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Growth, Physiological and Nutrient Uptake Traits of Crotalaria Cover Crops Influenced by Levels of Carbon Dioxide under Low Light Intensities

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Authors' contributions

This work was carried out in collaboration between all authors. Authors VCB and MKE designed and conducted the experiment, performed the statistical analysis and wrote the first draft of the manuscript. Author ZLH conducted nutrient analysis and reviewed the manuscript. Authors YL, ADQP, DA and AAFA contributed in designing and revising the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Crotalarias are tropical legumes grown as cover crops or green manure crops to improve soil fertility and reduce soil degradation. As understory plants in plantation crop systems, these cover crops receive elevated levels of [CO_2] and low irradiance. A greenhouse experiment was conducted to evaluate the effects of ambient (400 µmol mol^{-1}) and elevated (700 µmol mol^{-1}) levels of [CO_2] at

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low photosynthetic photon flux density (PPFD) of 100, 250 or 450 µmol m^{-2} s^{-1} on growth, physiological and nutrient use parameters of four Crotalaria species (*C. breviflora*, *C. mucronata*, *C. ochroleuca* and *C. spectabilis*). Elevated [CO_2] had little effect on growth, but increased NAR and nutrient use efficiency of N, Cu, Fe and Zn. PPFD had significant effects on growth, physiology and NUE. Increasing PPFD increased nutrient use efficiency of N and K, but reduced nutrient use efficiency of P and micronutrients. At low light intensities irrespective of [CO_2], intraspecific differences were observed in crotalaria for growth, physiology and nutrient uptake traits. Irrespective of [CO_2] levels at low PPFDs, *C. mucronata* was efficient in N, Ca, Cu, and Zn use efficiency and *C. spectabilis* was efficient in P, Ca, Mg, Fe, and Mn use efficiency.

Keywords: Nutrient use efficiency; net assimilation rate; nutrient transport; water use efficiency.

1. INTRODUCTION

Plantation crops such as cacao, coffee, tea and banana are established on recently cleared sloppy and infertile soils. Legume cover crops in early plantation establishment could reduce soil degradation due to soil erosion and leaching of nutrients [1,2,3]. Perennial legumes grown as cover crops can fix atmospheric nitrogen, increase soil organic matter, improve soil aggregation, enhance water holding capacity, improve biological activity, promote soil fertility and reduce weed infestation [1,3]. Crotalaria species are fast growing tropical cover crops that can yield 5 to 10 t/ha/yr dry matter and fix 60-200 kg N/ha/yr [3,4,5].

Survivability and persistence of cover crops such as crotalaria grown as understory plants depends largely on the amount of light reaching their canopies [6,7,8]. Cover crops grown with plantation crops experience low light intensities [8,9]. Shading reduces yields of most tropical legumes [7,8,10,11]. Inter-specific differences in shade tolerance of tropical cover crops have been reported [10,12,13,14,15,16]. In early stages of plantations, cover crops receive full sunlight but as the plantation develops the photosynthetic photon flux density (PPFD) is reduced as the trees grow. In legume cover crops growth and development, physiological processes and nutrient uptake and use efficiency are greatly reduced by decreases in PPFD [17,18,19,20].

Rising atmospheric [CO_2] levels, which are expected to double by the end of the century [21], and litter decomposition in plantation systems contribute to elevated [CO_2] at the cover crop level. Elevated [CO_2] has been shown to increase plant biomass and photosynthesis in many species [22,23,24,25]. Interspecific differences have been reported in growth, physiological parameters and nutrient use efficiency of legume cover crops grown at ambient (400 µmol mol^{-1}) and elevated (700 µmol mol^{-1}) levels of [CO_2] with low levels of PPFD (100, 250, and 450 µmol m^{-2} s^{-1}) [20]. In this study, overall, total dry biomass, root dry biomass, root/shoot ratio, and stem height were significantly influenced by levels of [CO_2] and PPFD. In all the cover crops tested, increasing levels of [CO_2] and PPFD increased RGR, NAR, WUE and SPAD, and decreased water flux (VO). There is limited information available on how increasing [CO_2] might affect growth, physiological processes and nutrient use efficiency in crotalaria species. Differences were found in the response of photosynthesis to varying irradiance and external [CO_2] levels in crotalaria [26]. However, the impact of [CO_2] and low irradiance on growth, physiology and nutrient use efficiency of different crotalaria species is unknown.

The objective of this research was to assess the effects of ambient (400 µmol mol^{-1}) and elevated (700 µmol mol^{-1}) levels of [CO_2] at low levels of PPFD (100, 250 or 450 ± 50 µmol m^{-2} s^{-1}) on growth, physiological and nutrient uptake parameters of four crotalaria species.

2. MATERIALS AND METHODS

2.1 Perennial Legume Cover Crops

Four perennial legume cover crops selected for this study were: *Crotalaria breviflora* DC. (Shortflower Rattlebox), *C. mucronata* Desv. (Smooth Crotalaria), *C. ochroleuca* G. Don (Slender Leaf Rattlebox) and *C. spectabilis* Roth (Showy Crotalaria). Table 1 lists the growth habits, strengths and limitations of these cover crops [3,4,27].

Cover crops used in this study are known to have unique characteristics that may be useful for limiting soil degradation and improving soil

fertility. *C. breviflora* is a non-climbing perennial shrub, native to Brazil. It can produce 3-5 t/ha/yr of dry matter (DM), fix 98-160 kg/ha/yr of nitrogen and is used mainly as a cover crop in tropical tree plantations. *C. mucronata* is a non-climbing perennial shrub, native from Africa to Asia. It is used as a cover crop producing 5-10 t/ha/yr of DM and fixing 80 to 160 kg/ha/yr of N. It is tolerant of shade and drought [27]. *C. ochroleuca* is a non-climbing, perennial shrub, native to tropical Africa. It is used as a cover crop and produces 10 t/ha/yr of DM [27]. *C. spectabilis* is a non-climbing shrub, native to tropical Asia. If used as a cover crop, it can produce 4-11 t/ha/yr of DM and fix 60 to 170 kg/ha/yr of N [27].

2.2 Growth Medium and Planting

Growth medium was prepared by mixing Perlite: Sand: Peat moss (2:2:1 volume basis) in cement mixer with required macro- and micro-nutrients to provide supplemental nutrients (mg/kg) of 600 N, 600 P, 240 K, 1012 Ca, 309 Mg, 500 S, 119 Fe, 0.7 B,17.5 Mn, 7 Cu, 7 Zn and 0.35 Mo to support good crop growth. Nutrients were applied as triple superphosphate, urea, calcium sulfate, dolomitic lime, osmocote 18N-6P_2O_5-12K_2O (The Scotts Company, Marysville, Ohio), and micronutrients as Scott's Micromix. For the study, one-gallon black plastic pots containing 2 kg of growth medium and possessing adequate bottom drainage were used. Into each pot 10 seeds of a crotalaria species were planted. Pots were weighed and water was applied as needed to maintain soil moisture at field capacity (-33 kPa) throughout the growth cycle. One pot without any plants was placed in each of three mini chambers to monitor the evaporative water loss.

2.3 Growth Conditions

Two glasshouses (18 m^2 each) with day/night temperatures of 30/28°C were used for plant growth. The first glasshouse contained ambient levels of [CO_2] (400 µmol mol^{-1}) and the second contained elevated levels of [CO_2] (700 µmol mol^{-1}) measured by WMA2 infrared gas analyzers (PP Systems, Amesbury, MA). When the [CO_2] level fell below 700 µmol mol^{-1}, [CO_2] was injected to the desired level. In each glass house, mini-chambers were constructed using PVC pipe (112 cm W x 120 cm L x 81cm H). These mini-chambers were covered with one or two layers of plastic shade cloth to achieve the desired PPFD levels of 100, 250 or 450 µmol m^{-2} s^{-1}.

Table 1. Common names, scientific names, growth habits, and strengths and limitations of cover crops used[1]

Common name	Scientific name	Growth habit[2]	Strength	Limitation
Shortflower rattlebox	*Crotalaria breviflora* DC.	N/S	Good vegetative cover. Reduces nematodes. Shorter than others.	No data
Smooth crotalaria	*Crotalaria mucronata* Desv., *Crotalaria pallida* Aiton	N/S	Good green manure crop. Reduces nematodes number	Pest and disease problems; Susceptible to Root-Knot nematode; Toxic to animals
Slender leaf rattlebox	*Crotalaria ochroleuca* G. Don, *C. brevidens* Benth.	N/S	Good green manure crop. Reduces nematodes. Edible vegetable in parts of Africa.	No data
Showy crotalaria	*Crotalaria spectabilis* Roth	N/S	Rapid development; Control of root-knot nematodes; Requires little attention after established. Reduces nematodes number	Toxicity to animals; High potential as a weed

[1]References: [3,4,27]
[2]N = Non-Climbing, C= Climbing, S =Shrub

2.4 Evaluation of Traits

2.4.1 Growth traits

In each pot after 14 days of growth, plants were thinned to 6 plants/pot. The removed plants were used as an initial harvest. Stem height and SPAD index were recorded after an additional 36 days of growth. A non-destructive method was used to estimate the chlorophyll content of the leaves using a SPAD meter (KonicaMinolta Chlorophyll Meter, Model 502, Ramsey, NJ, USA). After 36 days of growth, shoots (stems and leaves) were harvested, weighed, and total leaf area (cm^2) was measured using a LI-3100 Leaf Area Meter (Li-Cor Inc., Lincoln, NE). Stems and leaves were washed in deionized water, freeze-dried and the shoot dry biomass (SDB) was recorded. Root biomass was determined by removing roots from the growth medium, washing, blotting dry and weighing. A Comair Root Length Scanner (Hawker de Haviland, Melbourne, Victoria, Australia) was used to measure total root length and the roots were oven dried at 70°C for 5 days before the root dry biomass (RDB) was recorded.

2.4.2 Physiological traits

Specific leaf area (SLA), Relative Growth Rate (RGR) and Net Assimilation Rate (NAR) were determined as follows:

SLA, (cm^2/g) = [Total leaf area/plant, cm^2/Total leaf dry biomass/plant, g]

RGR = [ln (Wt_2/Wt_1) / (T_2-T_1)] Where Wt is total biomass (shoot + root), T is time in days, subscript 1(15 days) and 2 (36 days) refer to initial and final harvests.

NAR = [RGR/LAR] where LAR (cm^2/g) = [Total leaf area/plant, cm^2/Shoot+Root dry biomass/plant, g]

Water Flux (VO) and Water Use Efficiency (WUE) were calculated as follows:

Water Flux (VO) = {[TRANS / ($T_2 - T_1$)][lnRL_2 – lnRL_1)/(RL_2 – RL_1)]} / (2πRR); where TRANS is Transpiration, T is time in seconds, subscripts 1 and 2 refer to initial and final harvests and RR is the Root Radius (cm) = $(RFW / RL \times \pi)^{1/2}$ where RFW is root fresh biomass (cm^3)

Water Use Efficiency (WUE) = Shoot dry biomass g plant/Amount of water transpired, (g $plant^{-1}$). Total amount of water Transpired was calculated by subtracting the Evaporation from the total water loss during 36 days of growth.

2.4.3 Nutrient traits

Nutrient Uptake (U), influx (IN), transport (TR) and use efficiency (NUE) were determined as follows. Freeze-dried shoots (stems, leaves) were ground to pass through a 1-mm sieve and sent to University of Florida, Indian River Research and Education Center (UFL-IRREC) for elemental analysis. A 0.4 g plant sample was digested in 5 ml of concentrated nitric acid (14 N), and an inductively coupled plasma optical emission spectrometry ((ICP-OES, Ultima JY Horiba Inc. Edison, NJ, USA) was used to determine concentrations of elements in the digest following USEPA method 200.7 [28]. Total N in plant tissue was analyzed by combustion method using a CN Analyzer (Vario MAX CN Macro Analyzer, Elementar Analysensysteme GmbH, Hanau, Germany) [29].

Uptake (U) = Concentration of any given element (mg or µg) x Shoot Dry Biomass (g/plant)

IN = [(U_2 - U_1) / (T_2-T_1)] [(lnWr_2-ln Wr_1)/(Wr_2-Wr_1)], where U refers to elemental uptake in shoot (mmoles $plant^{-1}$), T is time in seconds, Wr is root dry biomass, and subscripts 1 and 2 refer to initial and final harvest time

TR = [(U_2 - U_1) / (T_2-T_1)] [(lnWs_2-ln Ws_1)/(Ws_2-Ws_1)], where Ws is shoot dry biomass

ER = [mg of Ws / mg or µg of any given element in shoot]

2.5 Statistical Analysis

Experiment was conducted under a split plot design where [CO_2] levels were the main plots, PPFD were the subplots and cover crops were the sub sub plots. Treatments were replicated three times. All data were analyzed using general linear model (GLM) procedures of SAS (Ver. 9.1, SAS Institute, and Cary, NC).

3. RESULTS AND DISCUSSION

3.1 Growth Traits

PPFD had significant effects on the growth traits of crotalaria, suggesting that low levels of PPFD reduced growth of all cover crop species (Table 2). With the exception of specific leaf area, all

other growth traits increased with increases in PPFD. Low PPFD (shading) is known to reduce yields of most tropical legumes [10,11]. Similar interactions have been reported between cover crops and PPFD to growth traits of many species of perennial legume cover crops [18,20]. Cover crop species that tolerate lower PPFD have a better chance of growing and persisting for a longer period as understory plants in agroforestry based plantation crops.

Increasing [CO_2] increased growth traits of all the species but there was a significant reduction in specific leaf area at the higher [CO_2]. Similar effects of increasing [CO_2] on growth traits of five perennial legume species have been reported [20]. Carbon dioxide levels and the interaction with PPFD and species had little effect on total dry biomass, root dry biomass, stem height, total root length and leaf area. In many species increases in growth at higher PPFD were not as large as would be predicted by the increase in photosynthesis [30,31,32].

Species had highly significant effects on most of the growth traits. *C. mucronata* had the highest total dry biomass, stem height and leaf area. It has been reported that plants with greater leaf area have greater potential for growth than those with smaller leaf area [33]. *C. breviflora* and

Table 2. The effect of [CO_2] and PPFD on shoot, root and leaf growth of Crotalaria leguminous cover crops

Species	PPFD (µmol m^{-2} s^{-1})	Total dry biomass (g $plant^{-1}$)	Root dry biomass (g $plant^{-1}$)	Root / shoot ratio	Stem height (cm $plant^{-1}$)	Total root length (cm $plant^{-1}$)	Total leaf area (cm^2 $plant^{-1}$)	Specific leaf area (cm^2 g^{-1})
400 µmol [CO_2] mol^{-1}								
C. spectabilis	100	0.50	0.05	0.11	19.83	560.1	121.4	373.8
	250	1.29	0.14	0.13	21.83	1474.9	212.0	260.7
	450	1.58	0.23	0.17	19.33	1318.6	262.9	266.6
C. breviflora	100	0.13	0.01	0.12	17.67	136.8	37.5	494.9
	250	0.40	0.06	0.15	21.00	520.5	75.5	328.2
	450	0.72	0.13	0.20	24.33	975.9	114.4	280.8
C. mucronata	100	0.55	0.04	0.09	15.00	591.1	168.8	434.9
	250	2.20	0.24	0.12	26.00	1417.3	470.6	349.7
	450	2.59	0.34	0.17	32.00	790.0	376.2	270.9
C. ochroleuca	100	0.23	0.02	0.10	18.33	239.5	62.2	466.1
	250	0.57	0.05	0.09	26.33	342.0	108.5	330.7
	450	1.09	0.15	0.16	29.67	1750.8	116.6	238.5
700 µmol [CO_2] mol^{-1}								
C. spectabilis	100	0.64	0.08	0.13	22.33	668.9	150.4	406.0
	250	1.42	0.20	0.16	26.33	1471.7	178.2	248.6
	450	1.35	0.19	0.17	20.00	1849.0	166.9	223.7
C. breviflora	100	0.17	0.03	0.19	17.67	152.3	47.5	512.4
	250	0.44	0.06	0.15	26.17	456.6	67.7	287.5
	450	0.69	0.12	0.22	22.00	641.1	76.3	197.4
C. mucronata	100	1.17	0.09	0.09	24.33	705.6	290.8	386.9
	250	1.85	0.24	0.14	28.33	981.1	318.1	313.8
	450	2.20	0.30	0.16	26.33	1202.3	246.3	227.8
C. ochroleuca	100	0.33	0.04	0.13	22.00	445.7	75.2	397.8
	250	0.65	0.07	0.12	22.67	683.7	102.9	287.7
	450	1.23	0.17	0.15	30.33	1214.7	120.1	222.8
Significance								
[CO_2] (C)		NS	NS	*	NS	NS	NS	**
PPFD (P)		**	**	**	**	**	**	**
Species (S)		**	**	**	*	**	**	**
$LSD_{0.05}$		1.43	0.19	0.11	14.5	1374	233.3	110.9

*, ** *Significant at 0.05 and 0.01 levels of probability, respectively. NS = Not significant*

C. ochroleuca had smaller leaf areas and produced the least amount of total and root biomass and root length. In other tropical perennial cover crops, it has been reported that cover crops with larger leaf area produced higher shoot and root biomass than cover crops with lower leaf area [17,20].

3.2 Physiological Traits

Carbon dioxide levels had significant effects on VO, WUE and NAR, but not on SPAD or RGR (Table 3). Previously it was found that increasing levels of [CO_2] increased RGR, NAR, WUE and SPAD, and decreased VO for five perennial legume cover crops [20]. WUE appears more efficient at high [CO_2], especially for *C. mucronata.* Doubling atmospheric [CO_2] reduced stomatal conductance in C_3 annual crop plants by 34% [22] and such effects are very much expressed in these cover crops.

PPFD had significant influence on SPAD, WUE, RGR and NAR. Increases in PPFD decreased the VO and increased WUE, RGR and NAR. SPAD index gives an indication of chlorophyll content and reducing light reduces SPAD readings. Similar effects of increasing PPFD and [CO_2] on physiological traits of tropical perennial legumes have been reported [20].

Table 3. The effect of [CO_2] and PPFD on, SPAD, water flux (VO), water use efficiency (WUE), RGR and NAR of perennial tropical leguminous cover crops

Species	PPFD (µmol m^{-2} s^{-1})	SPAD	Water Flux (VO) (cm^3 H_2O influx cm^{-2} of roots s^{-1}) (x 10^{-6})	WUE (g shoot / g trans) (* 10^{-3})	Relative growth rate (RGR) (g g^{-1} d^{-1}) (x 10^{-2})	Net assimilation rate (NAR) (g cm^{-2} d^{-1}) (x 10^{-4})
	400 µmol [CO_2] mol^{-1}					
C. spectabilis	100	31.5	60.82	1.41	7.77	3.24
	250	38.2	58.07	1.54	10.02	6.22
	450	45.6	50.39	2.03	10.52	6.35
C. breviflora	100	42.3	44.33	0.98	5.83	2.02
	250	47.7	36.41	1.47	8.70	4.65
	450	51.6	26.02	2.05	10.17	6.36
C. mucronata	100	38.6	76.23	1.70	10.29	3.40
	250	42.5	59.31	2.25	13.88	6.45
	450	42.0	59.74	2.19	14.25	10.19
C. ochroleuca	100	34.9	43.01	1.10	9.33	3.43
	250	42.4	34.18	1.77	11.51	5.97
	450	51.1	32.11	1.52	13.36	13.17
	700 µmol [CO_2] mol^{-1}					
C. spectabilis	100	42.7	35.94	2.19	8.38	3.61
	250	44.9	48.02	1.69	10.24	8.20
	450	40.8	46.00	1.71	10.12	8.64
C. breviflora	100	36.8	44.45	0.99	6.36	2.44
	250	39.6	30.77	2.05	8.95	5.82
	450	44.0	18.96	3.10	10.12	9.15
C. mucronata	100	41.8	61.23	2.86	11.73	4.68
	250	45.3	43.33	3.30	13.41	7.72
	450	45.6	50.41	2.92	13.55	12.01
C. ochroleuca	100	38.0	33.95	1.29	10.24	4.52
	250	52.7	30.97	2.01	11.93	7.79
	450	48.3	21.35	2.39	13.58	13.75
Significance						
[CO_2] (C)		NS	*	**	NS	**
PPFD (P)		**	NS	**	**	**
Species (S)		NS	**	**	**	**
$LSD_{0.05}$		15.7	55.80	1.01	2.85	4.57

*, ** *Significant at 0.05 and 0.01 levels of probability, respectively. NS = Not significant.*

Species had significant effects on VO, WUE, NAR and RGR, but not on SPAD. Irrespective of [CO_2] and PPFD, *C. mucronata* was the most efficient in VO and WUE reflecting the higher shoot dry matter accumulation.

3.3 Nutrient Uptake Traits

3.3.1 Nutrient concentrations

Concentrations of N, P and K were slightly higher than the standard reported concentrations for leguminous crops [17,18,34,35] and all other essential nutrients were at adequate levels (Table 4). The [CO_2] had significant effects on N, P, Ca, Cu, Fe, and Zn, which generally were higher at 400 µmol mol^{-1} [CO_2].

PPFD had significant effects on all nutrient concentrations except Fe. N, K and Fe decreased with increasing PPFD, and all other nutrient concentrations increased with increasing PPFD. Increasing PPFD from 200 to 400 µmol m^{-2} s^{-1} reportedly decreased the concentrations of most of the micronutrients, which was attributed to increased dry matter at the slightly higher PPFD causing dilution effects [17].

Species had significant effects on all nutrient concentrations except Zn, but each species had its own preference. *C. breviflora* was highest for Ca and Fe but low for K. *C. mucronata* was lowest for N, Ca, Cu, Fe and Zn. *C. ochroleuca* was highest for P, K, Mg and Cu. *C. spectabilis* was high for N, but low for P, Mg and Mn.

Table 4. The effect of [CO_2] and PPFD on nutrient concentration in Crotalaria leguminous cover crops

Species	PPFD (µmol m^{-2} s^{-1})	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
		----------mg g^{-1}----------					----------µg g^{-1}----------			
400 µmol [CO_2] mol^{-1}										
C. spectabilis	100	74.04	10.38	25.73	9.88	2.47	56.43	144.6	209.3	85.9
	250	73.27	10.63	22.37	12.74	3.44	63.53	149.8	325.9	111.7
	450	67.10	12.27	19.18	14.66	4.87	71.95	143.3	404.2	118.5
C. breviflora	100	72.00	10.17	22.31	13.08	3.81	60.74	158.7	278.6	115.2
	250	71.14	11.92	19.77	12.37	3.55	57.49	115.8	293.5	92.9
	450	70.84	14.49	17.93	16.06	4.36	72.00	138.7	344.8	94.6
C. mucronata	100	73.45	11.54	29.50	9.80	3.22	43.05	120.2	385.9	115.2
	250	64.06	12.58	21.77	11.51	3.61	45.15	107.4	485.6	95.5
	450	41.90	14.75	18.96	14.25	4.74	69.76	137.9	463.1	127.8
C. ochroleuca	100	65.48	14.04	26.85	11.88	3.61	64.43	131.8	373.8	79.5
	250	63.76	14.49	26.36	11.03	4.14	76.16	147.1	380.3	128.4
	450	57.91	13.07	22.53	10.50	4.47	67.52	118.4	302.9	90.1
700 µmol [CO_2] mol^{-1}										
C. spectabilis	100	74.08	9.18	23.99	11.25	3.04	44.55	108.6	278.4	92.0
	250	72.17	9.92	22.02	10.12	3.48	50.33	96.9	242.1	96.0
	450	69.83	10.33	23.61	9.79	4.11	48.68	77.7	236.9	88.8
C. breviflora	100		9.05	25.45	12.78	3.86	45.99	149.6	244.9	73.0
	250	63.89	9.14	16.73	12.26	3.48	41.71	133.0	249.8	70.4
	450	59.52	14.28	15.17	15.25	4.37	61.86	135.7	386.6	90.6
C. mucronata	100	60.03	12.53	25.08	10.29	3.79	33.53	105.4	410.3	75.1
	250	56.51	12.06	19.53	9.80	4.37	45.97	118.8	470.4	75.7
	450	46.65	14.16	18.66	10.95	4.92	60.54	117.4	409.2	89.6
C. ochroleuca	100	56.03	11.88	33.56	11.81	3.99	42.09	138.1	375.7	76.5
	250	65.82	12.96	24.82	9.11	4.04	45.43	125.0	306.9	73.4
	450	48.14	16.14	19.07	12.84	5.56	62.30	134.4	362.8	105.9
Significance										
[CO_2] (C)		**	*	NS	*	NS	**	**	NS	**
PPFD (P)		**	**	**	**	**	**	NS	*	*
Species (S)		**	**	**	**	**	*	**	**	NS
$LSD_{0.05}$		17.15	4.48	7.42	5.57	1.58	28.65	55.82	155.6	63.5

*, ** *Significant at 0.05 and 0.01 levels of probability, respectively. NS = Not significant*

Crotalaria generally had higher N concentrations (63.9 mg g^{-1}) than other tropical perennial leguminous cover crops (Calopo 51.8, Jack bean 43.5, Brazilian lucerne 49.6, White lead tree 57.3, Mucuna 51.4 mg g^{-1}) [20].

3.3.2 Nutrient uptake

Carbon dioxide levels and the interactions with PPFD and Species had no significant effects on nutrient uptake by these crotalaria species (Table 5). Overall, with exception of Mg uptake, increasing [CO_2] increased uptake of all the other nutrients. PPFD and Species were highly significant for nutrient uptake of all nutrients tested. In general, nutrient uptake levels increased as the PPFD increased. Highly significant effects of increasing PPFD from 200 to 400 µmol m^{-2} s^{-1} on uptake of macro-micronutrients have been reported [17]. changes in mineral composition of Joint Vetch, Calopo, Centro, Ea-Ea, Tropical Kudzu and Brazilian Lucerne grown in varying levels of shade (18 to 100% of daylight) in greenhouse conditions have been reported [36].

Uptake of all nutrients was highest for *C. mucronata* and lowest for *C. breviflora*. Significant variability in nutrient uptake among various cover crop species is associated with different growth habits, the amount of dry matter accumulated in the shoot and the specific demand of the plant for any particular nutrient [18,37]. The nutrient concentration values in the plants may have been higher for crotalaria, but the N uptake values were higher for Jack bean

Table 5. The effect of [CO_2] and PPFD on nutrient uptake in Crotalaria leguminous cover crops

Species	PPFD (µmol m^{-2} s^{-1})	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
		mg plant^{-1}					µg plant^{-1}			
400 µmol [CO_2] mol^{-1}										
C. spectabilis	100	33.9	4.75	11.61	4.36	1.12	25.7	65.9	94.1	39.4
	250	84.9	12.35	25.85	15.00	4.00	72.8	173.2	377.7	134.7
	450	90.5	16.55	25.84	19.97	6.57	96.2	191.7	544.0	160.3
C. breviflora	100	9.01	1.18	2.61	1.51	0.44	7.0	17.9	32.3	13.1
	250	24.3	4.17	6.91	4.29	1.24	20.1	40.3	102.7	33.2
	450	41.7	8.70	10.64	9.79	2.61	42.4	81.4	207.9	79.2
C. mucronata	100	33.1	6.16	15.31	5.10	1.67	22.0	62.9	203.0	36.6
	250	125.8	24.54	42.62	22.22	7.07	90.6	211.6	954.0	170.4
	450	98.9	32.43	42.65	30.72	10.24	154.5	286.6	1038.7	205.2
C. ochroleuca	100	13.7	2.91	5.37	2.45	0.73	13.5	26.9	77.1	16.4
	250	32.8	7.40	13.84	5.60	2.14	42.8	77.5	201.6	60.1
	450	53.9	12.20	21.14	9.85	4.18	63.1	111.3	282.7	84.3
700 µmol [CO_2] mol^{-1}										
C. spectabilis	100	42.1	5.22	13.64	6.38	1.73	25.4	61.7	158.3	51.8
	250	88.2	12.05	26.97	12.30	4.22	61.0	117.8	291.4	115.9
	450	81.0	11.78	27.38	11.60	4.63	55.1	91.3	275.3	100.8
C. breviflora	100		1.32	3.63	1.94	0.57	6.8	22.0	36.8	10.8
	250	24.2	3.49	6.33	4.69	1.32	15.9	50.5	95.1	26.8
	450	33.4	8.11	8.52	8.60	2.47	35.3	76.2	220.2	51.3
C. mucronata	100	61.9	13.28	25.03	11.40	4.34	36.2	113.3	457.7	79.9
	250	87.8	19.46	30.91	16.27	7.23	74.9	197.8	782.4	124.3
	450	85.2	27.05	34.93	20.09	9.86	119.7	227.3	812.9	176.1
C. ochroleuca	100	21.8	3.56	9.69	3.57	1.24	13.1	41.5	110.9	23.5
	250	37.8	7.61	14.55	5.39	2.39	26.1	70.9	183.1	43.4
	450	51.2	17.02	20.28	13.09	5.87	65.8	140.6	376.4	111.2
Significance										
[CO_2] (C)		NS	NS	NS	NS	NS	NS	NS	NS	NS
PPFD (P)		**	**	**	**	**	**	**	**	**
Species (S)		**	**	**	**	**	**	**	**	**
$LSD_{0.05}$		67.3	17.07	25.25	14.05	6.52	81.0	145.6	615.7	129.9

**, ** Significant at 0.05 and 0.01 levels of probability, respectively. NS = Not significant*

[20] (283.7 mg g^{-1} for Jack bean vs 82.9 mg g^{-1} for *C. mucronata*) because of the higher dry matter accumulation of Jack bean. This means that more N could be held by Jack bean and incorporation of Jack bean residues could provide higher available N for the associated plantation crops.

3.3.3 Nutrient Influx (IN)

The carbon dioxide level had little effect on Nutrient Influx (IN) of macro- or micro-nutrients (Table 6). Increasing PPFD significantly increased influx of P, Ca, Mg, Cu, Fe, Mn, and Zn, but did not affect N or K. The species of crotalaria significantly influenced influx of macro- and micro-nutrients. *C. mucronata* was consistently high, *C. breviflora* was always lower in influx of macro-micro nutrients.

3.3.4 Nutrient Transport (TR)

The [CO_2] had significant effects on Nutrient Transport of Mg, Cu, Fe and Zn (Table 7). With the exception of Mg, transport of all other nutrients decreased slightly with the increase in [CO_2] to 700 µmol mol^{-1}. PPFD had significant effects on nutrient transport of all nutrients except K. Increasing PPFD increased transport of P, Ca, Mg, Cu, Fe, Mn, and Zn. The transport rate of N was highest at 250 µmol m^{-2} s^{-1} and lower at 100 and 450 µmol m^{-2} s^{-1}. TR of macronutrients was reported to be significantly influenced by increasing PPFD from 200 to 400 µmol m^{-2} s^{-1} [18].

Table 6. The effect of [CO_2] and PPFD on nutrient influx (IN, pmol cm $root^{-1}$ sec^{-1}) in perennial tropical leguminous cover crops

Species	PPFD (µmol m^{-2} s^{-1})	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
400 µmol [CO_2] mol^{-1}										
C. spectabilis	100	6.00	0.38	0.70	0.23	0.11	0.96	2.91	3.94	1.50
	250	6.04	0.46	0.64	0.41	0.18	1.27	3.27	7.04	2.50
	450	6.52	0.54	0.68	0.46	0.27	1.67	3.74	10.30	2.43
C. breviflora	100	5.08	0.27	0.50	0.24	0.13	0.80	2.09	3.87	1.23
	250	4.55	0.37	0.48	0.27	0.13	0.86	1.88	5.05	1.31
	450	5.19	0.51	0.47	0.45	0.20	1.18	2.55	6.85	2.27
C. mucronata	100	5.62	0.57	0.97	0.32	0.17	0.81	2.83	10.58	1.55
	250	8.74	0.79	1.05	0.57	0.30	1.33	3.56	17.57	2.67
	450	8.23	1.48	1.47	1.17	0.64	3.56	8.64	26.36	4.58
C. ochroleuca	100	4.96	0.43	0.76	0.28	0.16	0.92	2.37	6.49	1.18
	250	7.00	0.73	1.00	0.43	0.26	1.54	3.87	10.00	3.48
	450	4.33	0.45	0.58	0.26	0.18	1.11	2.08	5.56	1.41
700 µmol [CO_2] mol^{-1}										
C. spectabilis	100	5.44	0.31	0.61	0.28	0.13	0.71	1.97	5.34	1.53
	250	5.61	0.36	0.61	0.29	0.16	0.91	1.96	5.26	1.73
	450	5.00	0.33	0.61	0.26	0.17	0.86	1.63	4.65	1.39
C. breviflora	100		0.18	0.43	0.17	0.10	0.46	1.63	2.46	0.68
	250	5.19	0.35	0.48	0.37	0.17	0.79	2.79	5.34	1.29
	450	5.39	0.65	0.49	0.50	0.25	1.34	3.03	10.00	1.86
C. mucronata	100	6.36	0.66	1.03	0.47	0.29	0.77	3.05	13.18	1.88
	250	9.69	0.96	1.22	0.62	0.45	1.81	5.39	21.71	2.91
	450	7.44	1.09	1.16	0.62	0.52	2.28	4.69	18.41	3.43
C. ochroleuca	100	5.70	0.39	0.82	0.30	0.17	0.70	2.49	6.72	1.22
	250	6.76	0.62	0.93	0.33	0.24	0.95	2.89	8.30	1.61
	450	5.75	0.75	0.74	0.48	0.34	1.50	3.77	9.70	2.45
Significance										
[CO_2] (C)		NS	NS	NS	NS	NS	NS	NS	NS	NS
PPFD (P)		NS	**	NS	**	**	**	**	*	**
Species (S)		**	**	**	**	**	**	**	**	**
$LSD_{0.05}$		5.82	0.81	0.93	0.60	0.46	2.06	4.69	19.17	3.63

**, ** Significant at 0.05 and 0.01 levels of probability, respectively. NS = Not significant*

Table 7. The effect of [CO_2] and PPFD on nutrient transport (TR, pmol g shoot^{-1} sec^{-1}) in perennial tropical leguminous cover crops

Species	PPFD (µmol m^{-2} s^{-1})	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
400 µmol [CO_2] mol^{-1}										
C. spectabilis	100	4857	311.0	605.3	225.1	92.2	0.82	2.37	3.51	1.22
	250	6084	440.9	666.0	371.7	164.2	1.16	3.12	6.93	2.01
	450	5778	479.4	592.2	443.3	241.6	1.37	3.09	8.90	2.19
C. breviflora	100	3872	241.0	417.2	228.9	111.9	0.71	2.03	3.55	1.28
	250	5172	394.6	516.1	310.5	147.9	0.93	2.10	5.39	1.49
	450	5899	548.6	534.4	468.4	209.2	1.33	2.88	7.31	2.31
C. mucronata	100	6201	457.2	919.5	294.7	160.4	0.82	2.61	8.55	1.29
	250	7356	652.4	894.4	459.1	238.1	1.15	3.09	14.20	2.14
	450	4944	776.8	794.1	577.2	316.7	1.80	3.99	13.80	2.33
C. ochroleuca	100	5149	498.8	748.2	321.2	161.2	1.12	2.57	7.42	1.33
	250	6097	625.8	906.4	365.1	227.6	1.64	3.54	9.29	2.58
	450	6329	646.2	882.8	400.1	281.2	1.63	3.25	8.42	2.11
700 µmol [CO_2] mol^{-1}										
C. spectabilis	100	5176	292.4	602.9	275.8	121.9	0.69	1.89	5.00	1.38
	250	6074	378.3	665.1	297.8	168.4	0.94	2.04	5.20	1.73
	450	5802	388.3	704.1	286.1	195.3	0.89	1.62	5.04	1.58
C. breviflora	100		227.5	502.7	242.1	121.5	0.57	2.06	3.34	0.86
	250	4788	310.2	447.3	317.5	148.8	0.69	2.49	4.71	1.13
	450	4928	536.9	448.8	438.9	208.0	1.14	2.81	8.15	1.61
C. mucronata	100	5827	553.7	862.2	352.8	217.1	0.73	2.58	10.34	1.57
	250	6203	602.7	770.1	378.9	279.0	1.12	3.31	13.31	1.80
	450	5161	714.3	741.2	422.0	318.6	1.50	3.30	11.72	2.15
C. ochroleuca	100	5118	457.2	1014.7	348.9	196.8	0.80	2.94	8.08	1.40
	250	6474	578.9	877.5	312.7	229.6	0.99	3.07	7.72	1.55
	450	5355	811.6	760.3	494.5	355.6	1.53	3.74	10.24	2.52
Significance										
[CO_2] (C)		NS	NS	NS	NS	*	**	*	NS	**
PPFD (P)		**	**	NS	**	**	**	**	**	**
Species (S)		**	**	**	**	**	**	**	**	**
$LSD_{0.05}$		1776	220.2	241.4	188.1	110.9	0.69	1.39	4.86	1.21

*, ** *Significant at 0.05 and 0.01 levels of probability, respectively. NS = Not significant*

Species had significant effects on Transport of all nutrients (Table 7). *C. mucronata* and *C. ochroleuca* had the highest values, *C. breviflora* and *C. spectabilis* had the lower values. Although the crotalaria transport values are similar, they are consistently higher than Jack bean [20]. It has been reported that only the crop species had significant effects on TR of micronutrients but levels of PPFD had no significant effects on TR [17].

3.3.5 Nutrient use efficiency

The [CO_2] had significant effects on nutrient use efficiency of N, Ca, Cu, Fe, and Zn (Table 8). With exception of Mg use efficiency, increasing [CO_2] increased nutrient use efficiency of all the other nutrients. PPFD had significant effects on all nutrient use efficiencies except Fe. Increasing PPFD increased the use efficiency of N and K, and decreased the efficiency of P, Ca, Mg, Cu, Mn and Zn. Species had significant effects on nutrient use efficiencies of all the nutrients. NUE of *C. mucronata* was highest for N, Ca, Cu, and Zn, but lowest for Mn. NUE of *C. spectabilis* was highest for P, Ca, Mg, Fe, and Mn, but lowest for N and Zn. *C. breviflora* and *C. ochroleuca* fell in between. Intraspecific variations for macro- and micro-nutrient use efficiencies are well documented in legume cover crops [17,18,20,38,39]. Crotalaria were not as nutrient use efficient as Jack bean (18.4 mg mg^{-1} for *C. mucronata* vs 24.7 mg mg^{-1} for Jack bean) [20].

Table 8. The effect of [CO_2] and PPFD on nutrient use efficiency (NUE, mg shoot mg element in shoot^{-1}) in perennial tropical leguminous cover crops

Species	PPFD (µmol m^{-2} s^{-1})	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
400 µmol [CO_2] mol^{-1}										
C. spectabilis	100	13.53	96.4	38.99	104.9	405.2	1.78	0.70	0.48	1.17
	250	13.66	99.9	44.78	80.9	304.7	1.65	0.67	0.31	1.05
	450	15.00	81.7	52.25	69.6	206.5	1.42	0.71	0.25	0.84
C. breviflora	100	13.90	98.3	45.03	77.8	262.7	1.65	0.65	0.36	0.91
	250	14.19	84.1	50.63	81.0	281.9	1.74	0.86	0.34	1.05
	450	14.16	69.2	55.90	62.8	230.4	1.39	0.72	0.29	0.80
C. mucronata	100	13.62	98.2	34.25	103.2	313.6	2.35	0.85	0.30	1.56
	250	15.63	79.5	46.12	87.2	277.9	2.25	0.94	0.21	1.15
	450	24.38	68.3	52.79	71.1	213.6	1.45	0.76	0.22	1.07
C. ochroleuca	100	15.41	71.3	39.55	86.4	284.6	1.56	0.77	0.27	1.26
	250	15.71	69.2	37.97	91.3	242.5	1.37	0.68	0.27	0.87
	450	17.38	76.8	44.60	95.4	224.3	1.49	0.85	0.33	1.11
700 µmol [CO_2] mol^{-1}										
C. spectabilis	100	13.50	109.1	41.78	89.4	328.7	2.25	0.92	0.36	1.12
	250	13.92	100.9	45.50	101.1	287.8	1.99	1.04	0.42	1.05
	450	14.39	97.8	42.47	104.9	260.0	2.26	1.34	0.42	1.16
C. breviflora	100		111.8	39.32	81.2	262.4	2.22	0.68	0.42	1.39
	250	15.66	109.9	59.83	82.6	288.3	2.42	0.76	0.40	1.43
	450	16.82	71.4	65.92	65.9	232.1	1.69	0.74	0.27	1.11
C. mucronata	100	17.04	80.0	40.71	97.7	267.9	3.06	0.95	0.24	1.33
	250	18.16	82.9	51.56	103.8	231.7	2.19	0.86	0.22	1.33
	450	21.61	70.7	53.81	91.9	205.0	1.68	0.87	0.25	1.12
C. ochroleuca	100	17.85	84.2	30.13	84.9	256.1	2.42	0.72	0.27	1.32
	250	15.22	77.2	40.32	110.4	249.0	2.26	0.82	0.33	1.38
	450	21.13	62.5	52.47	79.9	180.2	1.61	0.75	0.28	0.95
Significance										
[CO_2] (C)		**	NS	NS	*	NS	**	**	NS	**
PPFD (P)		**	**	**	**	**	**	NS	**	**
Species (S)		**	**	**	**	**	**	**	**	*
$LSD_{0.05}$		5.62	41.1	12.77	41.02	111.5	1.03	0.39	0.15	0.67

*, ** *Significant at 0.05 and 0.01 levels of probability, respectively. NS = Not significant*

4. CONCLUSION

Intraspecific variations in perennial legume cover crops crotalaria for growth, physiological and macro-micro nutrient uptake parameters were observed at ambient and elevated levels of [CO_2] and low to medium levels of PPFD. It is possible to find crotalaria species that could be useful as cover crops in the early stages of plantation crop establishment, when the PPFD's at canopy level are adequate. The findings of this study could facilitate development of shademanagement systems to improve growth, nutrient use efficiency and extend persistence of understory legume cover crops in early plantation development.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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