42	229.3±64.94
	DoT 15	3.47±0.85	6.14±2.1	8.97±2.69	0.64±0.08	52.7±16.73	84.62±30.67	188.57±13.4
% Change		+15.28	+45.49	+32.49	+4.91	-24.21	-72.87	-0.97
Treated	DoT 0	3.46±1.39	4.62±2.66	7.57±3.77	0.66±0.16	50.59±4.91	301.22±68.54	114.91±64.47
	DoT 5	3.89±1.26	6.11±2.54	9.35±3.52	0.75±0.17	53.15±17.12	321.01±75.01	32.22±16.03
	DoT 10	3.44±0.96	7.24±2.12	9.95±2.86	0.67±0.1	77.84±30.68	259.48±133.44	194.64±102.78
	DoT 15	4.16±0.88	7.47±1.59	10.85±2.29	0.76±0.14	69.91±18.36	165.32±164.79	177.54±10.28
% Change		+20.23	+61.68	+43.32	+15.15	+38.18	-45.11	+54.5
P value		NS	NS	NS	< 0.05	< 0.05	NS	NS

(NS- Not significant)

Effect of minerals in *Pongamia pinnata* under CO_2 treatment

Table 7 depicts the variations in minerals under elevated CO_2. Sodium increased under elevated CO_2 by 12.5 %, which is in accordance with the study conducted in bamboo by Guo *et al*. (2021). Under enriched CO_2 potassium increased by 15.62%, which was consistent with the reports of Guo *et al*. (2021). Calcium and magnesium increased by 30% and 108.5% respectively under enriched CO_2, which is in accordance with the study of Guo *et al*. (2021) where calcium and magnesium increased in bamboo species under excess CO_2.

Potassium and magnesium are essential plant nutrients that critically contribute to photosynthesis and long-distance transport of photo assimilates. Deficiency of either potassium or magnesium decreases the CO_2 assimilation. From the study of Reddy and Zhao (2005), it was found that cotton grown under elevated CO_2 was more susceptible to potassium deficiency and it affected the photosynthesis of plants.

Soil characteristics

Variations in soil characteristics under enhanced CO_2 is represented in table 8. Soil pH showed variations under enriched CO_2 (from 5.32 to 6.6). Similarly, soil carbon increased by 47.12% under elevated CO_2. Jastrow *et al*. (2005) reported that elevated CO_2 increases soil carbon. Increased soil carbon storage is a result of the delivery of carbon to soil which is due to enhanced plant growth under elevated CO_2 which might be due to the increased photosynthesis (Rogers *et al*., 1999).

An increase of 4.88% in soil moisture was observed in CO_2 treated chamber after 15 days, which is in accordance with the studies conducted in grass ecosystems (Nelson *et al*., 2004; Dermody *et al*., 2007), where elevated CO_2 enhanced soil moisture, which is a major factor attributing carbon assimilation.

Statistical analysis

The results of statistical analysis depicts that there is significant difference between the treatments in morphological parameters such as plant height, leaf length, leaf number, leaf area and biochemical parameters such as carotenoids, carbohydrates and calcium, which was represented as $p < 0.05$.

Table 7. Variations in minerals under elevated CO_2

Experimental condition	Day of Treatment (DoT)	Calcium (%)	Magnesium (%)	Sodium (%)	Potassium (%)
Control	DoT 0	1.68±0.11	0.24±0.20	0.39±0.124	0.56±0.44
	DoT 5	1.6±0.32	0.26±0.28	0.32±0.23	1.03±0.90
	DoT 10	1.16±0.46	0.34±0.25	0.52±0.09	0.88±0.63
	DoT 15	1.12±0.22	0.39±0.15	0.31± 0.30	0.82±0.89
% Change		-33.3	+62.5	-20.5	+46.4
Treated	DoT 0	1.2±0.34	0.14±0.06	0.16±0.01	0.32±0.14
	DoT 5	1.44±0.47	0.39±0.17	0.48±0.08	0.56±0.85
	DoT 10	0.85±0.24	0.292	0.37±0.05	1.41±0.23
	DoT 15	0.84±0.35	0.292±0.21	0.18±0.196	0.377±0.65
% Change		+30	+108.5	+12.5	+15.62
P value		< 0.05	NS	NS	NS

Table 8. Variations in soil characteristics of plants retained in control and CO_2 treated conditions.

Parameters	Control		% Change	Treated		% Change	P value
	DoT 1	DoT15		DoT 1	DoT15		
Soil pH	6.58±0.09	7.64±0.83	+16.1	5.32±1.36	6.6±0.94	+24.06	< 0.05
Soil moisture (%)	27.37±5.33	28±4.12	+2.3	24.36±3.28	25.55±28.08	+4.88	NS
Soil carbon (%)	2.6±1.41	6.53±2.42	+151.15	4.69±1.31	6.9±1.4	+47.12	NS

(NS- Not significant)

Conclusion

The percentage day flux of CO_2 inside the treated chamber was higher than that of control and standardization study. However, the percentage night flux of CO_2 inside the control was higher than CO_2 treated and that of the standardization study. Thus, the plants inside the treated chamber were assumed to have consumed a higher share of CO_2, but the respiratory attribution of CO_2 by them compared to control, was less. Resultant day flux of CO_2 in the treated chamber can be attributed to the plants growing within it and is confirmed from the results of the standardisation experiment. Uptake and assimilation of CO_2 by the plants are also evident from the increased morphological attributes of growth like plant height, stem thickness, leaf area, higher carbohydrate, mineral content, and soil carbon content. However increased Phenol content and carotenoid content under elevated CO_2 are indications of stress to which the plants are subjected to under higher levels of CO_2 supply. Thus, *Pongamia pinnata* can be promoted as an effective tree species in the perspective of carbon offset planting, considering their CO_2 sequestration efficiencies.

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Conflict of Interest

We don't have any conflicts of interest associated with this publication. As first author, I confirm that the manuscript has been read and approved by corresponding author and co-author.

References

Aguera, E., Ruano, D., Cabello, P. and Haba, P. 2006. Impact of atmospheric CO_2 on growth, photosynthesis and nitrogen metabolism in cucumber (*Cucumis sativus* L.) plants. *J. of Plant Physiology*. 163 : 809-817.

Ainsworth, E. A., Davey, P. A., Bernacchi, C. J., Dermody, O. C., Heaton, E. A., Moore, D. J. and Long, S. P. 2002. A metaanalysis of elevated CO_2 effects on soybean (*Glycine max*) Physiol growth and yield. *Global change Biol*. 8: 695-709.

Al-Rawahy, S. H., Sulaiman, H., Farooq, S. A., Karam, M. F. and Sherwani, N. 2013. Effect of O_3 and CO_2 levels on growth, biochemical and nutrient parameters of alfalfa (Medicago Sativa). *APCBEE Procedia*. 5: 288-295.

Amthor, J. S. 1991. Respiration in a future, higherCO_2 world. *Plant, Cell & Environment.* 14(1): 13-20.

Ayub, G., Zaragoza-Castells, J., Griffin, K. L. and Atkin, O. K. 2014. Leaf respiration in darkness and in the light under pre-industrial, current and elevated atmospheric CO2 concentrations. *Plant Science*. 226: 120-130.

Bernacchi, C. J., Calfapietra, C., Davey, P. A., Wittig, V. E., Scarascia Mugnozza, G. E., Raines, C. A. and Long, S. P. 2003. Photosynthesis and stomatal conductance responses of poplars to free air CO_2 enrichment (PopFACE) during the first growth cycle and immediately following coppice. *New Phytologist*. 159: 609-621.

Bohre, P., Chaubey, O. P., and Singhal, P. K. 2014. Biomass production and carbon sequestration by *Pongamia pinnata* (Linn) Pierre in tropical Env. *Int. J Bio-Sci Bio-Tech. 6*: 129-140.

Bryant, J. P., Chapin III, F. S. and Klein, D. R. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos*. 40: 357-368.

Bunce, J. A. 2001. Seasonal patterns of photosynthetic response and acclimation to elevated carbon dioxide in field-grownstrawberry. *Photosynthesis Res*. 68: 237-245.

Campbell, C. S., Heilman, J. L., McInnes, K. J., Wilson, L. T., Medley, J. C., Wu, G. and Cobos, D. R. 2001. Diel and seasonal variation in CO_2 flux of irrigated rice. *Agricultural and Forest Meteorology.* 108:15-27.

Campbell, D. E., and Young, R. 1986. Short-term CO_2 exchange response to temperature, irradiance, and CO_2 concentration in strawberry. *Photosynthesis Res*. 8:31-40.

Centritto, M., Magnani, F., Lee, H. S., and Jarvis, P. G. 1999. Interactive effects of elevated CO_2 and drought on cherry (*Prunus avium*) seedlings. Photosynthetic capacity and water relations. *The New Phytologist*. 141:141-153.

Cernusak, L. A., Winter, K., Dalling, J. W., Holtum, J. A., Jaramillo, C., Körner, C. and Wright, S. J. 2013. Tropical forest responses to increasing atmospheric CO2: current knowledge and opportunities for future research. *Functional Plant Biology.* 40 (6): 531-551.

Ceulemans, R. and Mousseau, M. 1994. Effects of elevated Atoms. CO_2 on woody plants. *New Phytologist*. *127*: 425-446.

Crous, K. Y., Zaragoza Castells, J. O. A. N. A., Ellsworth, D. S., Duursma, R. A., Loew, M., Tissue, D. T. and Atkin, O. K. 2012. Light inhibition of leaf respiration in field grown *Eucalyptus saligna* in whole tree chambers under elevated atmospheric CO_2 and summer drought. *Plant, Cell & Environment.* 35(5): 966-981.

Dermody, O., Weltzin, J. F., Engel, E. C., Allen, P. and Norby, R. J. 2007. How do elevated CO_2, warming, and reduced precipitation interact to affect soil moisture and LAI in an old field ecosystem. *Plant and Soil*. 301: 255-266.

Dlugokencky, E.J., Hall, B.D., Montzka, S.A., Dutton, G., Muhle, J. and Elkins, J.W. 2018. Atoms. composition in State of the Climate in 2017. vol. 99, (pp46–49). *Colarado: Bull. of the American Meteorological Society.*

Dong, J., Gruda, N., Lam, S. K., Li, X. and Duan, Z. 2018. Effects of elevated CO_2 on Nutral Qual. of vegetables: a review. *Frontiers in Plant Sci.* 9 : 924.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T. and Smith, F. 1956. Colorimetric method for determination ofsugars and related substances. *Analytical Chemistry.* 28 : 350-356.

Edwards, N. T., Tschaplinski, T. J. and Norby, R. J. 2002. Stem respiration increases in CO_2 enriched sweetgum trees. *New Phytologist*. 155 (2): 239-248.

Elvira, A. I. andVasenev, I. I. 2020. Changes in CO_2 Flux Conditions in the Spruce Forest of Various Ages in the Central Forest Reserve of Russia. *European Journal of Molecular & Clinical Medicine.* 7(7): 693-704.

Faria, T., Wilkins, D., Besford, R. T., Vaz, M., Pereira, J. S. and Chaves, M. M. 1996. Growth at elevated CO_2 leads to down-regulation of photosynthesis and altered response to high temperature in *Quercus suber* L. seedlings. *J. of Experimental Botany.* 47 : 1755-1761.

Ghasemzadeh, A., Jaafar, H. Z. and Rahmat, A. 2010. Elevated carbon dioxide increases contents of flavonoids and phenolic compounds, and antioxidant activities in Malaysian young ginger (*Zingiber officinale* Roscoe.) varieties. *Molecules*. 15(11): 7907-7922.

Guo, Z., Zhuang, M., Yang, L., Li, Y., Wu, S. and Chen, S. 2021. Differentiated mineral nutrient management in two bamboo species under elevated CO_2 environment. *Journal of Environmental Management*. 279 : 111-600.

Hendrix, D. L., Mauney, J. R., Kimball, B. A., Lewin, K., Nagy, J. and Hendrey, G. R. 1994. Influence of elevated CO_2 and mild water stress on non-structural carbohydrates in field-grown cotton tissues. *Agricultural and Forest Meteorology.* 70 : 153-162.

Ho, L. C. 1977. Effects of CO_2 enrichment on the rates of photosynthesis and translocation of tomato leaves. *Annals of Appl Biol*. 87(2) : 191-200.

IPCC, 2001: Climate Change 2001: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Third Assessment Rep of the Intergovernmental Panel on Climate Change, White, Eds., Cambridge University Press, Cambridge.

IPCC, 2007: Climate Change 2007. The Fourth Assessment Rep of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

IPCC, 2018: Climate change 2018. Integrated Assessment Models: What Are They and How Do They Arrive at Their Conclusions. Geneva.

Jach, M. E. and Ceulemans, R. 2000. Effects of season, needle age and elevated Atoms. CO_2 on photosynthesis in Scots pine (*Pinus sylvestris*). *Tree Physiol*. 20: 145-157.

Jain, V., Pal, M., Raj, A. and Khetarpal, S. 2007. Photosynthesis and nutrient composition of spinach and fenugreek grown under elevated carbon dioxide concentration. *Biologia Plantarum*. 51 : 559. doi:10.1007/s10535-007-0122-9.

Janani, S., Priyadharshini, P., Jayaraj, R. S. C., Buvaneswaran, C. and Warrier, R. R. 2016. Growth, physiological and biochemical responses of Meliaceae species-*Azadirachta indica* and *Melia dubia* to elevated CO_2 concentrations. *J. of Appl Biology & Biotechnology.* 4 : 052-060.

Jastrow, J. D., Michael Miller, R., Matamala, R., Norby, R. J., Boutton, T. W., Rice, C. W. and Owensby, C. E.2005. Elevated Atoms. carbon dioxide increases soil carbon. *Global Change Biol*. 11: 2057-2064.

Kallarackal, J. and Roby, T. J. 2012. Responses of trees to elevated carbon dioxide and climate change. *Biodiversity and Conser*.

Karnosky, D. F., Darbah, J. N., Sober, A., Riikonen, J., Kets, K., Nelson, N. and Percy, K. E. 2006. Ozone effects on growth and productivity of *Populus tremuloides*. *Proc. Workshop, Obergurgl, Tyrol, Austria.* Federal Res., and Training Centre for Forests, Vienna, Austria, pp. 325-329.

Kaul, M. 2010. Carbon storage and sequestration potential of selected tree species in India. *Mitigation and Adaptation Strategies for Global Change*. 15(5): 489-510.

Keutgen, N. and Chen, K. 2001. Responses of citrus leaf photosynthesis, chlorophyll fluorescence, macronutrient and carbohydrate contents to elevated CO_2. *J. of Plant Physiol*. 158: 1307-1316.

Kimball, B. A. 2010. Lessons from FACE: CO_2 effects and interactions with water, nitrogen and temperature. pp. 87-107. London: Imperial College Press.

Krishan, G., Srivastav, S. K., Kumar, S., Saha, S. K. and Dadhwal, V. K. 2009. Quantifying the underestimation of soil organic carbon by the Walkley and Black technique–examples from Himalayan and Central Indian soils. *Current Sci*. 96 :1133-1136.

Kull, O., Tulva, I. and Vapaavuori, E. 2003. Influence of elevated CO_2 and O_3 on Betula pendula Roth crown structure. *Annals of Botany.* 91: 559-569.

Lahive, F., Hadley, P. and Daymond, A. J. 2018. The impact of elevated CO_2 and water deficit stress on growth and photosynthesis of juvenile cacao (*Theobroma cacao* L.). *Photosynthetica*. 56: 911-920.

Lindsey, R. 2020. Climate change: atoms. carbon dioxide. National Oceanic and Atmospheric Administration: Copenhagen, Denmark.

Loreto, F., Velikova, V. and Di Marco, G. 2000. Respiration in the light measured by 12 CO_2 emission in 13CO_2 atmosphere in maize leaves. *Functional Plant Biology.* 28(11): 1103-1108.

Lotfiomran, N., Kohl, M. and Fromm, J. 2016. Interaction effect between elevated CO_2 and fertilization on biomass, gas exchange and C/N ratio of European beech (*Fagus sylvatica* L.). *Plants*. 5: 38.doi:10.3390%2Fplants5030038.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. *J. Biological Chemistry.* 193: 265-275.

Maji, A.2017. A Preliminary Study on Carbon Sequestration Potential of Few Road Side Plant Species of Pursurah Area of Hooghly district, West Bengal (India). *Research Journal of Pharmaceutical Biological and Chemical Sciences*. 8 (3): 2051-2056.

Malick, C. P. and Singh, M. B. 1980. Estimation of total phenols in plant enzymology and histoenzymology. *Plant Enzymology And Histoenzymology: A Text Manual.* Kalyani Publishers. New Delhi.

Mohamed, S. J., Jellings, A. J. and Fuller, M. P.2013. Positive effects of elevated CO_2 and its interaction with nitrogen on safflower physiology and growth. *Agronomy for Sustainable Develop.* 33: 497-505.

Nelson, J. A., Morgan, J. A., LeCain, D. R., Mosier, A. R., Milchunas, D. G. and Parton, B. A. 2004. Elevated CO_2 increases soil moisture and enhances plant water relations in a long-term field study in semiarid shortgrass steppe of Colorado. *Plant and Soil.* 259: 169-179.

Norby, R. J., DeLucia, E. H., Gielen, B., Calfapietra, C., Giardina, C. P., King, J. S. and De Angelis, P. 2005. Forest response to elevated CO_2 is conserved across a broad range of productivity. *Proceedings of the National Academy of Sci.* 102: 18052-18056.

Norby, R. J., Wullschleger, S. D. and Gunderson, C. A. 1996. Tree responses to elevated CO_2 and implications for forests. *Carbon Dioxide and Terrestrial Ecosystems.* 1-21.

Ormrod, D. P., Lesser, V. M., Olszyk, D. M. and Tingey, D. T. 1999. Elevated temperature and carbon dioxide affect chlorophylls and carotenoids in Douglas-fir seedlings. *Int. J. Plant Sciences*. 160 : 529-534.

Pal, M., Karthikeyapandian, V., Jain, V., Srivastava, A. C., Raj, A. and Sengupta, U. K. 2004. Biomass production and Nutr. levels of berseem (*Trifolium alexandrium*) grown under elevated CO_2. *Agric Ecosystems and Env*. 101 : 31-38.

Reddy, K. R. and Zhao, D. 2005. Interactive effects of elevated CO_2 and potassium deficiency on photosynthesis, growth, and biomass partitioning of cotton. *Field Crops Res.* 94 : 201-213.

Reddy, P.S., Rao, G.R. and Kumar, P.S. 2015. Soil organic carbon (SOC) changes under biodiesel plantations (Pongamia pinnata*). International Journal of Plant, Animal and Environmental Sciences*. 5(2): 132-139.

Rey, A. and Jarvis, P. G. 1997. Growth response of young Birch trees (*Betula pendula* Roth.) after four and a half

years of CO_2 exposure. *Annals of Botany*. 80: 809-816.

Rogers, H. H., Runion, G. B., Prior, S. A. andTorbert, H. A.1999. Response of plants to elevated Atoms. CO_2: Root growth, mineral Nutr., and soil carbon. In: *Carbon Dioxide and Env. Stress* (pp. 215-244). Academic Press.

Ruiz-Vera, U. M., De Souza, A. P., Ament, M. R., Gleadow, R. M. and Ort, D. R. 2021. High sink strength prevents photosynthetic down-regulation in cassava grown at elevated CO_2 concentration. *J. Experimental Botany*. 72 : 542-560.

Sanz-Saez, A., Erice, G., Aranjuelo, I., Nogues, S., Irigoyen, J. J. and Sanchez-Diaz, M. 2010. Photosynthetic down-regulation under elevated CO_2 exposure can be prevented by nitrogen supply in nodulated alfalfa. *J. Plant Physiology*. 167(18): 1558-1565.

Scott, P. T., Pregelj, L., Chen, N., Hadler, J. S., Djordjevic, M. A. and Gresshoff, P. M. 2008. Pongamia pinnata: an untapped resource for the biofuels industry of the future. *Bioenergy Research*. 1 (1): 2-11.

Sharma, A.R.U.N.A. and Sengupta, U.K. 1990. Carbon dioxide enrichment effects on photosynthesis and related enzymes in *Vigna radiata* L. Wilczek. *Indian J. of Plant Physiol.* 33: 340-346.

Shoaf, W. T. and Lium, B. W. 1976. Improved extraction of chlorophyll a and b from algae using dimethyl sulfoxide. *Limnology and Oceanography*. 21(6): 926-928.

Superales, J. B. 2016. Carbon Dioxide Capture and Storage Potential of Mahogany (*Swietenia macrophylla*) Saplings. *Int. J. of Env. Sci and Develop*. 7: 611.

Taub, D. R., Miller, B. and Allen, H. 2008. Effects of elevated CO_2 on the protein concentration of food crops: a meta analysis. *Global Change Biol*. 14: 565-575.

Taylor, G., Ranasinghe, S., Bosac, C., Gardner, S. D. L. and Ferris, R. 1994. Elevated CO_2 and plant growth: cellular mechanisms and responses of whole plants. *J. of Experimental Botany*. 1761-1774.

Tognetti, R., Longobucco, A., Raschi, A., Miglietta, F. and Fumagalli, I. 1999. Responses of two Populus clones to elevated Atoms. CO_2 concentration in the field. *Annals of Forest Sci*. 56: 493-500.

Urbonaviciute, A., Samuoliene, G., Sakalauskaite, J., Duchovskis, P., Brazaityte, A., Siksnianiene, J. B. and Baranauskis, K. 2006. The effect of elevated CO_2 concentrations on leaf carbohydrate, chlorophyll contents and photosynthesis in radish. *Polish J. of Env. Studies*.

Wang, X., Lewis, J. D., Tissue, D. T., Seemann, J. R. and Griffin, K. L. 2001. Effects of elevated atmospheric CO_2 concentration on leaf dark respiration of *Xanthium strumarium* in light and in darkness. *Proceedings of the National Academy of Sciences*. 98(5) : 2479-2484.

Zhang, Y. 2008. Leaf photosynthesis of *Betula albosinensis* seedlings as affected by elevated CO_2 and planting density. *Forest Ecol and Management*. 255: 1937-1944.

Ziska, L. H. and Bunce, J. A. 1994. Increasing growth temperature reduces the stimulatory effect of elevated CO_2 on photosynthesis or biomass in two perennial species. *Physiologia Plantarum*. 91(2): 183-190.