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Ambient and Elevated Carbon Dioxide on Growth, Physiological and Nutrient Uptake Parameters of Perennial Leguminous Cover Crops under Low Light Intensities

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Authors' contributions

This work was carried out in collaboration between all authors. Authors VCB and ME conducted the experiment, performed statistical analysis and wrote the manuscript. Author ZLH conducted the nutrient analysis and reviewed the manuscript. Authors YL, AQP, DA, AAFA and NKF contributed in designing and monitoring the experiment and revising the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Adaptability and optimum growth of cover crops in plantation crops is affected by the inherent nature of the cover crop species and the light intensity at canopy levels. Globally concentrations of atmospheric CO_2 are increasing and this creates higher photosynthesis and nutrient demand by crops as long as the light intensity is adequate. An experiment was undertaken to assess effects of ambient (400 µmol mol^{-1}) and elevated (700 µmol mol^{-1}) levels of [CO_2] on the growth and physiological parameters and nutrient use efficiency in five selected tropical perennial legume cover crops (Calopo/frisolla, Jack bean, Brazilian lucerne, Leucaena, and Mucuna) under low levels of photosynthetic photon flux density (PPFD; 100, 250, and 450 µmol m^{-2} s^{-1}). Overall, total dry biomass, root dry biomass, root/shoot ratio, and stem height were significantly influenced by levels of [CO_2] and PPFD and cover crop species. With some exceptions, these growth parameters showed significant interactions between cover crop species x [CO_2] and cover crop species x 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). With few exceptions, overall macro-micronutrient concentrations were significantly influenced by levels of [CO_2] and PPFD and species. Macro-micronutrient uptake levels were significantly influenced by cover crop species; however with few exceptions, levels of PPFD also had significant effects on uptake of all nutrients. Across crop species, increasing [CO_2] and PPFD increased uptake of all nutrients and this was a reflection of higher shoot dry matter accumulations at the higher levels of [CO_2] and PPFD. Nutrient influx (IN) of all the nutrients was significantly influenced by crop species. However, with few exceptions levels of [CO_2] and PPFD and their interactions had no effects on IN of nutrients. Cover crop species and levels of [CO_2] and PPFD and the interaction of PPFD x species had significant effects on nutrient transport (TR). Macro-micronutrient use efficiency was significantly influenced by levels of [CO_2], PPFD and crop species. Brazilian lucerne and Jack bean were efficient in nutrient use efficiency of N, K, Mg, Cu, Fe, and Mn; while Calopo and Leucaena were efficient in Zn use efficiency and Leucaena was efficient in P use efficiency.

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

1. INTRODUCTION

In the tropics, plantation crops such as cacao, coffee, tea and banana are invariably established with wide spacing on recently cleared sloppy land. Loss of vegetative cover causes massive soil degradation due to soil erosion and leaching of nutrients. Establishment of quick growing cover crops before planting and during early stages of establishment of these plantation crops prevents soil erosion, leaching of nutrients, and weed infestation and further improves soil fertility. Cover crops grown in the inter-row of plantation crops have controlled soil erosion, improved organic matter content, improved nutrient status, enhanced soil physical properties and reduced weed infestations [1,2,3,4,5,6]. Cover crop residues incorporated into the soil improve soil organic matter; this in turn improves the fertility of soil as well as its physical, chemical and biological properties [7]. In addition, soil organic matter improves the storage of carbon thereby helping to reduce the accumulation of atmospheric carbon dioxide [8].

Growth and development of cover crops are to certain degree genetically determined but are influenced by environmental variables such as rainfall, light quality and intensity, temperature, atmospheric [CO_2] and soil fertility [9,10]. Globally, the atmospheric carbon dioxide concentration [CO_2] is expected to double by end of this century from the current level of 400 µmol mol^{-1} [11,12]. Increased litter decomposition in plantation crops also contributes to higher [CO_2] at the ground level. Elevated atmospheric [CO_2] has contributed to increased biomass and net photosynthesis of plants under adequate light, nutrients and water [13,14,15,16]. As the tree crops mature, understory cover crops suffer from inadequate levels of photosynthetic photon flux density (PPFD) at their canopy level for their growth and development. In tropical regions, incoming PPFD is around 1800 µmol m^{-2} s^{-1} [17], but understory plants in rainforests, where tree density is very high, receive only 4-10% of the incoming photosynthetically active radiation (PAR) [18,19]. In agroforestry based plantation crops, cover crops receive full sunlight during early stages of plantation crops but as the plantation trees grow incoming PPFD reaching the cover crop canopy is reduced. Low PPFD reduces growth, development and nutrition of cover crops [15,20,21]. The ability of cover crops

to survive in plantation crops depends largely on the amount and quality of light reaching their canopies [9,10]. Many cover crops are very sensitive to low light intensity and in many instances will not survive longer than few years because they are suppressed by reduced light quality (intensity) due to increased canopy of plantation crops and companion shade trees [5,22,23]. Various degrees of shade tolerance among tropical forage legumes have been reported [22,23,24]. Cover crops that tolerate reduced PPFD have greater potential to survive longer, reduce soil degradation, improve soil C sequestration, and control weed infestations in plantation crops. Baligar et al. [15] evaluated independent short term effects of PPFD and [CO_2] on photosynthesis of perennial peanut (*Arachis pintoi*), calopo (*Calopogonium mucunoides*), jack bean (*Canavalia ensiformis*), leucaena (*Leucaena leucocephala*) and mucuna (*Mucuna pruriens*). In all these legume species, reducing PPFD from 1000 to 50 µmol m^{-2} s^{-1} reduced net photosynthesis (Pn) to less than 10% of the higher light level. Increasing external [CO_2] from 250 to 700 µmol mol^{-1} doubled Pn. In four Crotalaria species (*C. breviflora*, *C. mucronata*, *C. ochroleuca*, *C. spectabilis*) Baligar et al. [16] reported that increasing PPFD from 50 to 1500 µmol m^{-2} s^{-1} increased Pn by 21-fold, and increasing the external [CO_2] from 100 to 1000 µmol mol^{-1} increased Pn by 4.7 fold. Inter-specific differences in cover crops have been reported for shade tolerance [25,26,27,28], varying light intensities [15,16,20,21], and soil acidity tolerance [29,30]. Macro and micro nutrient use efficiency under various soil acidity and light intensities (shade levels) have been reported [21,24,31,32,33,34,35]. Information concerning the impact of low PPFD levels under increasing concentration of [CO_2] on growth and nutrition of tropical cover crops is lacking. 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 and physiological and nutrient uptake parameters of perennial leguminous cover crops.

2. MATERIALS AND METHODS

2.1 Perennial Legume Cover Crops

Five perennial legume cover crops selected for this study were: Calopo/frisolla (*Calopogonium mucunoides*), Jack bean (*Canavalia ensiformis*), Brazilian lucerne/Brazilian Stylo (*Stylosanthes guianensis*), Leucaena (*Leucaena leucocephala*), and Mucuna (*Mucuna pruriens*).

Cover crops used in this study are known to have unique characteristics that may be useful for halting soil degradation and improving soil fertility (Table 1). Jack bean is a climbing perennial herb, native from Mexico to Brazil. When used as a cover crop, it produces 1-6 t ha^{-1} yr^{-1} of DM and fixes 35-55 kg $ha^{-1}yr^{-1}$ of N. It is tolerant of shade and drought [36]. Calopo, a vigorous, twining perennial herb, native to tropical America, can produce 4-6 t ha^{-1} yr^{-1} of DM, fix 250-450 kg $ha^{-1}yr^{-1}$ of nitrogen, and is used mainly as a cover crop in tropical tree plantations [37]. Leucaena is a small perennial tree, native to the Yucatan Peninsula in Mexico. If used as a cover crop, it can produce 1-15 t ha^{-1} yr^{-1} of DM and fix 500 kg $ha^{-1}yr^{-1}$ of N. It is very tolerant of shade and drought [38]. Brazilian lucerne/Brazilian Stylo is an erect perennial herb, native to Central and South America. It is used as a cover crop and produces 5-20 t ha^{-1} yr^{-1} of DM and fixes 35-165 kg $ha^{-1}yr^{-1}$ of N. It is tolerant of soil acidity (Al, Mn) and low fertility and low P soils [39]. Mucuna is a vigorous, twining herb, native to southern China, which is used as a cover crop and can produce 2-12 t ha^{-1} yr^{-1} of DM and fix 50-330 kg $ha^{-1}yr^{-1}$ of N. It is easy to establish but lacks drought tolerance [40]. Growth habit and strengths and weaknesses of these cover crops are listed in Table 1.

2.2 Growth Medium and Planting

Growth medium was prepared by mixing Perlite: Sand: Peat moss (2:2:1 volume basis) in cement mixer along with required macro- and micronutrients to provide supplemental nutrients (mg kg^{-1}) 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. Nutrients were applied as Osmocote 18-6-12 (The Scotts Company, Marysville, Ohio), triple superphosphate, urea, calcium sulfate, dolomitic lime and Scott's Micromix. Ten seeds of each legume species were planted in each one-gallon black plastic pot possessing adequate bottom drainage and containing 2 kg of growth medium. Water was applied as needed to maintain soil moisture at field capacity (-33 kPa) throughout the growth cycle. One container without any plants was placed in each of the mini chambers to monitor the evaporative water loss.

2.3 Growth Conditions

Plants were grown in two glasshouses (18 m^2 each) with day/night temperatures of 30/28°C. One glasshouse contained ambient levels of

$[CO_2]$ (400 µmol mol^{-1}) and the second contained elevated concentration of $[CO_2]$ (700 µmol mol^{-1}) measured by WMA2 infrared analyzers (PP Systems, Haverhill, MA). When the $[CO_2]$ fell below 700 µmol mol^{-1}, CO_2 was injected to the desired levels. Plants were grown at three levels of PPFD: 100, 250 or 450 ± 50 µmol m^{-2} s^{-1}. Mini-chambers were constructed in each of the glasshouses of PVC pipe covered with one or two layers of plastic shade cloth to achieve the desired PPFD levels. The mini-chambers measured 112 cm W x 120 cm L x 81 cm H.

2.4 Evaluation of Plant Growth Parameters

After 14 days, plants were thinned (Calopo 10, Jack bean 3, Brazilian Lucerne 6, Mucuna 3 plants/pot). Removed plants were used as an initial harvest.

After an additional 36 days of growth, stem height and SPAD index were recorded. A SPAD meter (KonicaMinolta Chlorophyll Meter, Model 502, Ramsey, NJ, USA) was used as a non-destructive method to estimate the chlorophyll content of the leaves.

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 and freeze-dried and the shoot dry biomass (SDB) was recorded. The roots were removed from the soil, washed, blotted dry and weighed. Total root lengths were determined with a Comair Root Length Scanner (Hawker de Haviland, Melbourne, Victoria, Australia). Roots were oven dried at 70°C for 5 days until constant mass and the dry root biomass (RDB) was recorded.

Table 1. Common names, scientific names, growth habits, and strengths and limitations of cover crops used[1,2]

Common name	Scientific name	Growth habit[3]	Strength	Limitation
Calopo / Wild Ground nut / Frisolla	*Calopogonium mucunoides Desv.*	N/C/ H	Tolerant to soil acidity (Al), drought, water logging and moderate shade. Widely adaptable, erosion control	Weed potential, susceptible to root-knot nematode and cow pea virus, poor tolerance to heavy shade
Jack bean	*Canavalia ensiformis*	N/ H	Tolerant of drought, water logging and shade. Good erosion control. Good green manure.	Susceptible to many fungi and pests
Brazilian lucerne / Brazilian stylo	*Stylosanthes guianensis*	N/S	Adapt to acid infertile soils. Tolerant to drought. High N fixer. Good green manure, hay and pasture	Requires specific Rhizobium, seed shatter on ripening. Susceptible to Anthracnose.
Leucaena	*Leucaena leucocephala*	N/S	Tolerant of drought, multiple uses, highly productive	Poor in acid, infertile soils, susceptible to frost, weak seedling growth
Mucuna / Buffalo bean / Velvet bean	*Mucuna pruriens*	C/ H/S	Improves soil fertility. Resistance to pest and diseases Good green manure crop	Limited drought and shade tolerance, Needs non acidic and fertile soils.

[1]Cover crop seeds of Mucuna were obtained from: Sementes Pirai of Piracicaba, SP, Brazil and Calopo, Jack bean, Brazilian lucerne and Leucaena were obtained from Sementes Globo Rural Ltd, Goania, Go Brazil
[2]References: [10,36,37,38,39,40,41,42]
[3]N =Non Climbing, C= Climbing, H =Herb, S =Shrub

2.4.1 Specific Leaf Area (SLA), Relative Growth Rate (RGR) and Net Assimilation Rates (NAR) were calculated using the following formulas [21]

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 (14 days) and 2 (36 days) refer to initial and final harvest.

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

2.4.2 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 X π)1/2 where RFW is root fresh biomass (cm^3).

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

2.4.3 Nutrient uptake parameters: Nutrient uptake (U), influx (IN), transport (TR) and Nutrient Use Efficiency (NUE) are determined as follows: [21]

Dried stems and leaves were ground to pass through a 1-mm sieve and sent to University of Florida, Indian River Research and Education Center (UF-IRREC) for elemental analysis. Concentrations of elements in plant were determined by digesting 0.4 g plant samples in 5 mL of concentrated nitric acid (14 N), and concentrations of elements in the digested solution were determined using inductively coupled plasma optical emission spectrometry (ICPOES, Ultima JY Horiba Inc. Edison, NJ, USA) following USEPA method 200.7 [43]. Total N in plant tissue was analyzed by combustion method using CN Analyzer (Vario MAX CN Macro Analyzer, Elementar Analysensysteme GmbH, Hanau, Germany) [44].

Uptake (U) = Conc. of any given element (mg or µg) X Shoot Dry Biomass (g $plant^{-1}$)

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

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

2.5 Data Analysis

A split plot design was used with CO_2 concentrations as main plots, PPFD as subplots and cover crops as sub sub plots. Treatments were replicated three times. All data were analyzed using general linear model (GLM) procedures of SAS (Ver. 9.2, SAS Institute, Cary, NC).

3. RESULTS AND DISCUSSION

3.1 Shoot, Root and Leaf Parameters

Overall, total dry biomass, root dry biomass, root/shoot ratio, and stem height were significantly influenced by [CO_2] and PPFD and crop species (Table 2). With some exceptions, these growth parameters also showed significant interactions between cover crop species x [CO_2] and cover crop species x PPFD. Total root length was only significantly influenced by species. Baligar et al. [21] also reported similar interactions between cover crop species and growth parameters such as shoot and root weight, leaf area, specific leaf area, leaf mass/unit leaf area and levels of PPFD. Such significant interactions indicate that various cover crop species respond differently to levels of PPFD. Shading is known to reduce yields of most tropical legumes [45,46]. In Calopo, shading increased the shoot/root ratio [47].

Overall, these growth parameters (shoot and root biomass, root/shoot ratio, stem height, root length), and total leaf area (Table 2) in all crop species increased, but specific leaf area (SLA) decreased with increasing [CO_2] from ambient (400 µmol mol^{-1}) to elevated (700 µmol mol^{-1}) and increasing PPFD from 100 to 450 µmol m^{-2} s^{-1}. Increasing biomass due to elevated [CO_2]

has been recorded for C3 plants [48,49]. Doubling of atmospheric [CO_2] has been shown to increase plant biomass by almost 40% [49], however growth response in different plant species to increasing [CO_2] is not consistent. Leaf area per plant tends to increase with high CO_2 [13]. Overall, maximum total dry biomass (shoot + root), root biomass, root length and leaf area were produced by Jack bean and Mucuna. With higher leaf area, these crops might have higher photosynthetic rates than the other cover crop species tested thereby resulting in higher dry matter accumulations. Moss [50] reported that plants with a larger leaf area have greater potential for growth than those with smaller leaf area. Calopo and Brazilian Lucerne had smaller leaf areas and produced the least amount of total and root dry biomass and root length.

Table 2. The effect of [CO_2] and PPFD on shoot, root and leaf growth of perennial tropical 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}								
Calopo	100	0.29	0.02	0.07	27	348	82.6	433.3
	250	0.74	0.06	0.08	40	612	202.1	422.1
	450	1.24	0.11	0.09	47	847	287.8	329.9
Jack Bean	100	4.52	0.23	0.05	98	1532	909.2	317.3
	250	5.84	0.48	0.09	108	2155	915.7	281.8
	450	6.69	0.59	0.10	120	2677	1039.7	259.1
B. Lucerne	100	0.19	0.01	0.04	16	64	34.6	314.1
	250	0.22	0.01	0.07	13	103	38.1	247.9
	450	1.58	0.11	0.07	25	697	209.5	220.8
Leucaena	100	0.91	0.11	0.14	14	836	282.8	481.3
	250	1.38	0.21	0.18	21	1003	339.1	394.7
	450	1.60	0.30	0.23	24	934	294.6	312.7
Mucuna	100	4.25	0.27	0.07	125	2282	1373.3	526.3
	250	4.01	0.27	0.08	101	2415	1277.1	490.9
	450	3.12	0.36	0.13	102	2018	811.4	436.0
700 µmol CO_2 mol^{-1}								
Calopo	100	0.55	0.03	0.06	44	554	165.82	457.2
	250	1.00	0.06	0.07	58	709	234.51	382.4
	450	1.31	0.10	0.09	52	847	238.89	316.3
Jack Bean	100	7.78	0.53	0.07	126	2178	1554.81	386.0
	250	10.58	0.76	0.08	123	2188	1728.44	327.5
	450	6.40	0.82	0.17	137	2240	604.75	210.9
B. Lucerne	100	0.33	0.02	0.08	25	120	51.31	281.4
	250	1.14	0.07	0.06	29	518	157.05	238.9
	450	1.83	0.11	0.06	26	889	216.59	206.0
Leucaena	100	1.12	0.18	0.19	37	823	309.07	510.0
	250	2.19	0.48	0.28	51	987	393.20	395.1
	450	2.07	0.51	0.33	34	749	292.31	285.9
Mucuna	100	3.79	0.18	0.05	110	2448	1224.68	558.4
	250	4.23	0.26	0.07	108	2138	1135.08	432.4
	450	4.38	0.32	0.08	124	2456	1000.23	364.4
Significance								
CO_2 (C)		**	**	**	**	NS	NS	NS
PPFD (P)		*	**	**	*	NS	*	**
Species (S)		**	**	**	**	**	**	**
C x P		NS	NS	NS	NS	NS	NS	**
C x S		**	**	**	**	NS	NS	NS
P x S		NS	**	**	**	NS	**	**
C x P x S		NS	NS	NS	**	NS	**	NS
$LSD_{0.05}$		3.51	0.22	0.08	25.6	1693	705.3	98.1

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

Baligar et al. [20] reported that cover crops such as Sunn hemp, Cowpea and Lab-lab with larger leaf areas accumulated higher dry biomass in shoots and roots than cover crops with smaller leaf areas such as joint vetch, hairy indigo and crotalaria. Jack bean and Mucuna had the highest stem height and such growth tendency might help these plants to produce higher photosynthesis due to reduced mutual shading of leaves. Brown [51] reported that greater height benefits the plant by having the most efficient leaves in the most favorable position for increased photosynthesis. Irrespective of [CO_2] and PPFD, Jack bean and Mucuna recorded the longest root lengths. Such a root system might help the plant to absorb more water and nutrients by exploring a larger soil volume, and thus these cover crops could be suitable for infertile soils of tropical plantation crops. In all the crops, total dry biomass increased with increasing root dry biomass and root length. Fageria et al. [34] reported that in tropical legumes root dry biomass was a better indicator in determining shoot dry biomass than maximum root length. Baligar et al. [15,16] reported that increasing PPFD from 50 to 1000 and even up to 1500 µmol m^{-2} s^{-1} and increasing [CO_2] from 50 to 700 and up to 1000 µmol mol^{-1} increased net photosynthesis (Pn) in many perennial cover crops. Such increased photosynthesis at high PPFD and elevated [CO_2] might have contributed to improved cover crops growth parameters in the current study. Cover crop species that tolerate lower PPFD have a better chance of growing and persisting for a longer period of time as understory plants in an agroforestry based plantation system.

3.2 Physiological Parameters: Relative Growth Rate (RGR), Net Assimilation Rate (NAR), Rate of Water Flux (VO), Water Use Efficiency (WUE) and SPAD

Overall, RGR, NAR, and VO were significantly influenced by species, [CO_2] and PPFD and the interaction of [CO_2] x species (Table 3). The RGR and NAR were significantly influenced by interactions of [CO_2] x PPFD and [CO_2] x cover crop species. In all the cover crops, increasing [CO_2] and PPFD increased RGR, NAR, WUE and SPAD, and decreased VO. Increases in these physiological parameters is a reflection of the higher shoot dry matter accumulation of all cover crops with increasing [CO_2] and PPFD. The reduction in VO with increasing [CO_2] and PPFD is due to increased root length in all the species. Irrespective of [CO_2] and PPFD among cover crops, Jack bean and Mucuna were the most efficient in VO and WUE and this is reflected in their higher shoot dry matter accumulations. The physiological traits of these plants appear to be good indicators for selection of suitable cover crops that could acclimatize well to increasing [CO_2] and PPFD. Increasing [CO_2] increases C fixation by increasing water use efficiency [52]. High [CO_2] leads to increased Pn, plant growth and water use efficiency in C3 plant species [48,49]. Published information on the physiological response of these perennial legume cover crops to changing PPFD and [CO_2] is unavailable.

3.3 Macro-micro Nutrient Uptake Parameters

3.3.1 Nutrient concentrations

In all these cover crops, concentrations of N, P and K were slightly higher than the reported concentrations and all other remaining essential nutrients were at adequate levels (Table 4) [20,21,53,54].

With few exceptions, macro-micronutrient concentrations were significantly influenced of [CO_2] and PPFD and cover crop species. With exceptions of the concentrations of Cu and Mn, cover crop species x PPFD interactions significantly influenced nutrient concentrations. Overall the interaction effects of [CO_2] x PPFD or [CO_2] x cover crop species on concentrations of nutrients were not significant. Irrespective of PPFD in all cover crops, increasing [CO_2] slightly decreased all the nutrient concentrations; however irrespective of [CO_2], increasing PPFD only slightly decreased concentrations of K, Ca and Fe. In earlier research Baligar et al. [20] reported that crop species and PPFD had significant effects on micronutrient concentrations with the exception of Cu concentrations. Further they reported that increasing PPFD from 200 to 400 µmol m^{-2} s^{-1} decreased the concentrations of most of the micronutrients and they attributed this to increased dry matter at the slightly higher PPFD which caused dilution effects. Overall concentrations were in the order of N > K > Ca = P > Mg for macronutrients and Mn > Fe > Zn > Cu for micronutrients. Baligar et al. [20] reported a similar pattern of nutrient concentrations in other tropical cover crop species.

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}			
Calopo	100	33.7	51.1	1.83	6.8	2.37
	250	33.3	52.1	2.25	9.0	3.27
	450	33.1	54.8	3.10	10.4	4.48
Jack Bean	100	36.0	714.2	3.37	3.8	1.88
	250	41.6	600.4	3.53	4.4	2.85
	450	41.1	577.9	3.80	4.8	3.12
B. Lucerne	100	31.5	58.6	1.17	7.6	3.97
	250	35.9	30.1	0.98	8.2	4.77
	450	42.9	21.4	2.68	12.3	9.16
Leucaena	100	44.9	38.2	2.38	8.9	2.85
	250	57.5	32.2	2.91	10.0	4.05
	450	56.1	28.8	3.13	10.3	5.61
Mucuna	100	33.8	352.4	2.93	5.4	1.67
	250	30.4	356.6	2.96	5.2	1.65
	450	26.8	319.1	2.22	4.6	1.79
			700 µmol CO_2 mol^{-1}			
Calopo	100	34.8	49.8	2.60	8.4	2.81
	250	33.6	44.4	3.49	9.9	4.19
	450	30.3	49.4	3.56	10.5	5.82
Jack Bean	100	49.7	525.5	4.99	5.2	2.59
	250	44.5	449.1	6.02	5.8	3.55
	450	38.3	346.5	4.62	4.5	5.95
B. Lucerne	100	33.4	28.8	2.71	9.1	5.72
	250	38.3	20.6	3.68	11.6	8.37
	450	45.4	13.4	5.18	12.7	10.65
Leucaena	100	53.9	29.9	2.95	9.5	3.47
	250	60.0	31.2	3.51	11.2	6.23
	450	49.5	23.0	3.96	10.9	7.74
Mucuna	100	30.9	340.3	3.49	5.0	1.56
	250	26.1	328.1	3.47	5.4	2.03
	450	18.6	250.4	4.36	5.5	2.40
Significance						
CO_2 (C)		NS	**	**	**	**
PPFD (P)		NS	**	**	**	**
Species (S)		**	**	**	**	**
C x P		NS	NS	NS	**	*
C x S		**	**	*	*	**
P x S		**	*	*	**	**
C x P x S		NS	NS	NS	*	NS
$LSD_{0.05}$		11.5	200.0	2.48	2.07	2.17

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

3.3.2 Nutrient uptake

Macro- and micro-nutrient uptakes were significantly influenced by cover crop species however with few exceptions, levels of PPFD also had significant effects on uptake of all nutrients (Table 5). 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 [21,33]. Highly significant effects of increasing PPFD from 200 to 400 µmol m^{-2} s^{-1} and PPFD x crop species interactions on uptake of macro-micronutrients have been reported by Baligar et al. [20]. Across

the crop species, increasing [CO_2] and PPFD increased uptake of all nutrients and this reflects higher shoot dry matter accumulations. Wong [24] reported changes in mineral composition of Joint Vetch, Calopo, Centro, Ea-Ea, Tropical Kudzu and Brazilian Lucerne grown in varying levels of light (18 to 100% of daylight) in greenhouse conditions. In all the legumes the mean P, Mg, Cu, Fe and Zn content increased significantly with increasing PPFD. Accumulation of nutrients was in the order of N > K > P > Ca > Mg for macro nutrients and Mn > Fe > Zn >Cu for micronutrients. Similar trends in higher Mn and Fe uptake in other perennial legume cover crops have been reported [20,21].

Table 4. The effect of [CO_2] and PPFD on nutrient concentration in perennial tropical 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}										
Calopo	100	57.52	7.54	23.56	9.49	2.47	34.81	117.7	250.5	64.16
	250	55.56	8.84	24.25	11.05	2.50	36.90	97.7	303.0	63.02
	450	53.92	8.72	21.40	8.48	2.68	43.46	119.4	266.2	55.71
Jack Bean	100	56.64	7.60	25.31	11.93	1.71	21.46	90.1	224.9	58.36
	250	47.44	7.55	15.44	9.45	2.37	27.90	79.1	204.3	59.27
	450	41.56	11.68	17.03	13.99	4.13	32.77	123.4	360.8	78.13
B. Lucerne	100		6.21	19.34	10.21	2.06	30.26	102.3	127.0	64.79
	250	56.03	11.77	22.17	17.04	3.56	46.40	148.4	265.7	92.95
	450	50.13	8.97	21.11	12.94	2.56	39.56	95.0	207.9	94.56
Leucaena	100	62.48	8.08	26.10	8.58	2.83	49.22	112.9	245.3	65.06
	250	63.69	7.18	25.77	7.82	3.01	48.12	100.4	236.6	54.16
	450	61.55	7.68	24.09	6.85	3.30	56.56	100.5	247.9	60.55
Mucuna	100	50.25	14.36	21.98	13.57	2.93	65.83	131.9	321.0	91.29
	250	56.83	15.62	20.38	12.68	2.99	65.60	122.9	428.1	90.95
	450	54.44	16.07	23.39	9.29	3.17	74.03	106.1	309.8	81.86
700 µmol CO_2 mol^{-1}										
Calopo	100	50.15	6.97	24.02	11.50	2.47	26.90	85.6	275.1	45.13
	250	52.66	7.25	21.31	6.89	2.06	29.56	71.0	243.1	45.05
	450	41.39	9.42	19.57	7.51	2.63	35.3	65.1	218.2	50.47
Jack Bean	100	44.70	8.20	15.76	11.92	2.69	17.66	56.4	238.9	57.83
	250	43.49	8.22	13.33	7.74	2.67	19.38	59.4	200.8	53.73
	450	27.44	9.26	12.45	8.15	3.14	26.35	58.4	176.7	64.99
B. Lucerne	100	43.68	5.65	19.70	13.32	2.23	31.87	84.3	171.9	69.57
	250	45.11	8.34	20.71	12.96	2.51	29.77	83.8	206.5	82.15
	450	47.07	9.05	19.62	9.80	2.83	36.65	61.7	208.7	72.40
Leucaena	100	54.98	5.96	26.40	8.79	2.93	31.69	92.4	221.2	39.81
	250	51.28	6.46	20.80	6.05	3.76	36.86	86.5	255.1	47.42
	450	51.16	9.25	19.89	7.27	4.07	51.76	95.2	299.8	63.86
Mucuna	100	42.68	12.06	17.83	9.85	2.93	44.01	94.2	258.7	75.24
	250	48.43	14.08	17.57	8.87	2.82	47.70	109.3	289.9	79.37
	450		16.64	21.30	10.57	2.64	59.73	108.6	296.4	73.03
Significance										
CO_2 (C)		**	*	**	**	NS	**	**	*	**
PPFD (P)		**	**	**	*	**	**	NS	NS	NS
Species (S)		**	**	**	**	**	**	**	**	**
C x P		NS	NS	NS	**	NS	NS	NS	NS	NS
C x S		NS	NS	NS	NS	NS	NS	NS	NS	NS
P x S		**	*	**	**	**	NS	*	NS	*
C x P x S		NS	NS	NS	**	*	NS	NS	*	NS
$LSD_{0.05}$		15.30	4.96	8.28	6.04	1.69	26.00	60.83	178.6	36.88

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

Table 5. The effect of [CO_2] and PPFD on nutrient uptake in perennial tropical 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}										
Calopo	100	15.41	2.02	6.3	2.54	0.66	9.3	31.5	67.0	17.2
	250	37.26	6.17	16.5	7.67	1.70	23.0	65.9	204.3	43.7
	450	62.06	9.78	24.3	9.47	3.02	48.4	134.6	300.9	63.2
Jack Bean	100	243.94	32.55	109.4	50.92	7.30	92.3	385.2	964.9	249.6
	250	261.45	42.84	88.4	53.66	13.52	145.8	421.4	1171.9	330.0
	450	257.49	72.00	105.0	84.81	25.06	199.5	758.1	2174.4	479.4
B. Lucerne	100		1.05	3.6	1.95	0.36	5.3	16.5	23.2	12.3
	250	14.40	2.22	4.4	3.24	0.64	8.2	27.9	46.1	17.1
	450	73.93	12.74	31.1	18.76	3.67	56.8	135.3	304.4	137.1
Leucaena	100	49.66	6.48	21.1	6.90	2.29	40.9	89.4	202.1	51.1
	250	74.33	8.23	30.1	9.05	3.54	56.4	117.6	274.8	63.8
	450	78.58	10.01	30.9	8.97	4.35	72.4	131.9	322.4	79.5
Mucuna	100	201.41	58.22	86.6	54.29	11.67	261.0	513.1	1296.4	359.5
	250	217.72	58.25	75.9	46.71	10.92	243.6	458.9	1594.9	331.1
	450	152.12	44.88	64.4	24.83	8.75	205.6	291.3	855.9	226.0
700 µmol CO_2 mol^{-1}										
Calopo	100	26.20	3.64	12.5	5.99	1.29	14.0	44.5	143.2	23.5
	250	49.00	6.78	20.0	6.54	1.94	28.0	66.1	227.8	42.2
	450	50.46	11.38	23.9	9.28	3.18	43.4	80.8	276.7	62.0
Jack Bean	100	331.15	60.14	116.1	86.29	19.50	127.3	415.7	1752.9	421.0
	250	448.75	78.75	134.6	72.46	24.98	184.5	556.5	1847.3	522.9
	450	159.71	54.04	70.6	47.53	18.26	142.1	372.9	1104.7	359.8
B. Lucerne	100	9.66	1.68	5.8	3.94	0.67	9.6	25.8	51.4	20.9
	250	49.11	8.71	22.1	13.64	2.74	33.4	87.4	231.8	90.8
	450	80.70	15.69	33.6	16.91	4.90	63.4	106.5	366.5	125.8
Leucaena	100	51.80	5.59	24.8	8.22	2.76	30.0	86.5	208.4	37.4
	250	87.56	11.06	35.5	10.35	6.44	63.2	148.7	438.0	81.4
	450	81.13	14.33	31.4	11.43	6.31	80.1	149.9	461.1	97.7
Mucuna	100	158.93	42.63	64.7	34.57	10.40	152.1	331.2	954.0	268.4
	250	191.71	55.81	69.7	35.17	11.21	189.2	431.9	1147.1	315.6
	450		68.61	87.2	42.93	10.69	241.8	439.7	1214.5	299.9
Significance										
CO_2 (C)		NS	NS	NS	NS	**	NS	NS	NS	NS
PPFD (P)		NS	*	NS	NS	**	**	*	NS	*
Species (S)		**	**	**	**	**	**	**	**	**
C x P		NS	NS	NS	NS	NS	NS	NS	NS	NS
C x S		NS	NS	NS	NS	NS	NS	NS	NS	NS
P x S		NS	NS	NS	NS	NS	NS	NS	NS	NS
C x P x S		NS	*	NS	**	**	**	**	**	*
$LSD_{0.05}$		222.55	41.77	73.4	35.21	10.24	101.2	328.2	1008.0	229.9

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

3.3.3 Nutrient influx (IN) and transport (TR)

Nutrient influx (IN) of all the nutrients was significantly influenced by cover crop species (Table 6), however, with few exceptions, [CO_2] and PPFD and their interactions, had no significant effects on IN of nutrients, but overall increasing [CO_2] and PPFD increased IN for all the nutrients.

With few exceptions, cover crop species, [CO_2] and PPFD and interactions of PPFD x cover crop species had significant effects on transport (TR) of all the nutrients (Table 7). Earlier, Baligar et al. [21] reported that TR of macronutrients was significantly influenced by cover crop species and increasing PPFD from 200 to 400 µmol m^{-2} s^{-1} and their interactions. However another study by Baligar et al. [20] reported that only the crop species had significant effects on TR of

micronutrients but levels of PPFD had no significant effects on TR of micronutrients.

In this present study, irrespective of [CO_2], increasing levels of PPFD increased the TR for all the nutrients; but irrespective of PPFD, increasing [CO_2] only increased TR for P and Mg. TR for micronutrients was influenced minimally by [CO_2] or PPFD.

3.3.4 Nutrient use efficiency

With few exceptions, macro-micro nutrient use efficiency was significantly influenced by [CO_2] and PPFD and crop species (Table 8). With the exception of Ca use efficiency, interactions of PPFD x cover crop species had no significant effects on all other macronutrient use efficiencies. Irrespective of levels of PPFD,

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}										
Calopo	100	3.28	0.20	0.49	0.20	0.07	0.42	1.58	3.38	0.78
	250	4.47	0.30	0.68	0.28	0.12	0.77	2.00	6.07	0.97
	450	8.09	0.55	1.09	0.41	0.22	1.30	4.27	9.88	1.70
Jack Bean	100	7.29	0.48	1.20	0.58	0.10	0.65	2.67	6.25	1.75
	250	8.14	0.72	1.14	0.69	0.27	0.92	2.85	10.02	2.46
	450	4.74	0.72	0.77	0.73	0.33	1.00	4.01	13.03	2.25
B. Lucerne	100		0.41	1.06	0.56	0.17	0.99	3.63	4.75	2.15
	250	9.03	0.56	1.03	0.65	0.15	0.69	3.80	3.71	1.75
	450	8.22	0.71	1.23	0.76	0.26	1.56	4.26	8.65	3.44
Leucaena	100	6.35	0.37	1.08	0.34	0.19	1.28	2.89	7.47	1.45
	250	7.37	0.38	1.05	0.32	0.20	1.17	2.92	6.94	1.43
	450	8.48	0.49	1.19	0.33	0.25	1.67	3.47	8.55	1.62
Mucuna	100	6.77	1.05	1.02	0.68	0.23	2.10	3.70	13.03	2.53
	250	7.72	0.92	0.92	0.55	0.20	1.94	3.82	14.28	2.38
	450	4.55	0.66	0.74	0.30	0.15	1.46	2.39	6.99	1.52
700 µmol CO_2 mol^{-1}										
Calopo	100	4.06	0.26	0.68	0.31	0.11	0.48	1.57	5.33	0.76
	250	5.87	0.36	0.81	0.25	0.12	0.68	1.99	6.74	1.04
	450	6.14	0.60	1.02	0.42	0.22	1.09	2.35	9.60	1.56
Jack Bean	100	8.84	0.78	1.13	0.76	0.29	0.82	2.35	10.68	2.33
	250	7.51	0.78	0.89	0.59	0.31	0.88	2.89	10.33	2.36
	450	5.11	0.90	0.86	0.60	0.36	1.06	3.43	9.83	2.64
B. Lucerne	100		0.48	1.24	0.83	0.24	1.30	4.18	8.12	2.84
	250	8.61	0.79	1.46	0.91	0.28	1.12	4.14	9.44	3.36
	450	8.10	0.68	1.22	0.57	0.27	1.37	2.64	8.30	2.54
Leucaena	100	5.99	0.31	1.08	0.19	0.31	0.73	2.69	6.23	0.96
	250	9.52	0.55	1.38	0.40	0.55	1.62	4.23	12.42	1.83
	450	8.17	0.71	1.17	0.39	0.71	2.00	3.96	13.46	2.43
Mucuna	100	5.73	0.70	0.85	0.20	0.70	1.17	2.76	8.96	2.03
	250	5.90	0.82	0.93	0.18	0.82	1.33	3.52	9.45	1.97
	450		1.11	0.74	0.19	1.11	1.85	3.34	10.20	2.20
Significance										
CO_2 (C)		NS	NS	NS	NS	**	NS	NS	NS	NS
PPFD (P)		NS	**	NS	NS	**	**	NS	NS	*
Species (S)		*	**	**	**	**	**	*	*	**
C x P		NS	NS	NS	NS	NS	NS	NS	NS	NS
C x S		NS	NS	NS	NS	NS	NS	NS	NS	NS
P x S		NS	NS	NS	NS	NS	NS	NS	NS	NS
C x P x S		NS	NS	NS	NS	NS	NS	NS	NS	NS
$LSD_{0.05}$		7.81	0.83	1.08	0.64	0.26	1.65	4.65	13.01	2.17

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

overall use efficiency of all nutrients showed an increasing trend with increasing [CO_2]. Whereas irrespective of [CO_2], increasing PPFD increased nutrient use efficiency for only N, K, Ca and Fe and nutrient use efficiency for the other nutrients decreased with increasing PPFD. Brazilian lucerne and Jack bean were efficient in nutrient use efficiency of N, K, Mg, Cu, Fe, and Mn, Calopo and Leucaena were efficient in Zn use efficiency and Leucaena was efficient in P use efficiency. Interspecific variations for macro-micro nutrient use efficiency are well documented in legume cover crops [20,21, 55,56].

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}										
Calopo	100	3365	204.4	486.9	194.2	79.9	0.46	1.73	3.77	0.80
	250	4198	308.4	655.3	294.9	107.6	0.61	1.85	5.88	1.03
	450	4669	341.4	658.6	254.7	131.9	0.83	2.58	5.87	1.03
Jack Bean	100	2037	137.8	354.2	165.7	29.4	0.19	0.80	1.88	0.49
	250	1874	152.3	236.1	145.7	52.5	0.27	0.77	1.96	0.55
	450	1718	244.1	270.9	233.1	101.6	0.33	1.35	3.88	0.76
B. Lucerne	100		179.6	452.5	235.6	74.7	0.43	1.60	2.03	0.91
	250	4067	360.0	540.3	403.1	135.6	0.68	2.50	4.43	1.34
	450	5097	410.2	769.4	458.9	149.1	0.88	2.41	5.38	2.06
Leucaena	100	4606	273.4	698.1	223.8	119.7	0.82	2.09	4.70	1.03
	250	5187	265.6	756.9	223.6	140.9	0.87	2.05	4.94	0.95
	450	5134	293.5	724.8	201.9	159.1	1.05	2.12	5.34	1.10
Mucuna	100	2361	329.0	385.8	237.3	78.4	0.73	1.58	4.00	0.95
	250	2636	347.4	348.7	214.8	77.3	0.71	1.44	5.22	0.92
	450	2199	323.4	358.9	138.9	72.7	0.73	1.08	3.32	0.74
700 µmol CO_2 mol^{-1}										
Calopo	100	3575	227.9	610.4	287.8	99.4	0.43	1.52	5.03	0.68
	250	4347	272.9	628.7	199.1	97.0	0.54	1.46	5.14	0.79
	450	3623	375.1	611.9	230.7	131.4	0.69	1.43	4.93	0.95
Jack Bean	100	2016	181.5	267.4	201.9	68.6	0.19	0.62	2.68	0.60
	250	2203	196.7	248.6	140.7	74.9	0.23	0.72	2.41	0.60
	450	975	185.2	182.1	123.7	71.1	0.25	0.57	1.65	0.59
B. Lucerne	100	3052	191.7	528.1	348.9	95.4	0.53	1.59	3.25	1.12
	250	4331	359.6	710.3	432.6	138.9	0.64	2.00	5.08	1.70
	450	4926	429.5	735.9	358.9	170.7	0.85	1.62	5.58	1.63
Leucaena	100	4245	210.3	736.4	239.2	129.8	0.55	1.79	4.40	0.66
	250	4586	263.2	669.7	190.1	193.9	0.73	1.94	5.86	0.91
	450	4455	365.5	623.2	222.3	203.2	1.00	2.08	6.67	1.19
Mucuna	100	1884	260.3	297.6	161.4	73.6	0.46	1.04	3.04	0.74
	250	2273	321.9	308.7	154.4	75.7	0.53	1.31	3.59	0.83
	450		383.1	377.8	184.8	70.4	0.67	1.31	3.70	0.77
Significance										
CO_2 (C)		*	NS	NS	NS	**	**	**	NS	NS
PPFD (P)		**	**	*	NS	**	**	*	**	**
Species (S)		**	**	**	**	**	**	**	**	**
C x P		NS	NS	NS	**	NS	NS	NS	NS	NS
C x S		**	NS	*	NS	NS	NS	NS	NS	NS
P x S		**	**	**	**	**	NS	NS	*	**
C x P x S		**	*	NS	**	NS	NS	NS	*	**
$LSD_{0.05}$		1351	146.4	220.2	132.6	66.0	0.41	0.96	3.19	0.58

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

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}										
Calopo	100	17.40	132.9	42.61	106.2	406.6	2.95	0.86	0.41	1.57
	250	18.03	114.4	41.24	92.4	401.9	3.02	1.03	0.33	1.61
	450	18.64	115.1	46.74	118.6	374.7	2.32	0.84	0.38	1.80
Jack Bean	100	17.69	133.3	39.86	85.7	598.4	4.71	1.15	0.45	1.72
	250	21.88	161.5	85.09	128.9	545.2	3.67	1.31	0.66	1.82
	450	25.14	87.3	59.96	71.8	243.1	3.09	0.82	0.28	1.29
B. Lucerne	100		162.5	52.20	100.4	485.9	3.31	1.01	0.79	1.57
	250	17.85	97.1	46.91	65.8	400.2	3.26	0.78	0.65	1.36
	450	19.96	113.5	47.44	77.8	393.2	2.55	1.06	0.48	1.06
Leucaena	100	16.02	124.3	40.00	120.6	368.2	2.14	0.89	0.43	1.57
	250	15.71	141.3	38.81	128.5	332.7	2.09	1.00	0.42	1.87
	450	16.51	131.1	41.75	147.5	310.4	1.80	1.01	0.40	1.68
Mucuna	100	19.97	72.4	45.78	73.8	342.5	1.52	0.77	0.33	1.10
	250	17.79	64.0	49.18	79.3	338.5	1.53	0.68	0.23	1.12
	450	18.45	62.5	42.78	111.5	315.4	1.35	0.85	0.32	1.22
700 µmol CO_2 mol^{-1}										
Calopo	100	19.96	143.4	41.71	87.6	408.0	3.72	1.19	0.37	2.22
	250	19.00	137.9	47.23	147.2	485.3	3.41	1.42	0.41	2.22
	450	24.20	106.5	51.25	135.5	381.7	2.92	1.58	0.50	2.00
Jack Bean	100	22.94	122.8	64.67	86.7	371.8	5.69	1.90	0.43	1.76
	250	23.47	127.2	76.26	136.0	397.6	5.82	1.84	0.54	1.89
	450	36.95	109.9	80.84	125.5	322.7	3.83	2.02	0.63	1.55
B. Lucerne	100	22.89	179.3	51.64	76.2	450.7	3.16	1.19	0.58	1.44
	250	22.23	120.9	48.31	77.4	399.4	3.46	1.21	0.50	1.24
	450	21.28	110.9	51.03	102.4	355.6	2.77	1.63	0.49	1.39
Leucaena	100	18.21	169.4	37.98	114.9	341.1	3.19	1.10	0.45	2.53
	250	19.54	155.9	48.14	168.2	269.1	2.80	1.21	0.40	2.16
	450	19.58	108.4	50.42	138.6	246.3	1.99	1.07	0.34	1.59
Mucuna	100	23.72	83.4	56.13	102.4	342.3	2.32	1.08	0.39	1.33
	250	20.66	71.0	57.02	112.7	359.7	2.12	0.93	0.35	1.27
	450		61.0	47.01	94.9	379.7	1.68	0.93	0.34	1.38
Significance										
CO_2 (C)		**	NS	*	**	NS	**	**	NS	**
PPFD (P)		**	**	NS	**	**	**	NS	NS	*
Species (S)		**	**	**	**	**	**	**	**	**
C x P		NS	NS	NS	*	NS	NS	NS	NS	NS
C x S		NS	NS	NS	NS	NS	NS	**	NS	NS
P x S		**	*	*	*	NS	NS	NS	NS	NS
C x P x S		*	NS	NS	*	NS	NS	NS	NS	*
$LSD_{0.05}$		7.86	75.5	38.17	68.7	292.2	2.31	0.91	0.46	0.83

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

4. CONCLUSIONS

Inter-specific variations in perennial legume cover crops for growth, physiological and macro-micro nutrient uptake parameters were observed at ambient and elevated concentration of [CO_2] and low to medium levels of PPFD. From the obtained results it could be concluded that it is possible to find perennial legume cover crops that could be useful as cover crops in the early stages of plantation crop establishment, when the PPFD at canopy level is adequate to reduce soil erosion and loss of nutrients and improve fertility and quality of soils in the tropics. Findings of this study imply that it is vital in plantation cropping systems to manage canopy light levels of understory legume cover crops in order to improve their growth and nutrient use efficiency, and eventually this could lead to

longer persistence of understory legume cover crops.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Cunningham RK, Smith RW. Comparison of soil covers during cocoa establishment on clear-felled land. Trop Agric Trans. 1961;38:13-22.
2. Jordan D, Opoku AA. The effect of selected soil covers on the establishment of cacao. Trop Agric Trin. 1966;43:155-166.
3. Lau CH, Soong NK, Tan KS. Seasonal changes in humic substances in soils under different vegetative covers. In: Bachik AT, Pushparajah E, editors. Proceedings of the International Conference on Soils and Nutrition of Perennial Crops (ICOSANP), Kuala Lumpur. The Malaysian Society of Soil Science. 1985;199-207.
4. Opoku AA. Combined annual report for academic years 1968/69 and 1969/70. Agricultural Research Station, Kade. University of Ghana; 1970.
5. Willson KC. Coffee, cocoa and tea. New York: CABI Publishing; 1999.
6. Wood GAR, Lass RA. Cocoa, 4th edition. Oxford UK: Blackwell Science; 2001.
7. Stevenson FJ. Humus chemistry: Genesis, composition, reactions. New York: John Wiley & Sons; 1994.
8. Janzen HH. Carbon cycling in earth systems—a soil science perspective. Agric Ecosys Environ. 2004;104:399-417.
9. Baligar VC, Fageria NK. Agronomy and physiology of tropical cover crops. Journal of Plant Nutrition. 2007;30:1287-1339.
10. Fageria NK, Baligar VC, Jones CA. Growth and mineral nutrition of field crops, third edition. New York: Marcel Dekker Inc; 2011.
11. Bolin B. The Kyoto negotiation on climate change: A science perspective. Science. 1998;279:330–331.
12. IPCC (Intergovernmental Panel on Climate Change 2013). The physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM, editors. Cambridge, UK: Cambridge University Press. 2013;1535.
DOI: 10.1017/CBO9781107415324
13. Hogan KP, Smith AP, Ziska LH. Potential effects of elevated CO_2 and changes in temperature on tropical plants. Plant Cell Environ. 1991;14:763-778.
14. Amthor JS. Terrestrial higher–plant response to increasing atmospheric [CO_2] in relation to global carbon cycle. Global Change Biology. 1995;1:243–274.
15. Baligar VC, Bunce JA, Elson MK, Fageria NK. Irradiance, external carbon dioxide concentration and temperature influence photosynthesis in tropical cover crop legumes. Tropical Grasslands. 2010;44:24-32.
16. Baligar VC, Bunce JA, Elson, MK, Fageria NK. Photosynthetic photon flux density, carbon dioxide concentration and temperature influence photosynthesis in Crotalaria species. The Open Plant Science J. 2012;6:1-7.
17. Grace J, Lloyd J, Miranda AC, Miranda H, Gash JHC. Fluxes of carbon dioxide and water vapour over a C_4 pasture in south-western Amazonia (Brazil). Australian J Plant Physiol. 1998;25:519-530.
18. Miyaji KI, Da Silva WS, Alvim PdeT. Longevity of leaves of a tropical tree, *Theobroma cacao*, grown under shading, in relation to position within the canopy and time of emergence. New Phytologist. 1997;135:445–454.
19. Liang N, Tang Y, Okuda T. Is elevation of carbon dioxide concentration beneficial to seedling photosynthesis in the understory of tropical rain forest? Tree Physiology. 2001;21:1047-1055.
20. Baligar VC, Fageria NK, Paiva AQ, Silveira A, Pomella AWV, Machado RCR. Light intensity effects on growth and micronutrient uptake by tropical legume cover crops. Journal of Plant Nutrition. 2006;29:1959-1974.
21. Baligar VC, Fageria NK, Paiva A, Silveira A, De Souza JO Jr, Lucena E, Faria JC, Cabral R, Pomella AWV, Jorda J Jr. Light intensity effects on growth and nutrient use efficiency of tropical legume cover crops. In: Jose S, Gordon A, editors. Toward Agroforestry Design: An Ecological Approach. Advances in Agroforestry. 2008;4:67-79.

22. Wilson JR, Ludlow MM. The environment and potential growth of herbage under plantations. In: Shelton HM, Stur WW, editors. Forages for Plantation Crops. ACIAR (Australian Center for International Agricultural Research) Proceedings No 32. 1991;10–24.
23. Wong CC. Shade tolerance of tropical forages: review. In: Shelton HM, Stur WW, editors Forages for Plantation Crops. ACIAR (Australian Center for International Agricultural Research) Proceedings No 32. 1991;64–69.
24. Wong CC. Mineral composition and nutritive value of tropical forage legumes as affected by shade. MARDI (Malaysian Agricultural Research and Development Institute) Res Bulletin. 1990;18:135-144.
25. Wong CC, Sharudin MAM, Rahim H. Shade tolerance of some tropical forages for integration with plantations: II. Legumes. MARDI (Malaysian Agricultural Research and Development Institute) Research Bulletin. 1985;13:249-269.
26. Shelton HM, Humphreys SLR, Batello C. Pastures in the plantations of Asia and the Pacific: Performance and prospect. Tropical Grasslands. 1987;21:159-168.
27. Chong DT, Tajuddin I, Abd Samat MS. Productivity of cover crops and natural vegetation under rubber in Malaysia. In: Shelton HM, Stur WW, editors. Forages for Plantation Crops. ACIAR (Australian Center for International Agricultural Research) Proceedings, No 32. 2001;36-40.
28. Stur WW. Screening forage species for shade tolerance--a preliminary report. In: Shelton HM, Stur WW, editors. Forages for Plantation Crops. ACIAR (Australian Center for International Agricultural Research) Proceedings No 32. 2001;58-63.
29. Fageria NK, Baligar VC, Li YC. Differential soil acidity tolerance of tropical legume cover crops. Comm Soil Sci and Plant Analysis. 2009;40:1148-1160.
30. Fageria NK, Baligar VC, Li YC. Nutrient uptake and use efficiency by tropical legume cover crops at varying pH of an oxisol. J Plant Nutr. 2014b;37:294-311.
31. Fageria NK, Baligar VC. Evaluation of tropical legume cover crops for copper use efficiency. Amer J Plant Sci. 2014a;5:1236-1247.
32. Fageria NK, Baligar VC. Macronutrient-use efficiency and changes in chemical properties of an oxisol as influenced by phosphorus fertilization and tropical cover crops. Comm Soil Sci Plant Analysis. 2014b;45:1227-1246.
33. Fageria NK, Baligar VC, Moreira A, Moraes LAC. Soil phosphorous influence on growth and nutrition of tropical legume cover crops in acidic soil. Comm Soil Sci Plant Anal. 2013a;44:3340-3364.
34. Fageria NK, Ferreira EPB, Baligar VC, Knupp AM. Growth of tropical legume cover crops as influenced by nitrogen fertilization and rhizobia. Comm Soil Sci Plant Anal. 2013b;44:3103-3119.
35. Fageria NK, Baligar VC, Elson MK. Zinc requirements of tropical legume cover crops. Amer J Plant Sci. 2014a;5:1721-1732.
36. Duke JA. Handbook of legumes of world economic importance. New York: Plenum Press. 1981;345.
37. Chen CP, Aminah A. *Calopogonium mucunoides*. In: 't Mannetje, L and Jones, RM, editors. Plant Resources of South-East Asia, No. 4. Forages. Wageningen, The Netherlands: Pudoc Scientific Publishers. 1992;72-74.
38. Jones RJ, Brewbaker JL, Sorensson CT. *Leucaena leucocephala* (Lamk) de Wit. In: 't Mannetje L, Jones RM, editors. Plant Resources of South-East Asia No. 4. Forages. Wageningen, The Netherlands: Pudoc Scientific Publishers. 1992;150-154.
39. 't Mannetje L. *Stylosanthes guianensis* (Aublet) Swartz. In: 't Mannetje L, Jones RM, editors. Plant Resources of South-East Asia No. 4. Forages. Wageningen, The Netherlands: Pudoc Scientific Publishers. 1992;211-213.
40. Wulijarni-Soetjipto N, Maligalig RF. *Mucuna pruriens* (L.) DC. cv. group Utilis. In: Faridah Hanum I, van der Maesen LJG, editors. Plant Resources of South-East Asia No. 11. Auxiliary plants. Leiden, The Netherlands: Backhuys Publishers. 1997; 199–203.
41. Faridah Hanum I, van der Maesen LJG. Auxiliary plants. In: Faridah Hanum, I, van der Maesen LJG, editors. Plant Resources of South-East Asia (PROSEA). 1997;11: 161–163.
42. Cook BG, Pengelly BC, Brown SD, Donnelly JL, Eagles DA, Franco MA, Hanson J, Mullen BF, Partridge IJ, Peters M, Schultze-Kraft R. Tropical Forages: An interactive selection tool. CSIRO

Sustainable Ecosystems (CSIRO), Department of Primary Industries and Fisheries (DPI&F Queensland), Centro Internacional de Agricultura Tropical (CIAT) and International Livestock Research Institute (ILRI); 2005. Available:http://www.tropicalforages.info/key/Forages/Media/Html/index.htm (Accessed 22 July 2016)

43. USEPA. Trace elements in water, solids, and biosolids by inductively coupled plasma-atomic emission spectrometry. Washington, DC: USEPA, Office of Water; 1993.
44. Bremner JM. Nitrogen total. In: Sparks DL, editor. Methods of Soil Analysis, Part 3: Chemical Methods. Madison, WI: SSSA Book Series 5, Soil Sci Soc Amer. 1996;1085-1122.
45. Eriksen FI, Whitney AS. Growth and N fixation of some tropical forage legumes as influenced by solar radiation regimes. Agron J. 1982;74:703-708.
46. Fujita K, Matsumoto K, Ofosu-Budu GK, Ogata S. Effect of shading on growth and dinitrogen fixation of kudzu and tropical pasture legumes. Soil Sci Plant Nutr. 1993;39:43-54.
47. Ludlow MM, Wilson GL, Heslehurst MR. Studies on the productivity of tropical pasture plants. V Effects of shading on growth, photosynthesis and respiration in two grasses and two legumes. Australian J Agric Res. 1974;25:425-433.
48. Kimball BA. Carbon dioxide and agricultural yields: An assemblage and analysis of 430 prior observations. Agron J. 1983;75:779-788.
49. Poorter H. Interspecific variation in the growth response of plants to an elevated ambient CO_2 concentration. Vegetatio. 1993;104,105:77-97
50. Moss DN. Photosynthesis, respiration, and photorespiration in higher plants. In: Tesar MB, editor. Physiological basis of crop growth and development. Madison, WI: American Society of Agronomy and Crop Science Society of America. 1984;131-152.
51. Brown RH. Growth of the green plant. In: Tesar MB, editor. Physiological basis of crop growth and development. Madison, WI: Am Soc Agron and Crop Sci Soc. 1984;153-174.
52. Polley HW, Johnson HB, Marino BD, Mayeux HS. Increase in C3 plant water-use efficiency and biomass over Glacial to present CO_2 concentrations. Letters to Nature. 1993;361:61-64.
53. Reuter DJ, Robinson JB, editors. Plant analysis: An interpretation manual. Melbourne, Australia: Inkata Press; 1986.
54. Jones Jr JB, Wolf B, Mills HA. Plant analysis handbook. Athens, Ga.: Micro-Macro; 1991.
55. Fageria NK, Baligar VC, Clark RB. Micronutrients in crop production. Adv Agron. 2002;77:185–268.
56. Baligar VC, Fageria NK, He ZL. Nutrient use efficiency in plants. Communications in Soil Science and Plant Analysis 2001;32: 921–950.

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