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Carbon Dioxide Concentrations and Light Levels on Growth and Mineral Nutrition of Juvenile Cacao Genotypes

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Abstract

In many countries cacao (*Theobroma cacao* L.) is invariably grown as an understory crop in agroforestry types of cropping systems and subjected to low levels photosynthetic photon flux density (PPFD) due to presence of large number of upper story shade trees with poorly managed canopy structure. In recent years carbon dioxide concentration in the atmosphere is steadily increasing and it is unclear what impact this will have on performance of cacao grown under shade of upper story shade trees. A climatically controlled greenhouse experiment was undertaken to evaluate the effects of ambient and elevated carbon dioxide (400 and 700 μmol·mol^{-1}) and three levels of PPFD (100, 200, and 400 μmol·m^{-2}·s^{-1}) on growth, and macro- and micronutrient use efficiency of three genetically contrasting cacao genotypes (CCN 51, VB 1117 and NO 81). Intraspecific variations were observed in cacao genotypes for growth parameters at ambient to elevated carbon dioxide and low to adequate levels of PPFD. With the exceptions of total root length and leaf area, irrespective of carbon dioxide and PPFD levels, all three genotypes showed significant differences in all the growth parameters. For all the cacao genotypes, increasing PPFD from 100 to 400 μmol·m^{-2}·s^{-1} and carbon dioxide from 400 to 700 μmol·mol^{-1} increased overall growth parameters such as leaf, shoot and root biomass accumulation, stem height, leaf area, relative growth rate and net assimilation rate. Irrespective of carbon dioxide and PPFD, invariably genotypes differed significantly in macro-micronutrient uptake parameters such as concentration, uptake, influx, transport and use efficiency. With few exceptions, raising PPFD from 100 to 400 μmol·m^{-2}·s^{-1} and carbon dioxide from 400 to 700 μmol·mol^{-1} increased nutrient use efficiency for all the cacao genotypes. Elevated carbon dioxide and adequate PPFD are beneficial in improving cacao growth and mineral nutrient uptake and use efficiency.

Keywords

Relative Growth Rate, Net Assimilation Rate,
Mineral Nutrient Influx and Transport, Mineral Nutrient, Uptake Efficiency

1. Introduction

Cacao (*Theobroma cacao* L.) is native to the understory of Amazonian forests of South America, and botanically its characteristics are similar to shade adapted species rather than sun plants [1] [2] [3] [4]. Shade is better for growth and development of young cacao; however, heavy shade is detrimental to growth and production of mature and older trees [5] [6] [7] [8]. There is no universal agreement on the degree of shade required to maximize cacao production [9] [10] [11] [12]. Almeida and Valle [11] stated that cocoa is a C_3 species which prefers full sun, but is tolerant to moderate shading, due to its phenotypic plasticity acclimatization to moderate shade conditions. Depending on the climatic and ecological conditions of the area, cacao is cultivated under different cropping systems from full sun (open canopy) to managed and unmanaged multi-strata agroforestry, where it is planted together with various types of shade trees such as fruit, timber, firewood, and leguminous trees, or within thinned native forests [13]-[19]. Shade trees in multi-strata agroforestry systems of cacao planting are known to buffer cacao from climate changes, improve soil fertility and regulate pathogens and pests, thereby improving cacao sustainability and cacao production. Shade trees also provide other sources of income for farmers [6] [14] [20] [21] [22] [23].

In agroforestry systems, cacao is subjected to different light intensities, depending on the density of single or multi strata shade trees, types of shade trees and level of vegetative cover and extent of shade tree pruning adopted if any [10] [21] [24]. The intensity and quality of light falling on a cacao tree are known to affect its growth and yield but there is no universal agreement on the intensity and quality of light required to maximize its production [6] [10] [15] [25] [26]. The nature and density of leaf cover of upper story trees affect light reaching field grown understory plants and under such situations light could be low in PPFD with low R/FR ratio [27] [28].

In early stages of growth, cacao grows well under shade and needs shade to reduce nutrient and water stresses [2] [10] [25]. Lower yields in cacao grown under shade are mostly related to climatic conditions such as reduced light intensity, precipitation, temperature [20] [22] [23] [29] and therefore under such management systems it is advantageous to adopt cacao genotypes that can maintain desired productivity even under reduced light intensity.

Optimum cacao growth is achieved at 20% to 30% of full sunlight [1] [30] usually with adequate soil nutrient availability [31]. Under shaded plantations in Bahia Brazil, light intensity at noon above the cacao canopy ranged between 30 and 100% of the full sunlight and 4% - 10% at the ground level [32]. It has been reported that the PPFD for maximum net photosynthesis (PN) in cacao occurs at about 350 to 550 $\mu mol \cdot m^{-2} \cdot s^{-1}$, which is about 20% to 25% of the intensity of full sunlight [12] [33] [34] [35] [36]. In the cacao growing regions, Hutcheon [33] has shown that the maximum light saturation point in cacao was 250 - 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, which is about 15% of the full midday sunlight. Variations in morphological characteristics among cacao genotypes have been reported [4] [37] [38] [39] and these characteristics have been influenced by light level [1] [12] [40] [41] [42]. Plant traits could have great implications on the ability of plants to intercept and utilize solar radiation. Baligar *et al.* [40] reported that in juvenile cacao genotypes increasing PPFD from 65 to 190 $\mu mol \cdot m^{-2} \cdot s^{-1}$ increased shoot and root growth and net assimilation rate (NAR), however, PPFD of 1050 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was detrimental to early cacao growth. In seven genetically different cacao genotypes, Baligar *et al.* [43] reported that increasing PPFD from 100 to 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ increased shoot and root growth, relative growth rate (RGR) and net assimilation rates (NAR). Irrespective of levels of PPFD significant intraspecific differences between genotypes were observed for shoot and root growth.

In many plant species demands for mineral nutrients are greatly influenced by the light levels that reach the canopy [44] [45] [46] [47]. Baligar *et al.* [40] reported that in cacao genotype comum increasing PPFD from 65 to 1050 $\mu mol \cdot m^{-2} \cdot s^{-1}$ increased the nutrient use efficiency (NUE) for N, Na, S and Zn and decreased NUE for other nutrients. Recently the existence of intraspecific differences in cacao genotypes has been reported for macro-micro nutrient uptake and use efficiency [43], and these nutrient traits increased with increasing PPFD from 100 to 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$. However, information is limited on the influence of various light levels on NUE of cacao genotypes.

The present carbon dioxide concentration [CO_2] in the atmosphere is around 400 $\mu mol \cdot mol^{-1}$ and based on future emission scenarios, it could reach as high as 550 to 1370 $\mu mol \cdot mol^{-1}$ by the end of the 21st century [48] [49]. In older plantations, cacao is invariably subjected to low light levels coupled with increasing global atmospheric [CO_2] and reduced availability of soil nutrients and water that could have negative effects on growth and production potentials of cacao. Both light levels and photoperiod are of importance in determining plant responses to elevated [CO_2] [50]. Elevated [CO_2] has contributed to increased biomass of tropical plants [51] [52] [53] [54] and increased growth and development of cacao [12] [40]. Compared to row crops and temperate tree crops, information is very much lacking on the effects of low levels of PPFD and increasing levels of [CO_2] and their interactions on morphological development and NUE of cacao. Baligar *et al.* [40] reported that increasing external atmospheric

[CO_2] from 380 to 700 μmol·mol^{-1} increased root, shoot and leaf dry biomass, stem height, leaf area, shoot/root ratio, relative growth rate (RGR), and net assimilation rate (NAR) in Forestaro type cacao. Further in that study, increasing [CO_2] increased NUE for N, Na, Mg, Cu, Mn, and Zn and decreased NUE for other mineral nutrients. Baligar *et al.* [43] reported that in seven cacao genotypes, irrespective of PPFD levels, increasing [CO_2] from 400 to 700 μmol·mol^{-1} significantly increased shoot and root weight, root length, stem height, leaf area, specific leaf area, RGR, NAR, and macro-micro nutrient uptake and NUE.

Identification of plant traits for growth, physiological and NUE that are influenced by low PPFD at elevated [CO_2] can help to identify cacao genotypes that could perform well under a range of [CO_2] and light intensities. The objectives of this study were to evaluate the effects of ambient and elevated CO_2 concentrations at varying low to adequate light levels on growth, and macro- and micronutrient use efficiency in three genetically contrasting cacao genotypes.

2. Materials and Methods

2.1. Cacao Genotypes

Three cacao genotypes, CCN 51, VB 1117, and NO 81, were selected for this study. Pods of various cacao genotypes needed for this research were received from the MARS Center for Cocoa Science, Alimirante, Itajuipe, Bahia, Brazil. CCN 51 is a clone from Ecuador obtained from crosses of ICS 95 x IMC 6. ICS 95 is a Trinitario clone with parental contributions from lower Amazon Forestero and Criollo [55] [56], whereas IMC 67 is an upper Amazon Forestero collected from Peru [55]. VB 1117 is an Amelonado clone selected from Almirante, Bahia, Brazil for tolerance to witches' broom disease (*Moniliophthora pernicosa*). NO 81 is derived from crosses of IMC 47 × PA 107 [57]. Additional information on cacao clones used is given by Turnbull and Hadley [57] and Ahnert and Eskes [58]. Seeds used for this study were produced by self-pollination. Therefore, the full sib family plants generated by such seeds have, on average, similar traits to the parents, in this case, the clonal cuttings.

2.2. Growth Medium

The growth medium was prepared by mixing Perlite: Sand: Promix (2:2:1 volume basis) in a cement mixer. Supplemental nutrients were added at the time of mixing to provide 600 mg/kg N, 600 mg/kg P, 240 mg/kg K, 1012 mg/kg Ca, 309 mg/kg Mg, 119 mg/kg Fe, 0.7 mg/kg B, 17.5 mg/kg Mn, 7 mg/kg Cu, 7 mg/kg Zn and 0.35 mg/kg Mo. Pots were watered on a daily basis to maintain water content at the field water holding capacity.

2.3. Growth Conditions

Seeds were separated from cacao pods and seed coats were removed by hand. Seeds were soaked in 10% chlorine bleach (Sodium hypochlorite) for 2 min, rinsed twice in deionized-water, then soaked in 90% ethanol for 2 min and rinsed twice in DI-water. Seeds were germinated on sterile moist filter paper for 48 h at 25˚C ± 2˚C. Seeds with 5 mm radicals were planted in 3.8 L black plastic pots with adequate bottom drainage containing 2.2 kg of the growth medium. One seedling was planted in each pot. During the growth phase soil moisture was kept at field capacity (−33 kPa) by adding water every other day. On the 21st day of growth, an initial plant harvest was made for initial growth.

2.4. CO_2 and PPFD Treatments

For this study two air-conditioned greenhouses (13.5 m^2 each) with day/night temperatures of 30˚C/28˚C were used to conduct two independent experiments with ambient and elevated CO_2. One greenhouse contained near ambient [CO_2] (400 ± 50 μmol·mol^{-1}) and the other had elevated [CO_2] (700 ± 50 μmol·mol^{-1}). The CO_2 concentration of 700 μmol·mol^{-1} was controlled by a WMA2 infra-red gas analyzer (PP Systems, Amesbury, MA, USA) which injected CO_2 when the concentrations fell below the desired [CO_2]. Ambient night-time CO_2 concentration values were higher than 400 μmol·mol^{-1} at this site due in part to low wind speed and stable atmospheric conditions. Within each greenhouse, electrical fans continuously circulated the air at an air speed of 0.5 m·s^{-1} over the plants. Day-time air temperatures were maintained for 12 h per day beginning at 6 AM. The greenhouses transmitted approximately 60% of the incident PPFD daily. A data logger (21×, Campbell Scientific, Logan, UT, USA) recorded the PPFD, temperature and [CO_2] in both greenhouses at 30 s intervals. In each greenhouse two mini chambers were utilized to achieve different PPFD. Mini chambers were constructed with 2 cm (3/4 inch) diameter PVC pipe with overall dimensions of 60 W cm × 120 L cm × 81 H cm. To achieve different levels of light, tops and sides of mini chambers were covered with plastic shade cloth. A single-ply of Easy Gardener® sun screen fabric provided the minimum PPFD of 100 ± 20 μmol·m^{-2}·s^{-1}, a single-ply of black window screen provided medium PPFD of 200 ± 30 μmol·m^{-2}·s^{-1} and the absence of shade cloth was the third treatment which provided PPFD of 400 ± 40 μmol·m^{-2}·s^{-1}. On every 7th sunny day, at midday, PPFD were determined with a Li-Cor LI-190S Quantum sensor (Li-Cor Inc., Lincoln, NE, USA) in the mini chambers and in the greenhouses; these values do not represent PPFD of daily mean and do not include overcast days. Mini chambers were covered with mesh shade cloth so they had full air exchange with the greenhouse atmosphere. Plants in each mini chamber were rotated once a week to keep the light exposures constant.

2.5. Determination of Growth Parameters

The experiment was terminated after 90 days of growth in the mini-chambers. Stem height was recorded. Leaves were separated from stems and the leaf area was determined by a Li-Cor model 3100 leaf area meter (Li-Cor Inc., Lincoln, NE, USA). Roots and shoots were separated and washed with deionized water. Shoots were blotted dry, oven dried at 70˚C for 5 days and weighed. The roots were removed from the soil, washed, blotted dry and weighed. 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 and the dry root weights were recorded.

The following growth parameters were determined.

$$\text{Specific Leaf Area}\left(\text{SLA}, \text{cm}^2\cdot\text{g}^{-1}\right)=\frac{\text{Total leaf area}\left(\text{cm}^2/\text{plant}\right)}{\text{Total leaf dry weight}\left(\text{g}/\text{plant}\right)} \quad (1)$$

$$\text{Leaf Mass/Unit Leaf Area}\left(\text{LMA}, \text{g}\cdot\text{cm}^{-2}\right)=\frac{1}{\text{SLA}} \quad (2)$$

$$\text{Leaf Area Ratio}\left(\text{LAR}, \text{cm}^2\cdot\text{g}^{-1}\right)=\frac{\text{Total leaf area}\left(\text{cm}^2/\text{plant}\right)}{\text{Shoot}+\text{Root dry wt.}\left(\text{g}/\text{plant}\right)} \quad (3)$$

$$\text{Relative Growth Rate}\left(\text{RGR}, \text{g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}\right)=\frac{\ln\left(Wt_2/Wt_1\right)}{T_2-T_1} \quad (4)$$

where Wt is total weight (shoot + root), T is time in days, subscripts 1 and 2 refer to initial and final plant harvests.

$$\text{Net Assimilation Rate}\left(\text{NAR}, \text{g}\cdot\text{cm}^{-2}\cdot\text{d}^{-1}\right)=\text{RGR}/\text{LAR} \quad (5)$$

2.6. Determination of Nutrient Uptake Parameters

Dried shoot samples were ground to pass through a 1 mm mesh sieve. Chemical analysis of the shoot samples was performed at the Indian River Research and Education Center, University of Florida, Fort Pierce, FL, USA. Plant tissues (0.4 g) were digested in 5 mL of concentrated 14N HNO_3 [59]. The concentrations of macro- (P, K, Ca, and Mg) and micro-elements (B, Cu, Fe, Mn, and Zn) in the digested solution were analyzed using inductively coupled plasma optical emission spectrometry (ICPOES Ultima, J. Y. Horiba Group, Edison, NJ, USA) following USEPA method 200.7 [60]. Total N in the plant tissue was analyzed by combustion method using CN Analyzer (Vario MAX CN Macro Analyzer, Elementar Analysensysteme GmbH, Hanau, Germany) [61].

Nutrient uptake (U), influx (IN), transport (TR) and use efficiency (NUE) were calculated as follows:

$$\begin{aligned}\text{Uptake}\left(\text{U}\right)&=\text{Conc. of any given element}\left(\text{mg/g or µg/g}\right)\\&\quad\times\text{Shoot dry weight}\left(\text{g}/\text{plant}\right)\end{aligned} \quad (6)$$

$$\text{Influx}\left(\text{IN}\right)=\frac{\left(\left(U_2-U_1\right)/\left(T_2-T_1\right)\right)\left(\ln Wr_2-\ln Wr_1\right)}{Wr_2-Wr_1} \quad (7)$$

where U refers to elemental content in shoot (mmoles /plant), T is time in seconds, Wr. is total root length (cm/plant), and subscripts 1 and 2 refer to initial and final plant harvest times.

$$\text{Transport}\left(\text{TR}\right)=\frac{\left(\left(U_2-U_1\right)/\left(T_2-T_1\right)\right)\left(\ln Ws_2-Ws_1\right)}{Ws_2-Ws_1} \quad (8)$$

where U refers to elemental content in shoot (mmoles /plant), T is time in seconds, Ws is shoot dry weight (g/plant).

$$\text{Nutrient Use Efficiency}\left(\text{NUE}\right)=\frac{\text{mg of } Ws}{\text{mg of any given element in shoot}} \quad (9)$$

2.7. Statistical Analysis

A Split-Split plot design was used, where CO_2 treatments were the main plots, PPFD were the sub plots and genotypes were the sub-sub plots. Experimental units were replicated four times. Results were subjected to analysis of variance using general linear model (GLM) procedures of SAS (Ver. 9.1, SAS Institute, Cary, NC).

3. Results and Discussion

3.1. [CO_2] and PPFD Effects on Growth Parameters

Genotypic variations in growth parameters (shoot and root weight, root length, stem height, leaf area, specific leaf area, and RGR) were influenced by genotypes, [CO_2] and PPFD (**Table 1** and **Table 2**). Growth and development of cacao are profoundly influenced by genetic, physiological and morphological determinants and their interaction with environmental variables such as PPFD and [CO_2] [11] [12] [40]. With the exceptions of total root length and leaf area, irrespective of [CO_2] and PPFD, all genotypes showed significant differences in the growth parameters. Overall NO 81 genotype had higher values for all the growth parameters as compared to the other two genotypes. Cacao genotypes vary in morphological characteristics [37] [38] [39] and cacao morphological characteristics are influenced by the light level [1] [12] [40] [41] [42]. Elevated [CO_2] increases plant growth such as shoot and root biomass, leaf and root area, RGR and nutrient uptake; however, the magnitude of such responses is dependent on the availability of water and mineral nutrients, and environmental variables such as light and temperature [1] [36] [40] [41] [52] [53] [54] [62] [63] [64].

Irrespective of genotypes and PPFD, increasing [CO_2] from 400 to 700 $\mu mol \cdot mol^{-1}$ in-creased overall growth parameters, however, significant influences were observed for shoot and root biomass accumulations, plant height, leaf area and RGR. Effects of elevated [CO_2] on morphological and growth parameters of tropical plants has received considerably less attention than temperate plants. Increasing [CO_2] in tree species increased dry matter accumulation and mean mass per unit leaf [65]. Nine tropical plant species subjected to elevated (2× ambient) [CO_2] increased dry matter and P_N [66]. In seven C_3 crops and three weed species, Bunce [67] reported that increasing [CO_2] from 360 to 700 $\mu mol \cdot mol^{-1}$ increased dry mass, RGR and NAR.

Baligar *et al.* [40] grew cacao genotype comum for 57 days at CO_2 concentrations of 380 (ambient) and 700 $\mu mol \cdot mol^{-1}$ (elevated) and plants grown at 700 $\mu mol \cdot mol^{-1}$ had increased root, shoot and leaf dry masses, leaf area, stem height, root/shoot ratio and RGR as compared to plants grown at ambient [CO_2]. In a recent study, Baligar *et al.* [43] reported that shoot and root growth of seven genetically different cacao genotypes increased with increasing [CO_2] from 400 to 700 μmol·mol^{-1}. Results of the current study supported these earlier findings. Generally, C_3 plants respond positively to elevated CO_2 [68]. In the current study, increasing [CO_2] from 400 to 700 μmol·mol^{-1} reduced SLA. Bunce [69] reported increased growth in annual plants due to reduced LAR at elevated [CO_2]. Lahive *et al.* [64] reported that juvenile Amelonado cacao grown under elevated [CO_2] increased stem elongation, leaf fresh and dry biomass, and leaf

Table 1. The effect of [CO_2] and photosynthetic photon flux density (PPFD) on shoot and root growth of three cacao genotypes.

[CO_2] (μmol·mol^{-1})	PPFD (μmol·m^{-2}·s^{-1})	Dry Weights (g/plant)				Stem Height (cm/plant)	Root Length (cm/plant)	Root/ Shoot Ratio	RGR (g·g^{-1}·d^{-1}) (×10^{-2})	NAR (g·cm^{-2}·d^{-1}) (×10^{-4})
		Leaf	Shoot	Root	Total					
			CCN 51							
400	100	5.90	7.69	1.60	9.30	32.0	4211	0.21	3.49	1.54
	200	6.53	8.66	1.74	10.41	27.5	3262	0.20	3.64	1.76
	400	16.83	21.27	4.31	25.58	43.0	5917	0.21	4.64	2.49
700	100	7.39	9.55	2.15	11.70	41.8	4355	0.23	3.76	1.64
	200	11.54	15.27	2.85	18.12	45.5	5645	0.18	4.23	2.24
	400	23.02	32.80	7.41	40.21	60.3	5600	0.23	5.14	3.87
			VB 1117							
400	100	6.87	9.02	1.28	10.30	46.8	3942	0.14	3.86	2.02
	200	10.96	15.33	2.30	17.63	55.0	4745	0.15	4.48	3.28
	400	13.23	19.56	2.78	22.34	56.0	4851	0.14	4.71	3.91
700	100	11.37	15.95	1.81	17.76	76.5	5664	0.11	4.49	2.60
	200	16.77	24.24	2.96	27.19	84.5	6075	0.12	4.96	3.34
	400	22.75	35.27	5.16	40.43	99.0	4869	0.14	5.39	4.21
			NO 81							
400	100	7.49	9.57	1.59	11.16	48.5	5131	0.17	3.82	1.82
	200	11.23	14.50	2.07	16.57	48.5	5681	0.13	4.20	2.29
	400	22.07	29.08	4.61	33.70	63.3	5996	0.16	5.04	3.67
700	100	7.93	10.51	1.45	11.96	56	4804	0.14	3.87	2.00
	200	15.41	22.78	4.56	27.34	77.5	6443	0.20	4.81	3.48
	400	23.55	36.79	8.79	45.58	83.3	4233	0.24	5.38	5.01
Significance										
Genotype (G)		**	**	**	**	**	NS	**	**	**
[CO_2] (C)		**	**	**	**	**	NS	NS	**	**
PPFD (P)		**	**	**	**	**	NS	NS	**	**
$LSD_{0.05}$		6.19	9.33	2.43	11.16	24.9	897	0.09	0.62	1.26

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

Table 2. The effect of $[CO_2]$ and photosynthetic photon flux density (PPFD) on leaf parameters of three cacao genotypes.

$[CO_2]$ ($\mu mol \cdot mol^{-1}$)	PPFD ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Leaf Area (cm^2/plant)	Specific Leaf Area ($cm^2 \cdot g^{-1}$)	Leaf Mass/ Unit Area ($g \cdot cm^{-2} \times 10^{-3}$)	Leaf/ Shoot Ratio	Leaf Area Ratio ($cm^2 \cdot g^{-1}$)
CCN 51						
400	100	2143.2	362.7	2.77	0.77	230.5
	200	2163.0	329.1	3.05	0.75	206.7
	400	4783.2	285.4	3.52	0.79	187.4
700	100	2682.8	363.7	2.75	0.78	230.0
	200	3437.7	298.3	3.37	0.76	191.8
	400	5350.3	233.7	4.29	0.70	133.8
VB 1117						
400	100	1974.1	286.2	3.50	0.77	191.6
	200	2416.0	223.0	4.55	0.72	138.3
	400	2685.3	204.7	4.91	0.68	123.9
700	100	3079.0	270.5	3.70	0.71	173.4
	200	4040.9	241.8	4.16	0.69	149.2
	400	5173.2	228.9	4.41	0.65	130.0
NO 81						
400	100	2356.4	314.2	3.19	0.78	210.5
	200	3113.7	270.3	3.75	0.77	184.0
	400	4626.8	211.3	4.76	0.76	139.9
700	100	2342.7	302.5	3.37	0.75	198.7
	200	3904.7	250.1	4.03	0.67	140.7
	400	5004.6	212.2	4.77	0.64	110.7
Significance						
Genotype (G)		NS	**	**	**	**
$[CO_2]$ (C)		**	NS	**	**	**
PPFD (P)		**	**	NS	**	**
$LSD_{0.05}$		1694	65.2	1.01	0.10	50.2

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

area, compared to plants grown under the ambient averaged $[CO_2]$ of 400 $\mu mol \cdot mol^{-1}$.

In the present study at both $[CO_2]$ in all the genotypes, increasing PPFD from 100 to 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ increased growth parameters and the effects were significant for leaf, shoot and root biomass accumulation, stem height, leaf parameters, RGR and NAR, however PPFD effects were non-significant for root/shoot ratio, total root length and leaf mass/unit area. At both $[CO_2]$ in all the genotypes, increasing PPFD from 100 to 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ significantly increased leaf area and decreased SLA and LAR. This is a reflection of increased leaf and shoots plus root biomass with increasing PPFD. Baligar *et al.* [40] reported similar response in cacao leaf parameters by increasing PPFD from 65 to 1050 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and such response was observed in plants grown at [CO_2] of 330 and 700 $\mu mol \cdot mol^{-1}$. However, the higher PPFD of 1050 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was detrimental to the growth of cacao. Cacao genotypic variation in leaf production, leaf area and stem dry weight in response to light levels has been reported [38] [40] [41]. Van de Geijin and Dijkstra [50] stated that a complex interaction exists between plant nutrition, light level, daylength and growth enhancement by elevated [CO_2].

3.2. [CO_2] and PPFD Effects on Mineral Nutrient Uptake and Use Efficiency

3.2.1. Mineral Concentration and Uptake

Irrespective of [CO_2] and PPFD, invariably genotypes differed significantly in plant concentrations of all the essential macro-micro nutrients (**Table 3**). With the exception of B and Zn concentrations, overall CCN 51 in comparisons to other two genotypes had the highest concentration of all the nutrients. With the exception of Fe, irrespective of PPFD, increasing [CO_2] from 400 to 700 $\mu mol \cdot mol^{-1}$ decreased concentrations of all the nutrients, however the effect of [CO_2] was only significant for N, K, Ca, B, Cu, Mn and Zn concentrations. Irrespective of [CO_2], increasing PPFD from 100 to 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ decreased concentrations of all nutrients and this is a reflection of an increase in shoot dry matter with increasing PPFD. Concentrations of P and Mg were slightly higher but concentrations of other macro and micronutrients observed were comparable to concentrations reported in the literature [70] [71] [72].

Genotypes, irrespective of [CO_2] and PPFD, invariably differed significantly for up-take of all the essential macro-micro nutrients (**Table 4**). With exception of K, NO 81 genotype accumulated highest amount of all the nutrients, and this is reflection of its ability to accumulate highest amount of shoot dry matter compared to other genotypes. Irrespective of PPFD, increasing [CO_2] from 400 to 700 $\mu mol \cdot mol^{-1}$ increased the uptake of all the nutrients; however, the effect was significant only for uptake of P, K, Ca, Mg, Cu, and Fe. Baligar *et al.* [40] reported that in cacao genotype comum increasing [CO_2] from 380 to 700 $\mu mol \cdot mol^{-1}$ increased uptake of all essential nutrients and further they stated that increased nutrient uptake at higher [CO_2] is due to increased demand for mineral nutrients due to enhanced dry matter accumulations. In a recent study with seven cacao genotypes Baligar *et al.* [43] reported that increasing [CO_2] from 400 to 700 $\mu mol \cdot mol^{-1}$ significantly increased the uptake macro-micronutrients. In the current study irrespective of [CO_2] increasing PPFD from 100 to 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ significantly increased uptake of all the nutrients. The overall nutrient accumulation was in the order of N > K = Ca > Mg > P for macro nutrients and Mn > Zn > B > Fe > Cu for micronutrients.

Table 3. The effect of [CO_2] and photosynthetic photon flux density (PPFD) on mineral nutrient concentrations of three cacao genotypes.

[CO_2] (μmol·mol^{-1})	PPFD (μmol·m^{-2}·s^{-1})	N	P	K	Ca	Mg	B	Cu	Fe	Mn	Zn
		mg·g^{-1}					μg·g^{-1}				
						CCN 51					
400	100	23.3	4.2	20.4	17.2	7.2	41.1	15.3	28.0	88.6	35.7
	200	24.2	4.5	18.4	18.0	7.0	41.4	17.5	20.2	118.5	32.0
	400	20.3	4.1	16.3	18.4	7.9	32.0	17.1	28.7	61.1	30.6
700	100	22.2	4.4	21.1	17.9	6.2	36.7	19.4	27.2	91.5	36.2
	200	20.0	4.3	19.0	17.8	7.9	33.3	18.9	26.1	100.6	36.9
	400	12.6	3.8	12.6	14.7	7.3	16.0	11.1	31.8	36.3	20.9
						VB 1117					
400	100	21.6	3.2	20.2	11.8	7.1	46.7	12.7	20.9	139.4	54.0
	200	22.1	3.5	19.0	17.1	7.1	38.6	16.1	23.7	97.8	39.1
	400	20.6	3.2	17.8	15.3	6.5	29.9	13.4	23.6	82.5	35.2
700	100	19.7	3.3	20.2	14.8	6.4	36.3	14.4	26.9	78.0	33.1
	200	15.8	3.7	15.5	13.9	6.5	23.3	13.7	21.3	46.9	21.2
	400	12.8	3.6	13.2	12.6	5.9	14.4	10.4	21.3	37.0	21.7
						NO 81					
400	100	21.6	4.1	19.2	18.9	6.6	42.5	16.7	23.0	121.6	52.4
	200	21.0	4.3	16.3	18.3	6.1	34.2	14.6	24.2	90.2	38.7
	400	18.6	4.0	13.1	19.6	7.2	27.1	15.2	22.0	76.5	39.5
700	100	20.6	4.1	19.7	12.2	6.5	39.8	13.2	21.5	87.2	48.4
	200	15.1	4.3	15.6	18.0	7.3	26.1	14.1	28.5	60.2	33.1
	400	12.4	3.9	12.9	16.2	7.6	42.6	13.2	38.2	40.2	28.7
Significance											
Genotype (G)		**	**	**	**	*	NS	**	NS	NS	**
[CO_2] (C)		**	NS	**	*	NS	*	NS	*	**	**
PPFD (P)		**	NS	**	NS	NS	**	**	NS	**	**
$LSD_{0.05}$		5.4	1.3	4.0	7.7	2.0	36.9	6.7	16.0	63.2	20.9

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

3.2.2. Mineral Nutrient Influx (IN) and Transport (TR)

In South-Central America cacao is invariably grown on infertile soils under high temperatures and light intensities with lower soil moisture. Such climatic conditions might have major effects on plants ability to acquire (IN) and transport (TR) essential nutrients. However, very limited information is available on how increasing [CO_2] and PPFD affect macro-micronutrients IN and TR. In the current study, with the exception of IN for Mn, at all [CO_2] and PPFD, genotypes differed significantly for IN of all macro-micro nutrients (**Table 5**). Irrespective

Table 4. The effect of [CO_2] and photosynthetic photon flux density (PPFD) on mineral nutrient uptake of three cacao genotypes.

[CO_2] (μmol·mol^{-1})	PPFD (μmol·m^{-2}·s^{-1})	N	P	K	Ca	Mg	B	Cu	Fe	Mn	Zn
		mg/plant					μg/plant				
					CCN51						
400	100	179.6	32.6	156.3	131.2	55.5	305.6	117.6	211.7	662.2	281.4
	200	209.1	38.6	159.7	155.5	60.2	358.7	151.8	175.6	1030.8	279.7
	400	441.3	87.2	344.9	395.0	163.9	669.3	368.7	609.7	1361.4	660.3
700	100	212.5	42.3	200.2	169.8	59.2	345.6	184.2	255.9	868.0	337.9
	200	304.5	65.2	286.9	272.3	121.9	497.9	286.6	410.9	1491.5	560.1
	400	403.6	123.3	411.5	480.5	239.1	519.1	369.1	1058.9	1165.5	691.9
					VB 1117						
400	100	195.0	29.3	182.7	98.0	64.6	424.4	110.3	186.2	1200.4	468.4
	200	338.7	53.4	289.4	260.0	107.6	588.0	246.6	365.7	1504.9	592.7
	400	400.4	62.2	340.9	299.2	126.6	558.7	256.3	469.1	1575.9	683.5
700	100	312.8	53.1	320.7	236.3	101.9	577.3	230.2	426.8	1230.8	528.7
	200	383.3	88.7	376.9	334.0	157.0	562.7	330.9	511.1	1146.5	512.9
	400	439.6	128.0	459.3	440.0	210.1	494.1	365.4	767.8	1257.5	758.1
					NO 81						
400	100	205.8	38.4	184.2	179.3	63.2	407.9	158.0	217.3	1122.2	496.9
	200	306.6	63.6	241.2	268.2	90.1	477.8	215.9	361.3	1278.4	581.0
	400	534.6	117.5	377.2	566.8	210.4	766.9	439.1	653.0	2127.9	1115.2
700	100	215.8	42.3	204.5	126.9	68.1	412.6	139.1	223.8	912.2	498.6
	200	344.1	98.3	354.5	413.4	166.4	591.6	322.5	663.7	1367.0	758.0
	400	451.0	143.5	471.5	589.5	278.8	1514.9	483.8	1392.3	1449.9	1046.7
Significance											
Genotype (G)		*	**	**	**	**	NS	*	*	*	**
[CO_2] (C)		NS	**	**	**	**	NS	**	**	NS	NS
PPFD (P)		**	**	**	**	**	*	**	**	**	**
$LSD_{0.05}$		183.7	45.5	130.3	177.7	77.6	1235.8	170.4	486.9	1043.5	419.9

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

of PPFD, increasing [CO_2] from 400 to 700 μmol·mol^{-1} increased IN for all the nutrients, however the effect was significant only for IN of P, K, Ca, Mg, Cu, and Fe. Irrespective of [CO_2], increasing PPFD from 100 to 400 μmol·m^{-2}·s^{-1} significantly increased IN of all nutrients. Baligar *et al.* [40] reported that increasing [CO_2] from 380 to 700 μmol·mol^{-1} tended to increase IN in cacao for many of the essential nutrients; however, increasing PPFD from 65 to 1050 μmol·m^{-2}·s^{-1} tended to decrease IN for N, K, Ca, Mg, P, S, Cu and Fe.

At all [CO_2] and PPFD, genotypes differed significantly in TR of P, K, Ca, and Zn (**Table 6**). The [CO_2] significantly affected TR of N, P, Mg, Fe, Mn and Zn.

However, with the exception of TR for N and B, PPFD significantly affected TR for all the other mac-ro-micronutrients. Increasing [CO_2] increased TR for P, K, Mg and Fe only, however increasing PPFD slightly increased TR for P, Ca, Mg and Fe. Earlier, Baligar *et al.* [40] reported that in cacao genotype comum increasing [CO_2] from 400 to 700 μmol·mol^{-1} decreased TR for N, Ca, and Zn and increased TR for other elements. Such variations in IN and TR at varying [CO_2] and PPFD could be related to nature of genotypes and their interactions with [CO_2] and PPFD.

Table 5. The effect of [CO_2] and photosynthetic photon flux density (PPFD) on mineral nutrient influx by root length of three cacao genotypes.

[CO_2] (μmol·mol^{-1})	PPFD (μmol·m^{-2}·s^{-1})	N	P	K	Ca	Mg	B	Cu	Fe	Mn	Zn
		pmol cm·root^{-1}·s^{-1}					pmol cm·root^{-1}·s^{-1} (×10^3)				
						CCN 51					
400	100	0.79	0.06	0.25	0.21	0.15	1.98	0.11	0.25	0.74	0.24
	200	1.14	0.09	0.34	0.30	0.20	2.70	0.19	0.25	1.61	0.35
	400	1.89	0.15	0.51	0.53	0.36	3.02	0.32	0.65	1.36	0.52
700	100	1.04	0.10	0.36	0.31	0.17	2.40	0.21	0.37	1.32	0.39
	200	1.23	0.11	0.43	0.36	0.26	2.49	0.24	0.42	1.65	0.52
	400	1.63	0.19	0.56	0.65	0.52	2.37	0.28	0.90	1.22	0.51
						VB 1117					
400	100	1.30	0.09	0.45	0.20	0.26	3.92	0.13	0.32	1.84	0.66
	200	1.56	0.11	0.50	0.44	0.31	3.74	0.26	0.39	1.71	0.64
	400	1.85	0.13	0.55	0.50	0.34	3.71	0.26	0.52	1.95	0.72
700	100	1.48	0.11	0.55	0.38	0.27	3.57	0.23	0.53	1.70	0.51
	200	1.70	0.19	0.57	0.53	0.40	3.27	0.28	0.58	1.27	0.51
	400	2.23	0.31	0.88	0.82	0.69	3.42	0.42	1.07	1.43	0.77
						NO 81					
400	100	0.93	0.08	0.30	0.31	0.16	2.31	0.17	0.26	1.56	0.55
	200	1.57	0.16	0.46	0.50	0.29	3.02	0.24	0.45	1.30	0.66
	400	2.30	0.20	0.58	0.79	0.46	4.09	0.37	0.62	2.15	0.92
700	100	1.25	0.11	0.40	0.27	0.23	3.07	0.16	0.29	1.30	0.67
	200	1.37	0.14	0.50	0.52	0.34	3.01	0.26	0.48	1.54	0.70
	400	2.29	0.35	0.89	1.17	0.89	4.01	0.57	2.26	1.91	1.15
Significance											
Genotype (G)		*	**	**	**	**	**	*	**	NS	**
[CO_2] (C)		NS	**	**	**	**	NS	**	**	NS	NS
PPFD (P)		**	**	**	**	**	*	**	**	NS	**
$LSD_{0.05}$		0.32	0.03	0.08	0.12	0.05	0.48	0.07	0.16	0.44	0.16

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

Table 6. The effect of $[CO_2]$ and photosynthetic photon flux density (PPFD) on mineral nutrient transport of three cacao genotypes.

$[CO_2]$ ($\mu mol \cdot mol^{-1}$)	PPFD ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	N	P	K	Ca	Mg	B	Cu	Fe	Mn	Zn
		pmol g·shoot^{-1}·s^{-1}									
		CCN 51									
400	100	686.9	55.2	217.3	185.9	127.9	1.64	0.10	0.21	0.70	0.24
	200	748.8	61.7	204.3	203.2	128.6	1.73	0.12	0.16	0.98	0.22
	400	805.9	72.5	230.0	259.9	181.0	1.66	0.15	0.29	0.63	0.26
700	100	701.6	62.7	241.8	205.8	117.1	1.56	0.13	0.22	0.77	0.25
	200	719.3	69.5	246.3	231.3	168.4	1.59	0.15	0.24	0.95	0.29
	400	539.3	73.7	196.2	226.2	184.7	0.91	0.11	0.35	0.41	0.20
		VB 1117									
400	100	689.9	45.4	233.1	132.8	135.7	2.01	0.09	0.17	1.17	0.38
	200	824.2	58.0	254.0	227.2	154.8	1.90	0.13	0.22	0.95	0.32
	400	810.0	56.7	249.6	214.8	149.4	1.53	0.12	0.23	0.84	0.30
700	100	739.7	55.9	273.9	199.5	141.3	1.81	0.12	0.26	0.77	0.27
	200	655.1	69.0	231.8	204.4	157.4	1.27	0.13	0.22	0.50	0.19
	400	568.8	73.7	212.4	200.5	154.8	0.84	0.10	0.24	0.42	0.21
		NO 81									
400	100	683.0	57.0	218.5	216.1	124.3	1.80	0.12	0.18	1.01	0.37
	200	742.4	68.3	206.7	232.5	127.3	1.57	0.11	0.22	0.83	0.30
	400	781.6	76.5	195.7	291.7	177.4	1.48	0.14	0.23	0.82	0.36
700	100	663.5	58.5	228.4	140.5	124.4	1.71	0.09	0.17	0.74	0.35
	200	597.2	76.6	221.8	255.8	169.7	1.36	0.12	0.29	0.62	0.29
	400	545.0	78.6	204.0	253.1	196.9	2.46	0.13	0.43	0.46	0.27
Significance											
Genotype (G)		NS	**	**	**	NS	NS	NS	NS	NS	**
$[CO_2]$ (C)		**	**	NS	NS	*	NS	NS	**	**	**
PPFD (P)		NS	**	**	**	**	NS	**	**	**	*
$LSD_{0.05}$		238.8	24.8	56.4	95.8	50.0	2.08	0.06	0.16	0.56	0.16

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

3.2.3. Mineral Nutrient Use Efficiency (NUE)

Irrespective of $[CO_2]$ and PPFD, VB 1117 genotype was very efficient in NUE of absorbed P, Ca, Mg, B, Cu, Fe, Mn, and Zn, whereas No 81 was efficient in use of absorbed N and K (Table 7). However genotypic effects were significant only for NUE of P, K, Mg and Zn. In many crop cultivars, interspecific variation in NUE for macro and micro nutrients is well documented and such variations have been related to absorption, translocation, shoot demand, and dry matter production potentials per unit of nutrient absorbed [44] [73] [74] [75]. Irrespective of

Table 7. The effect of $[CO_2]$ and photosynthetic photon flux density (PPFD) on mineral nutrient use efficiency of three cacao genotypes.

$[CO_2]$ (μmol·mol^{-1})	PPFD (μmol·m^{-2}·s^{-1})	N	P	K	Ca	Mg	B	Cu	Fe	Mn	Zn
		mg shoot mg element in shoot^{-1}					mg shoot mg element in shoot^{-1} (×10^4)				
						CCN 51					
400	100	43.0	239.2	49.2	58.3	139.7	2.49	6.58	3.65	1.15	2.83
	200	41.3	224.9	54.7	56.3	143.7	2.42	5.73	5.04	0.85	3.23
	400	50.8	247.2	61.6	55.4	130.5	3.25	5.98	3.53	2.40	3.51
700	100	45.1	227.1	47.6	56.4	160.8	2.77	5.25	4.15	1.15	2.83
	200	50.1	238.7	53.0	56.3	129.8	3.04	5.32	3.94	1.02	2.78
	400	83.5	268.4	79.4	68.3	137.7	6.37	9.04	3.17	3.02	4.82
						VB 1117					
400	100	46.3	309.8	49.6	166.8	140.7	2.15	10.95	4.82	0.75	1.93
	200	45.3	291.1	52.8	59.0	142.2	2.60	6.24	4.27	1.05	2.59
	400	48.8	313.5	57.2	65.3	154.1	3.46	7.58	4.26	1.28	2.85
700	100	51.2	304.9	50.0	67.5	156.5	2.77	6.93	4.06	1.36	3.07
	200	63.8	276.5	64.7	72.6	154.7	4.31	7.57	4.79	2.16	4.75
	400	82.1	278.0	76.4	80.2	170.4	7.14	9.71	4.78	2.93	4.74
						NO 81					
400	100	46.4	250.0	52.3	53.4	151.9	2.38	6.06	4.62	0.94	1.93
	200	47.7	235.3	62.2	54.6	164.9	2.98	6.90	4.35	1.20	2.67
	400	54.3	252.1	76.9	52.2	142.0	3.82	6.75	4.70	1.45	2.72
700	100	48.6	246.7	51.0	113.4	154.1	2.54	9.40	4.72	1.16	2.20
	200	67.7	238.7	64.8	55.5	139.1	3.84	7.13	3.89	1.76	3.27
	400	84.4	260.5	78.3	63.5	133.3	5.79	7.73	2.79	2.84	3.58
Significance											
Genotype (G)		NS	**	**	NS	*	NS	NS	NS	NS	**
$[CO_2]$ (C)		**	NS	**	NS	NS	**	NS	NS	**	**
PPFD (P)		**	NS	**	NS	NS	**	NS	NS	**	**
$LSD_{0.05}$		25.4	88.7	14.9	126.4	39.3	2.68	7.01	2.44	1.72	1.58

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

PPFD, increasing $[CO_2]$ from 400 to 700 μmol·mol^{-1} increased NUE for all the nutrients but the effects were significant only for NUE of N, K, B, Mn and Zn. Baligar *et al.* [40] report that increasing $[CO_2]$ from 380 to 700 μmol·mol^{-1} increased NUE for N, Mg, Cu, Mn and Zn in cacao.

With the exceptions of Ca and Mg, irrespective of $[CO_2]$, increasing PPFD from 100 to 400 μmol·m^{-2}·s^{-1} invariably increased NUE for other nutrients but the effect was significant only for NUE of N, K, B, Mn and Zn. Baligar *et al.* [43]

reported that in seven genetically different cacao genotypes increasing PPFD from 100 to 400 μmol·m^{-2}·s^{-1} and increasing [CO_2] from 380 to 700 μmol·mol^{-1} increased NUE for all macro and micro nutrients. Generally, C_3 plants respond positively to increased [CO_2] above 370 μmol·mol^{-1} [65] [67] [68].

Loss of vegetative cover has increased light levels in cacao plantations and coupled with the anticipated increase in [CO_2] might alter the ability of cacao to utilize absorbed nutrients efficiently. Such changes might lead to higher fertilizer inputs to maintain yield potentials, thereby increasing the cost of cacao production. NUE values are useful in assessing the ability of cacao genotypes to use absorbed nutrients efficiently or non-efficiently. Such assessments are especially valuable in the identification of nutrient use efficient genotypes that are useful for degraded and low fertility tropical soils where adequate availability of essential nutrients is a constraint for growth and survivability of cacao. Cacao genotypes that have high NUE for essential nutrients under low PPFD, which is common for cacao grown under agroforestry systems, might be able to grow well and produce higher yields.

4. Conclusion

Cacao is invariably established as understory plant and is subjected to wide variations in light intensities depending upon nature and density of shade trees. Quality of light reaching cacao leaf canopy in greenhouse conditions is different than quality of light received by cacao grown under shade of upper story shade trees in field conditions. Therefore, the following conclusions are based on juvenile cacao genotypes subjected to ambient and elevated levels of [CO_2] at low to adequate levels of PPFD under greenhouse conditions. Intraspecific variations were observed in cacao genotypes for growth and nutrient uptake and use efficiency traits at varying levels of [CO_2] and PPFD. Increasing levels of [CO_2] and adequate PPFD are beneficial in improving cacao growth and mineral nutrient uptake and use efficiency. Overall, NO 81 genotype had a higher value for all the growth parameters and VB 1117 genotype was most efficient in nutrient use as compared to other two genotypes. Such information might be useful in identification of cacao genotypes suitable for heavy shaded cacao agroforestry or open canopy cacao cultivation systems.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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