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The Impact of Carbon Dioxide Concentrations and Low to Adequate Photosynthetic Photon Flux Density on Growth, Physiology and Nutrient Use Efficiency of Juvenile Cacao Genotypes

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Abstract: Cacao (*Theobroma cacao* L.) was grown as an understory tree in agroforestry systems where it received inadequate to adequate levels of photosynthetic photon flux density (PPFD). As atmospheric carbon dioxide steadily increased, it was unclear what impact this would have on cacao growth and development at low PPFD. This research evaluated the effects of ambient and elevated levels carbon dioxide under inadequate to adequate levels of PPFD on growth, physiological and nutrient use efficiency traits of seven genetically contrasting juvenile cacao genotypes. Growth parameters (total and root dry weight, root length, stem height, leaf area, relative growth rate and net assimilation rates increased, and specific leaf area decreased significantly in response to increasing carbon dioxide and PPFD. Increasing carbon dioxide and PPFD levels significantly increased net photosynthesis and water-use efficiency traits but significantly reduced stomatal conductance and transpiration. With few exceptions, increasing carbon dioxide and PPFD reduced macro–micro nutrient concentrations but increased uptake, influx, transport and nutrient use efficiency in all cacao genotypes. Irrespective of levels of carbon dioxide and PPFD, intraspecific differences were observed for growth, physiology and nutrient use efficiency of cacao genotypes.

Keywords: water use efficiency; nutrient uptake; influx and transport; net photosynthesis; stomal conductance; relative growth rate

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1. Introduction

Cacao (*Theobroma cacao* L) is native to the understory of the Amazonian forests of South America. As an understory plant, it has physiological characteristics similar to those of other shade-adapted species [1–4]. Growth and development of young cacao trees are better under shade; however, heavy shade is detrimental to growth and production of matured and older trees [5–8]. Cocoa is a C_3 species and prefers full sun, but is tolerant to moderate shading, due to its phenotypic plasticity for acclimatization in moderate shade conditions [9]. However, it does not tolerate dense shade, where pod production is low, even with adequate water levels and mineral nutrients availability in the soil. However, when the cacao tree is grown in full sun, there can be no limitations of water and mineral nutrients in the soil. In a long-term field study in Ghana, Amelonado cacao trees in full sun yielded three times as much as shaded trees; however, the economic life of unshaded trees did not last more than 10 years of intensive cropping due to infestation of diseases and insects and loss of needed soil nutrients [10]. There is no universal agreement on the

degree of shade required to maximize production of cacao grown under different tropical ecosystems of the world [9,11–13].

In the major cacao growing regions of South and Central America, cacao is often grown as an understory plant in agroforestry systems (AFSs) [9,14,15]. In AFSs, various types of managed and unmanaged single and multi-strata systems are used where cacao is planted together with different types of shade trees, such as timber, fruit, firewood and leguminous trees, and in some cases tree species retained from thinned native forests [16–22]. In these management systems, cacao is subjected to various levels of low light quantity and quality at its canopy level depending upon the density of single or multi-strata shade trees, the nature and level of vegetative cover and the extent of shade tree pruning [12,23,24]. Shade trees in multi-strata AFSs are known to moderate the microclimatic conditions thereby improving cacao sustainability and providing other sources of income for farmers [6,17,24–26].

The amount of light falling on a cacao tree is known to affect its growth and yield, and moderate shade tends to reduce water and nutrient stress [6,12,27]. Optimum growth of young cacao plants was achieved at 20% to 30% of full sunlight [1,28,29]. However, maximum yield of adult plants requires limited shade or full sun especially in areas of ecosystems with heavy cloud cover [8]. Maximum photosynthesis in cacao leaves occurs at a PPFD of 350 to 550 μmol m^{-2} s^{-1}, which is about 20% to 25% of the intensity of full sunlight [30–33]. In some young cacao genotypes, an increase of PPFD from 50 to 400 μmol m^{-2} s^{-1} increased the net photosynthetic rate (P_N) by about 50%, but further increases (up to 1500 μmol m^{-2} s^{-1}) had no effect, indicating that very little radiant energy is required to support efficient P_N in cacao [30].

In shaded cacao plantations in Bahia Brazil, light intensity at noon above the cacao canopy ranged between 30% and 100% of full daylight [34]. Niether et al. [24] reported that cacao received 39% of full sunlight in an agroforestry system in Bolivia. Increasing PPFD from 65 to 1050 μmol m^{-2} s^{-1} reduced the growth and concentrations of several macro–micro nutrients in cacao [35]. Depending on the photosynthetic characteristics of the shade tree canopy and its density, different levels of blue and red light are absorbed and/or transmitted; therefore, light reaching field grown understory plants could be low in photosynthetically active radiation and with low R/FR ratio [36,37].

The concentration of CO_2 [CO_2] in the atmosphere also affects growth of cacao. The present CO_2 concentration is around 400 μmol mol^{-1} and based on the Representative Concentration Pathway selected (RCP of 4.5 to 8.5) and future emission scenarios, CO_2 could reach as high as 550 to 1370 μmol mol^{-1} by the end of the 21st century [38,39].

Overall, elevated [CO_2] increases plant growth (shoot and root biomass, leaf and root area, RGR) and physiological parameters (photosynthesis, water use efficiency, and nutrient uptake), however, the magnitude of such responses is dependent on availability of water and nutrients, and environmental variables such as light and temperature [1,30,35,40–46]. In cacao, increasing [CO_2] increased shoot, root and leaf growth, macro–micro nutrient use efficiency, photosynthesis and water use efficiency (WUE) traits; however, the magnitude of such responses to increased [CO_2] in cacao depended on the levels of PPFD and genotypes involved [30,35,46,47].

Soils in the cacao growing regions of the world are often acidic, infertile and invariably deficient in nitrogen (N), potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), zinc (Zn) and iron (Fe), and that leads to severe essential nutrients deficiencies in cacao [11,12,29,48–52]. Increasing atmospheric [CO_2] coupled with low soil fertility and low irradiance subject cacao to severe nutrient stress and results in decline of yield potentials. Cacao has considerable genetic variation in morphological and physiological traits [4,53–55]. Such traits could be exploited in the selection of genotypes that have higher essential nutrient use efficiency under these abiotic stresses to generate cultivars more adapted to these conditions. Interactions between genotype and environmental factors may allow some genotypes to perform better in changing PPFD and CO_2 conditions. The objectives of this research were to assess the influence of ambient and elevated levels of [CO_2] and low to adequate photosynthetic photon density (PPFD) on the growth, and phys-

iological traits and macro–micro nutrient uptake, influx and transport and use efficiency in seven genetically contrasting cacao genotypes.

2. Materials and Methods

2.1. Cacao Genotypes

In total, 7 cacao genotypes (Catongo, Coca 3370/5, CCN 51, Amaz 15, LCT EEN 37/A, Na 33 and SCA 6) were used for this study. Pods of these genotypes were received from MARS Center for Cocoa Science (MCCS) Almirante, Itajuipe, Bahia, Brazil. Catongo is from the lower Amazon region of Brazil; Amaz 15, NA 33 and SCA 6 are from the upper Amazon region of Peru; whereas LCT EEN 37A and Coca 3370/5 are from the upper Amazon region of Ecuador; and CCN 51 is a hybrid from Ecuador. These genotypes have been widely distributed in most of the cacao producing countries and some have been commonly used as parental or as cultivars in cacao breeding programs. Genetic background, origin and diseases resistance of these genotypes are covered in Bartley [56], Turnbull and Hadley [57], and Ahnert and Eskes [58]. Seeds were produced by self-pollination of plants. In the case of self-incompatible plants, they were obtained by the mixture of Herrania and cacao pollen, which helps to break self-incompatibility. Therefore, the self-pollinated family plants generated by such seeds have, on an average, similar traits to the parents, in this case, clonal cuttings. Findings of this study had a good scientific interest, showing differences between different genetic populations.

2.2. Plants and Growth Medium

Growth medium was prepared containing sand: perlite: peat moss (2:2:1 volume) supplemented with essential nutrients (mg/kg) 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, USA), triple superphosphate, urea, calcium sulphate, dolomitic lime and Scott's Micromix. Cacao seeds were removed from the pods, surface-sterilized with 10% bleach 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. Seeds with 2 mm radicle were planted in 3.8 L black plastic pots with adequate bottom drainage containing 2.2 kg of the growth mixture. One seedling was planted in each pot. Soil moisture was maintained near field capacity (−33kPa) by adding water every other day. An initial plant harvest was collected at 21 days after planting. The remaining plants were grown for additional 90 days.

2.3. CO_2 and PPFD Treatments

The experiment was conducted in two glasshouses (18 m^2 each) at Beltsville, MD and plants were grown with day/night temperatures of 30/28 °C. In the first glasshouse, ambient [CO_2] of 400 ± 50 µmol mol^{-1} was maintained and in the second glasshouse elevated [CO_2] of 700± 50 µmol mol^{-1} was maintained throughout the growth period. In the second glasshouse if [CO_2] fell below 700 µmol mol^{-1} a WMA4 CO_2 analyzer (PP Systems, Amesbury, MA, USA) injected the desired amount of CO_2. After 55 days of growth, plants were swapped from one glasshouse to the other and [CO_2] levels were readjusted in each glasshouse as per the treatments. Within each glasshouse, electrical fans continuously circulated the air at an air speed of 0.5 m s^{-1} over the plants. Daytime air temperatures were maintained for 12h per day beginning at 6 AM. The greenhouses transmitted approximately 60% of the incident PPFD daily. A data logger (21x, Campbell Scientific, Logan, UT, USA) recorded the PPFD, temperature and [CO_2] in both glasshouses at 30–s intervals.

In both glasshouses, plants were grown at three levels of photosynthetic photon flux density (PPFD) (100 ± 20, 200 ± 20 and 400 ± 20 µmol m^{-2} s^{-1}). To achieve these three levels of irradiance, mini-chambers were constructed with 2 cm (3/4 inch) diameter PVC pipe with overall dimensions of 114 cm W × 119 cm L × 81 cm H (45″ × 47″ × 32″). To achieve three different levels of PPFD, the tops and sides of the mini chambers were

covered with three types of plastic mesh shade cloth: a single-ply of 70% smoke blue sun screen fabric (Easy Gardener, Waco TX) for low PPFD (100 μmol m^{-2} s^{-1}), a single-ply of black fiberglass window screen (New York Wire, Mt. Wolf, PA, USA) for medium PPFD (200 μmol m^{-2} s^{-1}) and a single-ply of 22% white shade cloth (National Tool Grinding, Inc, Erie, PA, USA) for high PPFD (400 μmol m^{-2} s^{-1}). Each mini chamber was covered with mesh shade cloth so they have full air exchange with the environment. In each mini chamber the plants were rotated once per week to keep the light exposures consistent. The light levels in each mini chamber were measured at mid-day with a LI-190S quantum sensor (Li-Cor Inc., Lincoln, NE, USA). All experimental units were replicated three times and each experimental unit had a control pot with no plant in order to quantify evaporation.

2.4. Determination of Plant Physiological Parameters

A week before plant harvest, net photosynthesis [P_N, μmol CO_2 m^{-2} s^{-1}], stomatal conductance [gs, mmol H_2O m^{-2} s^{-1}], internal leaf CO_2 [Ci, μmol mol^{-1}] and rate of transpiration [E, mmol H_2O m^{-2} s^{-1}] were measured on the fully expanded sixth leaf from top of each plant using a CIRAS-2 Portable Photosynthesis System (PP Systems, Amesbury, MA, USA). The artificial light source was adjusted to the PPFD of the treatments (100, 200 and 400 μmol m^{-2} s^{-1}). The CO_2 flux was adjusted to 400 or 700 μmol mol^{-1} depending on treatment. The leaf chamber temperature was constant at 30°C. Readings were recorded after 15 min of equilibration. A SPAD meter (Konica Minolta Chlorophyll Meter, Model 502, Ramsey, NJ, USA) was used to determine SPAD index which could be useful to estimate the chlorophyll content of the leaves.

Water Use Efficiency (WUE) was determined by the following equations:

Total Water Use Efficiency, $WUE_{Total} = g$ shoots dry wt. $plant^{-1}$/g H_2O transpired $plant^{-1}$ over 90 days of growth

Instantaneous water use efficiency, $WUE_{Inst} = P_N/E$, μmol CO_2/mmol H_2O

Intrinsic water use efficiency, $WUE_{Intr} = P_N/gs$, μmol CO_2/mmol H_2O

Where P_N (μmol CO_2 m^{-2} s^{-1}) is net photosynthetic rate, E (mmol H_2O m^{-2} s^{-1}) is transpiration rate, and gs (mmol H_2O m^{-2} s^{-1}) is stomatal conductance. These parameters were obtained from CIRAS-2 portable photosynthesis system measurements.

2.5. Determination of Plant Growth Parameters

After a growth period of 90 days, plants were harvested. Shoots were divided into stems and leaves and weighed. Total leaf area (cm^2) was measured using a LI-3100 leaf area meter (Li-Cor Inc., Lincoln, NE, USA). Shoots were washed in deionized water, freeze-dried and dry weight was recorded. The roots were removed from the soil, washed, blotted dry and weighed. Root lengths (cm $plant^{-1}$) were determined with a Comair Root Length Scanner (Hawker de Haviland, Melbourne, Victoria, Australia) and the roots were oven-dried at 70°C for 5 days and the dry weights were recorded.

Additional growth parameters were calculated by the following formulas:

Leaf area ratio (LAR, cm^2/g) = [total leaf area, cm^2/shoot+ root dry wt, g]

Specific Leaf Area (SLA, cm^2/g) = [Total leaf area/plant, cm^2/Total leaf dry wt./plant, g]

Leaf mass/unit leaf area (LMA, g/cm^2) = [1/SLA]

Root/shoot ratio (R/S) = [Wr/Ws], where Wr is root dry wt. and Ws is shoot dry wt.

Root Radius (RR, cm) = $(RFW/RL \times \pi)^{1/2}$ where RFW is root fresh wt. (cm^3)

Relative growth rate (RGR, g g^{-1} day^{-1}) = [ln $(Wt_2/Wt_1)/(T_2-T_1)$], where Wt is total wt. (shoot+root), T is time in days, subscripts 1 and 2 refer to initial and final plant harvest.

Net assimilation rate (NAR, g cm^{-2} day^{-1}) = [RGR/LAR]

2.6. Determination of Nutrient Uptake Parameters

Dried stems and leaves were ground together to pass through a 1 mm sieve and sent to University of Florida, Indian River Research and Education Center (UF-IRREC), Fort Pierce, FL, USA. for macro–micro nutrient analysis. Plant samples of 0.4 g were digested in 5 mL of concentrated nitric acid (14 N), and macro–micro nutrient concentrations in

the digested solutions were determined by using inductively coupled plasma optical emission spectrometry (ICPOES, Ultima JY Horiba Inc. Edison, NJ, USA) [59]. Total N in the plant tissue was analyzed by combustion method using a CN Analyzer (Vario MAX CN Elementar Analysensysteme GmbH, Hanau, Germany) [60].

Nutrient uptake (U), influx (IN), transport (TR) and nutrient use efficiency ratios (ER) were calculated using the following formulas:

Uptake (U) = (Conc. of any given element) × shoot dry wt.

Influx (IN) = $[(U_2 - U_1)/(T_2 - T_1)]$ $[(\ln Wr_2 - \ln Wr_1)/(Wr_2 - Wr_1)]$, where U refers to elemental content in shoot (mmol/plant), T is time in seconds, Wr is root dry wt., and subscripts 1 and 2 refer to initial and final plant harvest times.

Transport (TR) = $[(U_2 - U_1)/(T_2 - T_1)]$ $[(\ln Ws_2 - \ln Ws_1)/(Ws_2 - Ws_1)]$, where Ws is shoot dry weight.

Nutrient Use Efficiency (NUE) = [mg of Ws/mg of any given element in shoot]

2.7. Statistical Analysis

Experiment was split plot design with [CO_2] as main plots, PPFD as subplots and genotypes as sub-sub plots and experimental units were replicated three times. All data were analyzed for statistical significance by ANOVA in SAS (Ver. 9.3, SAS Institute, Cary, NC, USA).

3. Results and Discussion

3.1. Growth Traits

Irrespective of [CO_2] and PPFD, significant intraspecific differences between cacao genotypes were observed for total and root wt., root length, stem height, leaf area, specific leaf area, relative growth rate (RGR) and net assimilation rates (NAR) (Table 1). Overall, Amaz 15 genotype had higher total and root growth parameters than any of the other genotypes studied. Genetic, physiological and morphological determinants and their interactions with environmental variables such as levels of PPFD and [CO_2] profoundly influence the growth, development and nutrient use efficiency of cacao [9,30,35,46]. Variation in morphological characteristics among cacao genotypes has been reported [4,53–55] and these morphological characteristics are known to be influenced by levels of PPFD and [CO_2] [1,13,35,45,46,61].

Table 1. The effect of [CO_2] and photosynthetic photon flux density (PPFD) on shoot and root growth, leaf growth, relative growth rate (RGR) and net assimilation rate (NAR) of seven cacao genotypes.

CO_2 (µmol mol^{-1})	PPFD (µmol m^{-2} s^{-1})	Total Dry Weight (g/plant)	Root Dry Weight (g/plant)	Root/ Shoot Ratio	Stem Height (cm/plant)	Total Root Length (cm/plant)	Leaf Area (cm^2/plant)	Specific Leaf Area (cm^2 g^{-1})	RGR (g g^{-1} d^{-1}) (× 10^{-2})	NAR (g cm^{-2} d^{-1}) (× 10^{-4})
Catongo										
400	100	7.59	0.91	0.136	34.33	3594	1712	316.8	2.899	1.287
	200	7.87	1.11	0.167	32.33	3642	1391	266.3	2.930	1.662
	400	6.50	0.83	0.146	29.17	3342	1087	243.9	2.730	1.635
700	100	12.64	2.02	0.190	41.00	4265	2063	268.7	3.696	2.290
	200	15.44	2.61	0.204	45.17	4787	2277	246.8	3.884	2.808
	400	14.16	2.17	0.180	39.33	4566	1616	207.6	3.770	3.271
Coca 3370										
400	100	7.28	1.06	0.174	30.50	2661	1499	300.1	2.884	1.414
	200	10.89	1.61	0.172	34.50	4257	1926	264.8	3.336	1.886
	400	13.34	1.80	0.155	35.33	4104	2225	245.1	3.558	2.156
700	100	12.78	1.70	0.154	38.00	4196	2396	279.9	3.318	1.827
	200	19.87	2.31	0.129	48.67	5045	3454	267.9	3.758	2.167
	400	21.07	2.33	0.116	44.00	4938	3479	244.9	3.829	2.296

Table 1. *Cont.*

CO_2 (µmol mol^{-1})	PPFD (µmol m^{-2} s^{-1})	Total Dry Weight (g/plant)	Root Dry Weight (g/plant)	Root/ Shoot Ratio	Stem Height (cm/plant)	Total Root Length (cm/plant)	Leaf Area (cm^2/plant)	Specific Leaf Area (cm^2 g^{-1})	RGR (g g^{-1} d^{-1}) (× 10^{-2})	NAR (g cm^{-2} d^{-1}) (× 10^{-4})
CCN 51										
400	100	5.55	0.72	0.140	23.83	2072	1278	344.1	2.436	1.077
	200	8.04	0.94	0.130	30.50	2735	1722	301.5	2.977	1.405
	400	9.32	1.19	0.137	32.00	2752	1697	280.0	3.116	1.731
700	100	13.82	1.96	0.164	34.17	4205	2705	300.7	3.530	1.797
	200	16.68	2.13	0.149	42.33	4552	3092	288.8	3.721	1.995
	400	23.60	2.46	0.118	49.33	5925	3578	231.6	4.109	2.741
Amaz 15										
400	100	8.45	1.01	0.135	32.83	3006	1706	284.3	3.105	1.542
	200	11.66	1.53	0.149	34.00	4214	2212	271.3	3.457	1.911
	400	10.50	1.21	0.128	30.33	3400	1765	248.8	3.215	1.878
700	100	19.90	2.78	0.163	48.17	5682	3270	262.3	4.043	2.473
	200	23.84	2.97	0.141	54.50	5848	3616	241.9	4.229	2.780
	400	27.58	3.99	0.168	53.67	6436	3120	192.9	4.401	3.896
LCT EEN 37A										
400	100	3.51	0.42	0.145	15.50	1548	740	268.4	2.101	1.0221
	200	4.99	0.51	0.113	21.50	1875	841	231.2	2.561	1.544
	400	4.25	0.43	0.127	20.33	1833	708	215.1	2.287	1.426
700	100	15.64	1.73	0.124	39.50	4807	3155	288.4	3.909	1.978
	200	16.32	2.07	0.142	39.83	4627	2481	237.4	3.923	2.594
	400	18.81	2.09	0.125	44.00	5576	2731	210.9	4.099	2.858
Na 33										
400	100	7.34	0.86	0.134	34.83	3008	1284	256.6	2.848	1.654
	200	9.20	0.95	0.113	34.33	3541	1644	251.3	3.082	1.739
	400	5.77	0.63	0.114	27.17	2522	925	236.3	2.515	1.575
700	100	8.95	0.85	0.103	36.00	2575	1666	264.6	3.335	1.793
	200	21.51	2.80	0.144	53.33	5724	3009	225.9	4.292	3.058
	400	24.04	2.61	0.122	54.00	6375	2852	186.6	4.431	3.753
SCA 6										
400	100	6.81	0.77	0.126	28.67	2317	1418	293.2	3.207	1.560
	200	6.77	0.80	0.134	27.17	2457	1235	257.9	3.189	1.744
	400	9.46	1.13	0.140	29.00	3327	1412	221.4	3.564	2.391
700	100	14.64	1.97	0.156	44.50	4263	2297	258.4	4.085	2.624
	200	18.22	2.30	0.146	46.33	4169	2696	237.0	4.322	2.918
	400	22.68	2.93	0.147	51.00	5899	3194	222.1	4.549	3.337
Significance										
Genotype (G)		**	**	**	**	**	**	**	**	**
$[CO_2]$ (C)		**	**	NS	**	**	**	**	**	**
PPFD (P)		**	**	NS	**	**	*	**	**	**

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

In the current study with exception of root/shoot ratio, all the growth traits of shoots and roots in cacao genotypes were significantly influenced by the level of $[CO_2]$. Irrespective of PPFD levels, increasing $[CO_2]$ from 400 to 700 µmol mol^{-1} increased all growth traits except SLA which decreased with increasing $[CO_2]$. In many perennial tropical legume cover crops Baligar et al. [62,63] reported that increasing $[CO_2]$ from ambient (400 µmol mol^{-1}) to elevated (700 µmol mol^{-1}) increased growth traits (dry biomass of shoot, leaf and roots, RGR and NAR). Generally, C_3 plants respond positively to increased $[CO_2]$ above 370 µmol mol^{-1} [64–66]. In the current study, increasing $[CO_2]$ significantly increased total leaf

area in all the genotypes. Lahive et al. [46] reported increased leaf area in Amelonado cacao genotype grown at elevated [CO_2], however, in a recent study, Hebbar et al. [47] found no significant differences in leaf area between cacao grown at 400 and 700 µmol mol^{-1} [CO_2]. In the current study, increasing [CO_2] from 400 to 700 µmol mol^{-1} increased average root dry weight and root length by 0.97 to 2.32 g $plant^{-1}$ and 2962 to 4974 cm $plant^{-1}$ respectively. At elevated [CO_2], it seems that allocation of carbon fixed by photosynthesis to the roots is as high as that to the shoots. Elevated [CO_2] often increases the R/S ratio and fine-root proliferation [43].

In all the cacao genotypes studied, all the growth parameters were significantly influenced by levels of PPFD. Shade tolerant species including cacao are known to respond positively to elevated [CO_2], however such enhanced growth response is also governed by light levels [35,46,67,68]. Irrespective of levels of [CO_2], increasing PPFD from 100 to 400 µmol m^{-2} s^{-1} increased growth traits (total and root weight, stem height, root length, total leaf area, RGR and NAR). However specific leaf area (SLA) was reduced with increasing PPFD indicating that increasing PPFD increases the thickness of the leaves. In cacao genotypes, heavier shade may increase leaf area [19]. Such an adaptation seems to maximize the photon capture capacity of the leaves [45]. Irrespective of [CO_2], increasing PPFD from 100 to 400 µmol m^{-2} s^{-1} increased average root weight and root length by 1.34 to 1.84 g $plant^{-1}$ and 3443 to 4357 cm $plant^{-1}$, respectively. This indicates an increased allocation of carbon fixed through photosynthesis to roots at higher PPFD. Aerial morphological characteristics could have great implications on the ability of plants to intercept and utilize solar radiation and these characteristics in cacao are influenced by level of irradiance [1,9,13,35,45,61].

3.2. Physiological and Water Use Efficiency Traits

Significant intraspecific differences were observed for SPAD index, net photosynthesis (P_N), stomatal conductance (gs), internal CO_2 (Ci) and transpiration (E) irrespective of levels of [CO_2] and PPFD (Table 2). Amaz 15 had higher P_N than any other cacao genotype at all levels of [CO_2] and PPFD evaluated. This genotype also had the highest leaf area per plant. Increasing [CO_2] from ambient to 700 µmol mol^{-1} has been shown to increase P_N in C_3 plants [44]. In the current study irrespective of PPFD levels, increasing [CO_2] from 400 to 700 µmol mol^{-1} resulted in a significant increase in P_N of all cacao genotypes from an average of 2.47 to 3.41 µmol CO_2 m^{-2} s^{-1}. In an earlier study with 1.5-year-old cacao plants, increasing [CO_2] from 370 to 680 µmol mol^{-1} resulted in a 33% increase in P_N [30]. In cacao genotype Amelonado, increasing [CO_2] from 460 to 735 µmol mol^{-1} increased P_N by 56% [46]. Recently Hebbar et al. [47] in an open top camber study with cacao reported an increase in P_N of 29% by increasing [CO_2] from 400 to 700 µmol mol^{-1}. Increasing photosynthesis with increasing [CO_2] reported in these studies is typical of responses observed in other C_3 plants [69,70].

Levels of aerial [CO_2] have significant effects on gs activity. In all the cacao genotypes studied, irrespective of PPFD levels, increasing [CO_2] from 400 to 700 µmol mol^{-1} resulted in a significant reduction in gs from an average of 19.5 to 12.6 mmol H_2O m^{-2} s^{-1}. In leaves of annual C_3 plants, doubling of [CO_2] reduced gs by 34% [69]. In an earlier study with cacao genotypes, Baligar et al. [30] reported around a 65% reduction in gs by increasing [CO_2] from 370 to 700 µmol mol^{-1}. Such a large decrease in gs led to a substantial reduction in E, which could improve cacao water status and drought resistance. Elevated [CO_2] has been shown to reduce E and gs in most C_3 plants [69]. However, Lahive et al. [46] reported that CO_2 concentrations of ambient (average of 466 µmol mol^{-1}) and elevated (average of 725 µmol mol^{-1}) did not have an effect on gs in cacao genotype Amelonado. Stomatal conductance (gs) plays a vital role in regulating P_N, transpiration (E), leaf temperature and plant water stress tolerance [13,71,72].

Irrespective of the levels of PPFD, increasing [CO_2] from 400 to 700 µmol mol^{-1} resulted in a significant reduction in transpiration (E) from an average of 0.267 to 0.172 mmol m^{-2} s^{-1}. A large decrease in gs, as observed in the current study, with increasing [CO_2]

could lead to reduced E and such changes could improve water status and drought resistance of cacao. Baligar et al. [30] reported that increasing [CO_2] from 85 to 850 µmol mol^{-1} significantly decreased E from 0.66 to 0.16 mmol m^{-2} s^{-1} in three cacao genotypes.

It has been widely reported that maximum photosynthesis (P_N) in cacao occurs at PPFD of 350 to 550 µmol m^{-2} s^{-1} [30–33]. The limited PPFD received at cacao canopy levels might be the reason for lower yields in agroforestry systems [73]. In the seven cacao genotypes in the current study, irrespective of [CO_2], increasing levels of PPFD from 100 to 400 µmol m^{-2} s^{-1} resulted in significant increases in P_N from an average of 2.67 to 3.41 µmol m^{-2} s^{-1}. In an earlier study with three genetically differing cacao genotypes, Baligar et al. [30] reported that increasing PPFD from 50 to 400 µmol m^{-2} s^{-1} significantly increased P_N. However, P_N at 50 µmol m^{-2} s^{-1} of PPFD was about two-thirds of the maximum 3 µmol CO_2 m^{-2} s^{-1} indicating that cacao needs very little radiant energy to support its P_N. Higher rates of P_N, thicker leaves and high rates of E have been observed in certain cacao genotypes when grown in full sunlight rather than under shade [1]. Increasing PPFD from 100 to 400 µmol m^{-2} s^{-1} reduced specific leaf area from an average of 284.7 to 227.7 cm^2 g^{-1}, such increases in leaf thickness might contribute to higher P_N. However, exposure of leaves to extremely high light for longer periods may lead to photoinhibition and lower P_N [34,41,42]. Baligar et al. [35] reported that PPFD of 1050 µmol m^{-2} s^{-1} was detrimental to shoot, root and leaf growth of cacao seedlings. In all the cacao genotypes studied irrespective of levels of [CO_2], increasing levels of PPFD from 100 to 400 µmol m^{-2} s^{-1} resulted in significant increases in gs and E from an average of 15.33 to 19.35 mmol H_2O m^{-2} s^{-1} and 0.215 to 0.258 mmol H_2O m^{-2} s^{-1}, respectively. In an earlier short-term study, Baligar et al. [30] reported that the gs was not significantly affected by PPFD over the observed range of 50 to 400 µmol m^{-2} s^{-1}; however, there was a slight increase in E, but the relationship between E and PPFD was not significant. Under artificial shade, the quality of the PPFD that reaches the canopy of cocoa leaves is very different from the quality of the PPFD that reaches the canopy of cocoa trees leaves grown in field conditions and shaded by tree species. In field conditions, there is an attenuation of both the intensity and the quality of the light available for cocoa photosynthesis, depending on the greater or lesser absorbance and/or transmittance of electromagnetic light, mainly in the blue and red bands, which crosses canopy strata of different shade tree species. Depending on the photosynthetic characteristics of the shade tree canopy, different levels of PPFD blue and red light are absorbed and/or transmitted, which can affect net photosynthesis differently from cocoa grown under artificial shade. Therefore, obtained results of P_N are based on cacao genotypes subjected to ambient and elevated levels of [CO_2] under various levels of artificial shade.

Table 2. The effect of [CO_2] and photosynthetic photon flux density (PPFD) on photosynthesis and its components, and water use efficiency of seven cacao genotypes.

CO_2 (µmol mol^{-1})	PPFD (µmol m^{-2} s^{-1})	SPAD Index	Photosynthesis (µmol CO_2 m^{-2} s^{-1})	Stomatal Conductance (mmol H_2O m^{-2} s^{-1})	Internal CO_2 (µmol mol^{-1})	Transpiration (mmol H_2O m^{-2} s^{-1})	WUE_{Total} (g shoot/g trans.) ($\times 10^{-3}$)	WUE_{Inst} ¥ (µmol CO_2/mmol H_2O)	WUE_{Intr} ¥ (µmol CO_2/mmol H_2O)
					Catongo				
400	100	42.3	2.64	20.66	157.6	0.291	8.34	9.17	0.132
	200	42.8	1.75	15.36	158.3	0.213	6.22	8.71	0.125
	400	42.4	3.02	25.02	222.8	0.341	23.80	8.90	0.126
700	100	37.7	3.15	10.26	122.3	0.143	18.42	22.03	0.328
	200	42.1	4.34	16.39	192.2	0.218	10.60	20.23	0.269
	400	42.2	5.82	22.57	205.5	0.291	27.75	19.99	0.258

Table 2. *Cont.*

CO_2 (µmol mol^{-1})	PPFD (µmol m^{-2} s^{-1})	SPAD Index	Photosynthesis (µmol CO_2 m^{-2} s^{-1})	Stomatal Conductance (mmol H_2O m^{-2} s^{-1})	Internal CO_2 (µmol mol^{-1})	Transpiration (mmol H_2O m^{-2} s^{-1})	WUE_{Total} (g shoot/g trans.) ($\times 10^{-3}$)	WUE_{Inst} ¥ (µmol CO_2/mmol H_2O)	WUE_{Intr} ¥ (µmol CO_2/mmol H_2O)
Coca 3370									
400	100	43.8	3.21	20.19	106.4	0.284	9.53	11.51	0.163
	200	40.2	2.17	15.88	128.3	0.218	5.51	9.85	0.140
	400	40.2	3.27	24.96	127.1	0.321	7.34	10.02	0.133
700	100	41.9	2.63	12.07	283.1	0.170	19.77	16.18	0.233
	200	46.4	3.83	14.61	190.1	0.207	99.22	19.10	0.267
	400	40.0	3.46	12.66	190.8	0.175	30.54	19.87	0.274
CCN 51									
400	100	42.1	2.19	18.02	236.6	0.267	12.29	9.47	0.146
	200	42.9	1.85	12.58	132.9	0.179	5.26	9.64	0.139
	400	39.4	1.80	20.13	351.2	0.285	5.91	5.96	0.085
700	100	42.2	2.52	8.47	189.3	0.129	15.61	19.58	0.296
	200	45.9	4.52	14.21	135.7	0.192	18.26	22.71	0.313
	400	42.3	3.99	14.88	182.0	0.198	38.23	20.17	0.268
Amaz 15									
400	100	44.4	3.14	22.26	126.6	0.299	10.87	10.78	0.149
	200	40.3	2.90	17.77	81.1	0.238	6.12	12.22	0.166
	400	39.5	3.44	22.41	132.3	0.308	7.33	11.19	0.154
700	100	40.7	3.74	12.44	161.5	0.171	15.20	21.94	0.306
	200	43.0	4.31	14.00	109.7	0.187	11.49	23.31	0.325
	400	38.7	4.25	16.57	222.2	0.216	18.20	20.15	0.270
LCT EEN 37A									
400	100	43.5	3.57	42.11	323.7	0.552	5.57	7.76	0.106
	200	44.1	1.83	11.71	127.7	0.173	15.12	10.48	0.173
	400	38.8	4.16	31.75	190.0	0.422	6.09	9.99	0.135
700	100	43.1	4.14	13.54	141.8	0.190	16.67	21.82	0.305
	200	43.3	2.78	11.34	213.3	0.153	11.49	17.78	0.242
	400	45.4	4.83	17.27	158.3	0.222	30.68	21.23	0.273
Na 33									
400	100	42.1	1.47	11.58	133.4	0.166	14.26	10.30	0.154
	200	38.6	2.14	13.86	109.6	0.196	5.36	10.79	0.158
	400	36.9	1.11	11.83	233.2	0.172	4.29	8.44	0.118
700	100	36.1	2.03	7.64	261.1	0.116	20.66	15.07	0.234
	200	42.3	1.94	7.15	142.5	0.105	8.90	20.79	0.310
	400	34.6	3.10	12.71	181.3	0.168	12.42	19.82	0.257
SCA 6									
400	100	44.8	1.51	10.70	153.6	0.157	9.25	9.32	0.136
	200	42.6	1.77	13.65	150.2	0.194	8.32	9.08	0.130
	400	43.7	2.92	26.40	177.2	0.334	6.07	8.74	0.113
700	100	43.3	1.39	4.73	235.9	0.073	18.85	19.17	0.320
	200	41.7	2.41	9.35	239.7	0.132	12.83	17.48	0.249
	400	36.2	2.51	11.72	246.3	0.158	29.97	16.22	0.217
Significance									
Genotype (G)		*	**	**	NS	**	NS	NS	NS
$[CO_2]$ (C)		NS	**	**	NS	**	**	**	**
PPFD (P)		**	*	**	NS	**	NS	NS	NS

*, ** Significant at 0.05 and 0.01 levels of probability, respectively. NS = Not significant. ¥ Instantaneous water use efficiency, WUE_{Inst} = P_N/E, (µmol CO_2/mmol H_2O); Intrinsic water use efficiency, WUE_{Intr} = P_N/gs, (µmol CO_2/mmol H_2O).

Intraspecific differences in WUE traits (WUE_{Total}, WUE_{Inst} and WUE_{Intr}) between the cacao genotypes were observed but the differences were not significant (Table 2). Amaz 15 had the highest WUE_{Inst} and WUE_{Intr}, which is a reflection of its high P_N compared to the other cacao genotypes. In eight contrasting cacao genotypes, variations in WUE_{Inst} and WUE_{Intr} were negatively related to specific leaf area [4]. In the current study, all three WUE traits increased with decreasing specific leaf area. In the seven cacao genotypes studied, increasing PPFD and $[CO_2]$, increased WUE traits. Increasing $[CO_2]$ from 400 to 700 µmol mol^{-1} caused significant increases in all three water use efficiency traits (WUE_{Total}, WUE_{Inst} and WUE_{Intr}). Such significant increases in WUE_{Inst} and WUE_{Intr} traits at elevated $[CO_2]$ could be related to increased P_N and reduced gs and E [13,74]. Lahive et al. [46] reported significantly greater intrinsic water use efficiency (WUE_{Intr}) in plants grown at elevated CO_2 (average of 725 µmol mol^{-1}) and related such an increase to higher P_N, as there was no difference in the measured gs between ambient and elevated CO_2. In open top chambers, elevated $[CO_2]$ up to 700 µmol mol^{-1} increased P_N by 27% and resulted in high cacao biomass accumulation, and thus improved whole plant WUE [47]. Further Hebbar et al. [47] concluded that higher WUE at elevated $[CO_2]$ was due to high P_N rather than reduced water loss through stomata (E). In the current research with seven contrasting cacao genotypes, increasing $[CO_2]$ from 400 to 700 µmol mol^{-1} significantly increased P_N but gs and E were reduced significantly. Based on these findings, it is concluded that increasing P_N and decreasing gs and E at elevated $[CO_2]$ substantially contributes to the significant increases in WUE_{Inst} and WUE_{Intr} [30,46,74]. Enhanced WUE_{Intr} at elevated $[CO_2]$ is related to maintenance of higher plant water potential (Ψ) through reduced gs and greater fine root production [43]. Reduced gs in elevated $[CO_2]$ may alter plant responses to drought and improve WUE [75].

Irrespective of levels of $[CO_2]$, increasing levels of PPFD from 100 to 400 µmol m^{-2} s^{-1} increased WUE_{Total}, but there were no changes in WUE_{Inst}. Increasing PPFD from 100 to 400 µmol m^{-2} s^{-1} slightly reduced WUE_{Intr} from an average of 0.22 to 0.19 µmol CO_2 mmol H_2O^{-1}. This is a reflection of increases of gs from average of 15.33 to 19.35 mmol H_2O m^{-2} s^{-1} and moderate increases in P_N from average of 2.67 to 3.41 µmol m^{-2} s^{-1}. In other crops, it has been reported that relationships between WUE_{Total} and WUE_{Inst} may be either positive or negative [76]. Increases or decreases in WUE traits with varying PPFD and $[CO_2]$ are determined by increases or decreases of P_N, gs, and E [4,13,46]. As occurrences of drought episodes are becoming more common in tropical cacao regions [77–79], selection of cacao genotypes with high WUE under increasing levels of $[CO_2]$ would be beneficial in sustaining yield potential of cacao in current and future drought prone areas.

3.3. Nutrient Use Efficiency Traits

3.3.1. Nutrient Concentrations and Uptake

Cacao genotypes, irrespective of levels of $[CO_2]$ and PPFD, showed significant differences in macro–micro nutrient concentrations (Table 3). Overall, LCT EEN 37A, compared to the other genotypes, had the highest concentrations of P, K, Ca, Cu and Fe. The concentrations of P, Ca, Mg and Mn were slightly higher, but concentrations of other macro and micronutrients were comparable to the concentrations reported in the literature [50,80,81]. In all the genotypes tested, irrespective of PPFD, increasing $[CO_2]$ from 400 to 700 µmol mol^{-1} significantly reduced macro–micro nutrient concentrations; however, the effect of increasing $[CO_2]$ on Zn concentration was non-significant. This is a reflection of increased dry matter in the shoots (Table 1) of all cacao genotypes with increasing $[CO_2]$ which created dilution effects on the nutrient concentrations. The decline in concentrations of all macro–micro nutrients in cacao genotypes with increasing levels of $[CO_2]$ differed slightly from the conclusion drawn by Dong et al. [82] from meta-analysis of vegetable crops. They concluded that elevated $[CO_2]$ enhanced yield in vegetable crops but decreased the concentration of nitrate, Mg, Fe, and Zn by 18.0, 9.2, 16.0 and 9.4%, respectively, and increased the concentration of Ca by 8.2%. However, the concentration of P, K, S, Cu and Mn in that study were not affected by elevated $[CO_2]$. In Amelonado cacao,

Lahive et al. [46] reported that leaf N content decreased at elevated [CO_2]. In mango leaves, elevated levels of [CO_2] reduced concentrations of several minerals [83]. With the exception of N, Cu and Mn, and irrespective of levels of [CO_2], increasing PPFD from 100 to 400 µmol m^{-2} s^{-1} reduced concentrations of the other macro and micronutrients. However, the effects were only significant for concentrations of N, K, Ca, Mg and Mn. In several tropical perennial cover crop legumes, increasing [CO_2] from 400 to 700 µmol mol^{-1} slightly decreased all the macro–micro nutrient concentrations. However, increasing PPFD from 100 to 450 µmol m^{-2} s^{-1} only slightly decreased concentrations of K, Ca and Fe [62]. In another study with perennial legume cover crops, Baligar et al., [84] reported that increasing PPFD from 200 to 400 µmol m^{-2} s^{-1} significantly 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.

Uptake of all macro–micro nutrients were significantly influenced by genotypes and Amaz 15 had the highest nutrient uptake (Table 4). Overall, increasing levels of [CO_2] from 400 to 700 µmol mol^{-1} and PPFD from 100 to 400 µmol m^{-2} s^{-1} significantly increased uptake of all the macro–micro nutrients. In cacao genotype comum, Baligar et al. [35] reported that increasing [CO_2] from 380 to 700 µmol mol^{-1} increased uptake of all essential nutrients and further stated that such an increase in nutrient uptake at higher [CO_2] is due to increased demand for mineral nutrients due to enhanced dry matter accumulation. The overall nutrient accumulation in the current study was in the order of N > Ca >K >Mg > P for macro nutrients and Mn > Zn > Fe > B > Cu for micronutrients.

3.3.2. Nutrient Influx (IN) and Transport (TR)

In most of the cacao growing regions, cacao is often grown in infertile acidic soils and is subjected to the high temperature and radiation common with low soil moisture levels. Such climatic stresses could have major effects on the ability of plants to influx (IN) nutrients from soil through the roots and to transport (TR) these essential nutrients to shoots. In addition to these stresses, increasing atmospheric concentrations of [CO_2] could aggravate rates of IN and TR by increasing transpiration losses and photosynthesis. However, very limited information is available on how increasing levels of [CO_2] and low to adequate levels of PPFD affect IN and TR of macro–micro nutrients in cacao. In the current study, IN for all macro and micro nutrients were significantly influenced by genotypes, [CO_2] and PPFD (Table 5). Irrespective of levels of [CO_2] and PPFD, cacao genotype SCA 6 had higher IN of all macro–micro nutrients. Based on these findings SCA 6 could be a superior genotype to use as rootstock in establishing new plantations in infertile soils under changing climatic conditions. In the current study, irrespective of levels of PPFD, IN for all macro–micro nutrients increased significantly by increasing [CO_2] from 400 to 700 µmol mol^{-1}. It has been previously reported in cacao genotype comum that increasing [CO_2] from 380 to 700 µmol mol^{-1} tended to increase IN for many of the essential nutrients [35]. In the current study, increasing PPFD from 100 to 400 µmol m^{-2} s^{-1} significantly increased IN for all nutrients irrespective of levels of [CO_2]. Baligar et al. [35] found a similar result, but also that increases in PPFD to 1050 µmol m^{-2} s^{-1} tended to decrease IN for N, K, Ca, Mg, P, S, Cu and Fe. Increased plant influx (IN) of more nutrients from the growth medium helps meet increased demand by increased shoot biomass accumulation.

With the exceptions of K, Ca and Cu, transport (TR) for the other macro–micro nutrients were significantly influenced by cacao genotypes (Table 6). SCA 6 was superior in transport of N, Ca, Fe, and Mn and Coca 3370 was superior in TR for Mg, B and Mn. Overall, with a few exceptions, TR for all the macro–micro nutrients were significantly increased by increasing [CO_2] from 400 to 700 µmol mol^{-1} and PPFD from 100 to 400 µmol m^{-2} s^{-1}. In cacao genotype comum, Baligar et al. [35] reported that increasing [CO_2] from 380 to 700 µmol mol^{-1} decreased TR of N, Ca, and Zn, and increased TR for other elements. Such variations in IN and TR at varying levels of [CO_2] and PPFD could be related to nature of genotypes and their interactions with levels of [CO_2] and PPFD.

Table 3. The effect of [CO_2] and photosynthetic photon flux density (PPFD) on macro–micro nutrient concentrations of seven 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}			
Catongo											
400	100	27.31	4.48	15.40	15.87	6.80	34.30	24.15	61.90	77.54	40.83
	200	28.92	4.26	14.53	15.79	6.77	34.83	22.47	57.63	62.91	36.75
	400	31.46	5.04	15.54	15.36	6.34	32.68	24.88	95.58	55.72	51.21
700	100	25.84	4.00	15.82	14.86	6.26	28.38	18.81	25.66	63.10	33.31
	200	24.15	3.65	14.03	13.81	6.57	23.86	18.14	18.00	49.46	34.98
	400	26.97	3.71	12.99	12.94	5.57	24.54	17.11	19.54	44.68	36.48
Coca 3370											
400	100	27.00	3.82	14.70	14.82	8.04	30.76	21.11	56.73	110.06	58.68
	200	25.46	4.02	15.02	15.13	8.35	33.51	19.90	54.62	73.60	40.85
	400	28.15	3.91	12.83	14.09	7.50	29.28	21.21	72.55	63.10	41.48
700	100	26.21	4.15	12.92	13.95	7.03	24.20	17.39	18.73	67.50	41.66
	200	25.15	4.45	13.22	13.71	7.89	24.32	18.87	21.72	61.34	48.28
	400	24.54	3.85	11.23	11.84	6.20	19.32	17.27	34.04	36.67	46.01
CCN 51											
400	100	28.85	4.33	13.64	14.35	6.39	30.29	23.14	60.94	74.38	54.68
	200	27.68	4.47	14.82	15.64	7.24	30.77	22.56	44.73	66.95	47.11
	400	29.71	4.20	14.94	14.03	6.25	27.05	25.80	52.43	43.86	41.47
700	100	24.94	4.33	13.93	16.48	6.80	24.94	21.98	21.77	79.65	76.98
	200	24.69	3.63	12.80	13.89	6.53	20.53	17.58	26.86	54.54	50.82
	400	24.91	3.73	11.48	13.56	6.34	23.82	19.11	40.12	47.74	54.03
Amaz 15											
400	100	24.83	4.15	13.49	13.62	6.94	26.66	17.35	45.41	68.37	46.03
	200	25.17	4.28	14.45	15.57	7.82	31.27	19.75	43.78	61.22	49.74
	400	25.52	3.69	11.92	13.70	6.73	29.14	19.65	31.74	47.84	71.18
700	100	23.31	4.14	13.19	14.26	7.55	23.56	16.61	14.38	68.17	45.67
	200	22.02	3.77	11.29	12.04	6.74	19.07	14.54	20.07	47.75	43.13
	400	21.73	3.48	10.22	11.24	5.98	19.44	14.73	15.46	47.13	40.99
LCT EEN 37A											
400	100	26.70	4.57	17.73	16.84	6.76	30.61	24.52	77.09	74.45	57.56
	200	30.72	5.13	16.91	16.16	7.83	32.85	41.88	108.84	59.46	58.52
	400	32.58	4.75	15.98	15.25	7.43	31.79	36.45	80.44	45.79	45.27
700	100	22.39	4.06	14.27	13.66	6.41	22.39	16.83	30.97	56.90	47.76
	200	24.37	3.57	13.58	13.46	5.79	20.62	15.62	36.18	54.53	48.44
	400	23.39	4.14	11.46	14.54	6.62	20.47	17.68	46.68	42.68	45.84
Na 33											
400	100	23.11	3.81	13.79	15.87	6.16	31.05	20.51	67.26	85.89	66.47
	200	26.13	3.95	13.28	17.77	6.62	34.37	22.94	83.68	64.51	61.70
	400	28.70	3.68	14.51	14.48	6.08	34.70	21.31	87.95	46.13	60.22
700	100	23.84	3.71	15.05	16.80	6.50	30.02	18.35	53.72	63.89	51.03
	200	22.35	3.59	11.19	14.74	5.87	17.49	17.79	44.94	51.63	49.01
	400	20.85	3.58	10.47	12.87	5.76	21.79	17.81	47.56	39.90	63.92
SCA 6											
400	100	24.71	3.40	13.51	13.69	6.28	29.44	16.05	57.65	75.17	44.33
	200	25.53	3.40	14.04	15.53	6.93	31.24	19.76	82.19	69.80	62.89
	400	28.23	3.65	13.02	13.91	6.53	33.44	23.59	95.41	62.92	43.49
700	100	22.08	3.30	13.55	14.41	6.33	22.29	15.78	32.26	67.81	46.56
	200	22.21	2.92	10.22	11.88	5.46	18.79	14.00	34.43	50.48	36.46
	400	23.31	3.60	11.44	13.36	6.19	23.67	17.57	50.73	45.28	55.06
Significance											
Genotype (G)		**	**	**	**	**	*	**	**	*	**
[CO_2] (C)		**	**	**	**	**	**	**	**	**	NS
PPFD (P)		*	NS	**	**	*	NS	NS	NS	**	NS

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

Table 4. The effect of [CO_2] and photosynthetic photon flux density (PPFD) on macro–micro nutrient uptake of seven 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			
Catongo											
400	100	181.9	29.68	101.9	105.4	45.18	229.1	161.1	417.9	513.7	273.3
	200	193.7	28.33	98.1	106.0	45.69	235.3	150.1	380.1	416.4	252.7
	400	178.4	28.59	88.0	87.1	35.96	185.3	140.9	541.6	315.6	290.3
700	100	273.8	42.21	167.2	157.5	66.29	299.2	198.9	287.3	664.8	354.4
	200	305.3	45.46	169.9	169.9	81.61	301.8	222.3	215.9	608.9	456.2
	400	317.7	43.79	152.9	153.4	66.65	295.7	203.5	243.8	523.8	446.5
Coca 3370											
400	100	168.6	23.64	91.5	92.8	50.79	193.5	133.2	365.3	695.8	363.9
	200	236.4	37.28	140.2	140.5	77.85	311.6	184.8	499.3	683.4	379.0
	400	326.9	45.56	148.2	164.7	87.26	337.9	245.5	855.8	738.7	484.3
700	100	290.4	46.02	143.8	153.7	77.97	268.9	192.3	195.4	759.5	462.4
	200	427.6	74.69	223.3	229.9	134.66	411.3	318.5	419.4	1055.5	840.9
	400	447.9	70.49	208.1	214.7	115.23	353.7	323.4	607.4	675.6	889.6
CCN 51											
400	100	137.8	20.65	64.2	69.0	30.70	150.6	115.1	322.4	349.0	257.9
	200	196.8	31.84	105.0	111.4	51.50	217.9	161.0	320.8	475.9	334.3
	400	238.3	33.56	121.7	111.7	50.47	217.1	202.1	390.3	343.9	331.2
700	100	295.5	51.38	166.1	197.0	81.72	295.0	262.4	262.6	929.6	874.9
	200	360.7	52.37	185.6	200.9	95.23	299.6	257.3	401.6	803.5	734.4
	400	521.5	79.71	240.4	285.4	136.42	515.1	405.8	888.1	1036.3	1150.2
Amaz 15											
400	100	184.5	30.67	100.1	100.9	51.36	195.8	128.5	342.4	504.5	342.4
	200	254.8	42.82	143.8	157.2	78.54	311.4	195.6	446.2	614.6	502.8
	400	234.8	34.19	109.6	126.6	62.87	243.6	179.8	320.3	444.5	705.7
700	100	399.3	71.40	226.4	245.4	130.08	406.2	286.9	247.0	1174.0	785.6
	200	459.9	79.81	234.8	252.1	142.20	404.9	304.8	418.9	998.8	880.4
	400	509.1	81.96	239.9	264.1	140.27	454.1	344.7	366.8	1111.3	983.2
LCT EEN 37A											
400	100	82.1	13.38	51.5	51.2	20.41	90.4	69.5	216.3	231.3	162.5
	200	137.7	23.46	76.2	71.5	34.44	144.9	193.9	491.6	249.9	264.4
	400	118.9	18.20	60.2	56.7	26.58	123.8	121.0	276.5	186.0	173.9
700	100	311.9	56.55	198.8	189.9	89.18	310.3	233.7	429.1	792.1	664.2
	200	346.0	50.47	188.8	189.2	82.05	288.0	223.7	524.1	767.6	684.7
	400	387.4	68.72	189.4	239.2	110.06	337.8	287.8	743.6	703.7	777.9
Na 33											
400	100	149.2	24.50	88.9	102.3	40.04	202.4	132.8	410.2	576.1	424.9
	200	213.5	32.14	108.9	146.0	54.84	282.9	183.9	650.1	541.4	495.1
	400	140.9	18.42	73.6	72.3	30.73	172.5	103.5	361.6	229.7	293.7
700	100	193.6	29.74	120.4	135.2	52.68	241.4	147.2	425.7	524.4	411.3
	200	411.1	66.21	207.1	269.6	109.15	326.9	321.6	773.0	974.4	920.9
	400	443.2	76.07	222.4	272.1	122.75	462.6	375.5	1005.5	842.9	1436.6
SCA 6											
400	100	149.2	20.43	81.69	82.63	37.87	177.2	96.8	348.3	454.9	266.6
	200	151.2	20.28	83.63	93.33	41.69	190.1	118.1	496.9	423.0	366.7
	400	232.6	30.07	108.20	114.97	54.32	280.7	188.7	707.4	515.0	355.8
700	100	279.7	41.96	171.99	183.14	80.41	282.5	201.2	407.8	864.1	593.8
	200	352.3	45.76	162.28	187.20	86.23	296.9	221.0	556.5	799.7	582.1
	400	453.2	70.70	224.24	261.73	122.61	465.4	342.1	1017.3	916.0	1091.9
Significance											
Genotype (G)		**	**	**	**	**	**	**	**	**	**
[CO_2] (C)		**	**	**	**	**	**	**	NS	**	**
PPFD (P)		**	**	**	**	**	**	**	**	NS	**

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

Table 5. The effect of [CO_2] and photosynthetic photon flux density (PPFD) on macro–micro influx by root length of seven 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})			
Catongo											
400	100	0.98	0.07	0.19	0.21	0.14	1.62	0.19	0.61	0.73	0.31
	200	1.04	0.07	0.18	0.21	0.14	1.64	0.18	0.54	0.58	0.27
	400	1.00	0.07	0.17	0.18	0.12	1.36	0.18	0.82	0.46	0.34
700	100	1.53	0.10	0.34	0.32	0.22	2.19	0.25	0.42	0.97	0.41
	200	1.58	0.10	0.32	0.32	0.25	2.03	0.26	0.30	0.83	0.49
	400	1.69	0.10	0.29	0.29	0.20	2.03	0.24	0.33	0.72	0.49
Coca 3370											
400	100	1.19	0.07	0.23	0.24	0.21	1.84	0.22	0.69	1.33	0.56
	200	1.26	0.09	0.27	0.27	0.24	2.21	0.22	0.71	0.96	0.43
	400	1.80	0.11	0.29	0.32	0.28	2.45	0.30	1.20	1.05	0.56
700	100	1.62	0.11	0.29	0.31	0.25	1.99	0.24	0.29	1.11	0.55
	200	2.12	0.17	0.39	0.41	0.39	2.66	0.35	0.51	1.35	0.87
	400	2.29	0.16	0.37	0.39	0.34	2.35	0.36	0.82	0.89	0.93
CCN 51											
400	100	1.07	0.07	0.17	0.21	0.14	1.53	0.19	0.76	0.77	0.42
	200	1.37	0.09	0.26	0.28	0.21	1.99	0.24	0.58	0.89	0.49
	400	1.69	0.10	0.30	0.29	0.21	1.99	0.31	0.77	0.65	0.49
700	100	1.63	0.13	0.33	0.39	0.26	2.12	0.31	0.37	1.35	1.06
	200	1.89	0.12	0.35	0.38	0.29	2.04	0.29	0.53	1.09	0.82
	400	2.31	0.16	0.38	0.45	0.35	2.94	0.39	1.00	1.17	1.09
Amaz 15											
400	100	1.24	0.09	0.24	0.25	0.20	1.75	0.19	0.61	0.91	0.49
	200	1.38	0.11	0.28	0.31	0.25	2.25	0.24	0.63	0.88	0.59
	400	1.39	0.09	0.23	0.27	0.22	1.99	0.24	0.48	0.70	0.89
700	100	1.89	0.15	0.38	0.41	0.36	2.47	0.30	0.30	1.45	0.81
	200	2.13	0.16	0.39	0.42	0.38	2.41	0.31	0.50	1.20	0.89
	400	2.22	0.16	0.37	0.41	0.36	2.58	0.33	0.41	1.25	0.92
LCT EEN 37A											
400	100	0.68	0.05	0.16	0.17	0.11	1.06	0.14	0.54	0.54	0.30
	200	1.13	0.08	0.22	0.22	0.17	1.61	0.36	1.11	0.56	0.46
	400	0.95	0.06	0.17	0.17	0.13	1.30	0.23	0.64	0.38	0.28
700	100	1.69	0.14	0.39	0.37	0.28	2.25	0.28	0.61	1.12	0.78
	200	1.89	0.12	0.37	0.37	0.26	2.07	0.26	0.74	1.09	0.81
	400	1.89	0.15	0.33	0.42	0.31	2.17	0.31	0.96	0.89	0.80
Na 33											
400	100	0.97	0.07	0.21	0.25	0.15	1.74	0.19	0.75	0.99	0.61
	200	1.29	0.09	0.23	0.32	0.19	2.24	0.25	1.08	0.84	0.65
	400	1.05	0.06	0.19	0.19	0.13	1.71	0.16	0.83	0.45	0.47
700	100	1.48	0.10	0.33	0.38	0.24	2.44	0.24	0.85	1.05	0.68
	200	1.88	0.14	0.34	0.44	0.29	1.93	0.32	0.91	1.15	0.89
	400	1.89	0.15	0.34	0.41	0.31	2.59	0.35	1.12	0.94	1.25
SCA 6											
400	100	1.37	0.08	0.27	0.28	0.21	2.16	0.20	0.86	1.11	0.51
	200	1.34	0.08	0.26	0.31	0.22	2.21	0.24	1.16	1.00	0.68
	400	1.71	0.09	0.28	0.31	0.23	2.71	0.31	1.35	0.99	0.54
700	100	1.95	0.13	0.43	0.45	0.32	2.56	0.31	0.74	1.54	0.85
	200	2.50	0.14	0.41	0.47	0.35	2.72	0.34	1.02	1.46	0.87
	400	2.48	0.17	0.44	0.51	0.38	3.37	0.41	1.43	1.28	1.28
Significance											
Genotype (G)		**	**	**	**	**	**	**	**	**	**
[CO_2] (C)		**	**	**	**	**	**	**	NS	**	**
PPFD (P)		**	**	NS	**	**	*	**	*	**	*

*, ** 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 macro–micro nutrient transport of seven cacao genotypes.

CO_2 (µmol mol^{-1})	PPFD (µmol m^{-2} s^{-1})	N	P	K	Ca	Mg	B	Cu	Fe	Mn	Zn
		pmol g shoot^{-1} s^{-1}									
Catongo											
400	100	699.9	50.39	138.7	150.1	102.3	1.16	0.14	0.43	0.53	0.22
	200	743.1	47.54	130.4	149.3	101.9	1.17	0.13	0.39	0.42	0.19
	400	768.4	53.95	131.6	138.1	89.7	1.04	0.13	0.63	0.35	0.26
700	100	826.2	56.80	180.9	170.9	116.6	1.18	0.13	0.22	0.52	0.22
	200	803.2	53.77	165.1	163.9	127.1	1.03	0.13	0.15	0.42	0.25
	400	879.2	53.47	149.9	151.3	105.5	1.04	0.12	0.17	0.37	0.25
Coca 3370											
400	100	681.6	41.64	131.5	138.6	121.0	1.04	0.12	0.39	0.75	0.32
	200	738.5	51.64	156.0	159.7	143.1	1.29	0.13	0.42	0.56	0.25
	400	879.2	54.02	141.3	158.3	136.6	1.19	0.15	0.59	0.51	0.27
700	100	751.5	53.14	131.9	146.1	118.2	0.91	0.11	0.14	0.51	0.25
	200	811.1	64.40	152.0	158.9	149.1	1.03	0.13	0.19	0.52	0.33
	400	810.3	57.00	132.3	140.4	119.7	0.83	0.13	0.29	0.31	0.33
CCN 51											
400	100	633.6	39.88	101.4	121.1	84.2	0.88	0.11	0.39	0.45	0.25
	200	742.5	52.45	139.9	155.2	115.2	1.07	0.13	0.32	0.48	0.26
	400	831.8	51.00	147.8	142.9	102.5	0.97	0.15	0.38	0.32	0.24
700	100	785.9	61.00	157.4	188.7	126.0	1.02	0.15	0.18	0.66	0.52
	200	822.1	53.56	151.8	166.1	127.0	0.88	0.13	0.23	0.47	0.36
	400	915.3	61.56	150.2	177.8	136.2	1.14	0.15	0.38	0.45	0.43
Amaz 15											
400	100	684.5	51.47	134.6	138.2	113.5	0.97	0.11	0.33	0.51	0.27
	200	767.8	58.74	158.7	172.5	140.5	1.26	0.13	0.35	0.49	0.33
	400	726.7	46.86	121.6	143.3	113.9	1.07	0.13	0.25	0.36	0.46
700	100	804.0	64.18	163.2	176.4	152.7	1.06	0.13	0.13	0.61	0.34
	200	797.4	61.40	145.9	155.6	142.8	0.90	0.12	0.19	0.45	0.33
	400	812.0	58.31	136.2	149.5	129.9	0.94	0.12	0.15	0.46	0.33
LCT EEN 37A											
400	100	490.2	35.11	118.7	124.9	77.0	0.78	0.10	0.41	0.39	0.22
	200	709.6	52.33	140.6	139.7	107.9	1.01	0.23	0.69	0.36	0.29
	400	662.5	41.56	116.6	119.5	90.2	0.89	0.16	0.44	0.25	0.19
700	100	766.7	62.42	175.8	168.4	128.8	1.01	0.13	0.27	0.51	0.35
	200	835.5	54.32	165.6	165.3	115.8	0.92	0.12	0.32	0.48	0.36
	400	838.2	66.65	146.2	186.3	138.9	0.96	0.14	0.43	0.39	0.35
Na 33											
400	100	573.3	41.70	121.1	146.7	90.7	1.03	0.11	0.44	0.58	0.36
	200	710.4	47.35	126.9	176.8	105.8	1.24	0.14	0.59	0.46	0.36
	400	640.1	35.01	113.5	120.4	79.8	1.04	0.10	0.50	0.27	0.28
700	100	699.8	48.00	158.0	178.5	112.3	1.16	0.12	0.41	0.49	0.32
	200	824.9	59.38	147.3	193.2	126.3	0.84	0.14	0.42	0.49	0.39
	400	798.3	61.61	142.8	174.5	128.3	1.09	0.15	0.46	0.39	0.53
SCA 6											
400	100	685.0	40.64	133.0	139.4	102.5	1.08	0.10	0.42	0.55	0.25
	200	702.6	40.37	137.5	157.9	113.1	1.14	0.12	0.61	0.51	0.37
	400	869.7	49.20	141.9	154.5	117.4	1.36	0.16	0.75	0.50	0.28
700	100	786.6	52.28	172.2	183.2	131.1	1.03	0.12	0.29	0.63	0.35
	200	837.1	48.70	136.3	158.5	119.0	0.91	0.12	0.33	0.49	0.29
	400	921.4	63.86	161.0	186.9	142.1	1.22	0.15	0.51	0.46	0.47
Significance											
Genotype (G)		**	*	NS	NS	**	*	NS	**	**	**
[CO_2] (C)		**	**	**	**	**	*	NS	**	NS	**
PPFD (P)		**	*	*	*	**	NS	**	NS	**	NS

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

3.3.3. Nutrient Use Efficiency

With the exception of Mn, all cacao genotypes in this study, irrespective of levels of [CO_2] and PPFD, showed significant differences for NUE of all the other essential nutrients (Table 7). The existence of interspecific variations in NUE of macro and micro nutrients have been well documented for field, horticultural and perennial legume crops [62,63,85–88]. Variations in the growth and uptake and nutrient use efficiency among crop cultivars have been related to absorption, translocation, shoot demand, and dry matter production potentials per unit of nutrient absorbed [85,86]. In agroforestry systems, cacao is grown as an understory plant and subjected to rising [CO_2] and low levels of PPFD. Under such situations cacao genotypes that have high nutrient use efficiency for essential nutrients might be able to grow well and produce higher yields. Deficiencies of P, Ca, Mg, Zn and Fe have been widely reported in soils of cacao growing regions of the world [11,48,50]. Cabala-Rosand et al. [50] state that under field conditions the most common deficiencies noted in cacao are N, K, Zn, Fe and B. P is also a limiting nutrient in almost all soils under cacao [49]. Genotypes that have high NUE for any of these nutrients could improve the sustainability and productivity of cacao grown in nutrient deficient soils under agroforestry systems. Amaz 15 was most efficient in NUE for N, K, Ca, B, Cu and Fe and SCA 6 was most efficient for P and K. Since Amaz15 had the longest root length among the cacao genotypes tested, this probably helped it to acquire more nutrients. Barber [89] states that the quantity of a nutrient taken up by a plant depends on the configuration and growth rate of the roots. Irrespective of levels of PPFD, increasing [CO_2] from 400 to 700 µmol mol^{-1} significantly increased NUE for all nutrients. In cacao Comum, Baligar et al. [35] reported that NUE for N, Mg, Cu, Mn and Zn increased with increasing [CO_2] from 380 to 700 µmol mol^{-1}. Irrespective of levels of [CO_2], increasing PPFD significantly affected NUE for K, Ca, Mg, B and Mn. With the exceptions of N and Fe, increasing levels of PPFD from 100 to 400 µmol m^{-2} s^{-1} increased NUE for all other nutrients. %clearpage

Table 7. The effect of [CO_2] and photosynthetic photon flux density (PPFD) on macro–micro nutrient use efficiency (NUE) of seven 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^{-1}						mg shoot mg element^{-1} ($\times 10^4$)			
Catongo											
400	100	36.64	225.8	65.68	63.39	147.6	2.92	4.17	1.66	1.30	2.48
	200	34.70	237.9	69.06	64.09	148.4	2.92	4.48	1.93	1.65	2.76
	400	31.90	199.4	65.34	65.25	157.8	3.09	4.06	1.16	1.82	1.96
700	100	38.72	251.6	63.67	67.89	161.8	3.60	5.39	5.61	1.62	3.02
	200	41.53	278.1	75.12	74.38	154.9	4.20	5.68	5.95	2.07	2.87
	400	37.31	272.1	77.24	78.29	181.1	4.10	5.88	7.15	2.25	2.79
Coca 3370											
400	100	37.12	268.3	68.02	67.67	125.8	3.27	4.78	1.96	0.93	1.70
	200	39.28	249.4	67.24	66.21	120.8	3.01	5.03	1.99	1.36	2.45
	400	35.69	259.7	77.94	72.05	134.2	3.43	4.72	1.50	1.63	2.45
700	100	38.28	241.4	77.91	72.07	142.4	4.15	5.75	1.22	1.56	2.42
	200	40.30	231.3	77.07	74.78	128.0	4.20	5.39	5.52	1.64	2.10
	400	41.19	261.8	89.69	86.28	162.7	5.22	5.79	3.04	2.74	2.20
CCN 51											
400	100	34.69	231.5	73.65	69.91	157.3	3.36	4.54	3.88	1.36	1.84
	200	36.23	228.9	67.75	64.22	138.4	3.30	4.46	2.31	1.49	2.13
	400	33.92	242.6	66.93	72.79	160.5	3.72	4.12	2.53	2.39	2.44
700	100	40.10	231.9	72.30	60.80	147.9	4.02	4.61	5.90	1.28	1.38
	200	40.63	276.5	78.26	72.04	153.4	4.89	5.69	3.77	1.84	1.99
	400	40.44	268.5	87.37	74.67	159.6	4.37	5.27	2.58	2.13	1.86

Table 7. *Cont.*

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^{-1}						mg shoot mg element^{-1} ($\times 10^4$)			
						Amaz 15					
400	100	40.30	245.1	74.18	73.78	145.2	3.86	5.79	2.31	1.48	2.17
	200	39.77	237.0	70.65	64.27	128.7	3.27	5.23	2.42	1.66	2.35
	400	39.24	274.6	83.95	73.26	148.7	3.62	5.10	3.41	2.09	1.52
700	100	43.18	248.5	75.97	71.01	135.0	4.43	6.23	6.98	1.49	2.33
	200	45.43	267.4	88.61	83.42	149.5	5.30	6.88	5.51	2.09	2.49
	400	46.58	287.7	98.15	89.34	168.2	5.31	6.84	6.51	2.12	2.60
						LCT EEN 37A					
400	100	37.48	225.5	57.68	59.53	148.9	3.29	4.22	1.54	1.38	1.80
	200	32.55	196.9	59.22	62.49	128.6	3.06	2.54	0.97	1.79	1.71
	400	31.56	214.4	62.76	67.34	144.8	3.16	3.48	3.51	2.26	2.21
700	100	44.72	247.3	70.29	73.32	156.3	4.56	5.96	3.29	1.76	2.09
	200	41.04	281.4	74.51	74.78	173.1	4.89	6.41	3.23	1.84	2.12
	400	42.89	242.1	87.86	69.45	151.2	4.92	5.79	2.33	2.36	2.21
						Na 33					
400	100	43.36	263.8	72.64	63.22	162.9	3.23	4.89	1.83	1.25	1.55
	200	38.37	255.8	75.40	56.95	151.3	2.95	4.45	1.30	1.58	1.70
	400	35.51	275.2	69.11	71.28	164.9	2.96	4.88	1.71	2.24	1.76
700	100	41.99	275.1	67.63	59.79	154.1	3.38	5.54	2.05	1.61	1.98
	200	45.18	280.3	89.83	69.01	170.6	5.73	5.82	3.18	1.94	2.04
	400	48.19	285.7	96.05	78.54	174.4	4.65	5.71	2.28	2.59	1.98
						SCA 6					
400	100	40.55	298.8	74.55	73.21	159.9	3.44	6.26	1.79	1.33	2.30
	200	39.39	294.3	71.30	64.75	145.4	3.26	5.08	1.23	1.49	1.83
	400	35.60	276.7	76.83	72.10	153.3	3.00	4.55	2.02	1.61	2.39
700	100	45.30	306.8	74.11	69.89	158.7	4.49	6.47	3.56	1.49	2.22
	200	45.17	351.9	98.11	84.87	184.6	5.44	7.22	3.15	1.99	2.79
	400	43.38	280.3	87.71	75.36	163.1	4.33	5.73	2.04	2.25	1.83
						Significance					
Genotype (G)		**	**	**	**	**	**	**	*	NS	**
$[CO_2]$ (C)		**	**	**	**	**	**	**	**	**	*
PPFD (P)		NS	NS	**	**	**	*	NS	NS	**	NS

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

4. Conclusions

Under glasshouse conditions, elevated $[CO_2]$ increased growth, physiology, nutrient uptake and use efficiency; however, low light decreased growth, photosynthesis and nutrient uptake of cacao genotypes. Intraspecific differences were found in the genotypes such that AMAZ 15 was the highest for many parameters and LCT EEN 37A was often the lowest. Na 33 had high Fe uptake which could be a problem on Fe limited soils, but further testing is needed. Higher WUE in increasing levels of $[CO_2]$ should be considered in selection of cacao genotypes useful for drought prone areas to maintain cacao sustainability and improve yields.

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