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Light Intensity Effects on the Growth, Physiological and Nutritional Parameters of Tropical Perennial Legume Cover Crops

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Abstract: In the early stages of the establishment of plantation crops such as cacao, perennial legume cover crops provide vegetative cover to reduce soil and nutrient loss by erosion. Light intensity at cover crop canopy levels greatly influences their adaptability and optimum growth. As tree crops mature, understory cover crops suffer from inadequate light intensity. A greenhouse experiment was undertaken with nine perennial legume cover crop species (Calopo, Ea-Ea, Jack Bean, Lab-Lab, Mucuna ana, Mucuna preta, Cowpea, Black Pigeon Pea and Mixed Pigeon Pea) to assess the effects of three photosynthetic photon flux densities (PPFDs, μmol m^{-2} s^{-1}) 180 (inadequate light), 450 (moderate light) and 900 (adequate light) on growth, physiological and nutrient uptake parameters. PPFD had highly significant effects on leaf, shoot and root growth parameters and increasing the light intensity from 180 to 900 μmol m^{-2} s^{-1} increased all growth parameters with the exception of specific leaf area. In all the legume cover crops, increasing the light intensity significantly increased the net assimilation rates (NAR), SPAD index and net photosynthesis (P_N) and its components, stomatal conductance (gs), transpiration (E) and vapor pressure deficit (VPD). Cover crop species, PPFD and their interactions significantly affected water flux (Vo) and various water use efficiency parameters (WUE_{TOTAL}, WUE_{INST} and WUE_{INTR}). Increasing the PPFD increased the WUE in all of the cover crops. Species and PPFD had highly significant effects on the uptake of macro- and micronutrients. Overall uptakes of all nutrients were increased with increases in the PPFD from 180 to 900 μmol m^{-2} s^{-1}. With few exceptions, the nutrient use efficiency (NUE) of the nutrients was significantly influenced by species, PPFD and their interactions. Except for Mn, increasing the PPFD from 180 to 900 μmol m^{-2} s^{-1} increased the NUE for all the nutrients.

Keywords: nutrient and water use efficiency; nutrient uptake and transport; net photosynthesis

1. Introduction

Fast growing cover crops before and during the early stages of establishment of widely spaced perennial crops such as cacao, coffee, oil palm and rubber can reduce soil erosion, reduce nutrient losses, suppress weeds and improve soil health [1]. Establishment of fast growing cover crops can reduce soil loss by erosion, minimize nutrient loss by leaching and increase organic matter and N content, thereby restoring soil productivity [1–6].

When cover crops are grown under plantation crops, the quality and quantity of light reaching the cover crop canopy are important factors that limit the growth, development and persistence of cover crops. Cover crops are grown as understory plants hence they do not receive full sunlight and as upper story trees grow the amount of photosynthetic photon flux density (PPFD) received at the cover crop canopy is reduced. Cover crops that tolerate low irradiance have a better chance of growing and persisting under plantation crops. In tropical regions, incoming PPFD is around 1800 µmol m^{-2} s^{-1} [7] but understory plants may receive only 4–10% of the incoming PPFD [8,9]. Canopies of shade trees and cacao together reduce the PPFD at the cover crop canopy level. Therefore, in tropical plantation crop production, there is an increased interest in finding shade tolerant cover crops. Limited information is available on inter- and intra-specific differences in tropical cover crops for tolerance to shade [10–12]. Shading is known to reduce the yields of many tropical legumes and heavy shade can affect their survivability in plantation crops [13,14]. However, the ability of many tropical legume cover crop species to grow at low light intensity is unknown.

Very limited published evidence exists in the areas of tropical perennial legume cover crops' response to low to adequate light intensities [15–17]. When cover crops are grown under plantation crops, the growth of the cover crops is influenced by the amount of PPFD reaching the cover crop [4]. Baligar et al. [15,16] reported that in nine perennial legume cover crop species PPFD significantly affected growth and macro-micronutrient use efficiency. In these studies, interspecific differences in growth and nutrient use efficiency were influenced by levels of PPFD and overall increasing PPFD from 200 to 400 µmol m^{-2} s^{-1} increased all the growth and nutrient uptake traits. In five tropical perennial legume cover crops (Calopo, Jack Bean, Mucuna, White Lead Tree and Perennial Peanut) reducing the PPFD from 1000 to 50 µmol m^{-2} s^{-1} reduced photosynthesis to less than 10% of the higher light level [17]. Similarly, in four Crotalaria cover crop species increasing the PPFD from 50 to 1500 µmol m^{-2} s^{-1} increased photosynthesis by 21-fold [18]. Limited attention has been given to understanding the growth, physiology and nutrition of various cover crops under varying light intensities. An experiment was undertaken in the greenhouse to assess the effects of PPFD on the growth, physiological and nutritional parameters of nine tropical perennial leguminous cover crops.

2. Materials and Methods

2.1. Legume Species

Nine perennial cover crop species were selected for the experiment: Calopo (*Calopogonium mucunoides* Desv.), Ea-Ea (*Desmodium heterocarpon* (L.) DC.), Jack Bean (*Canavalia ensiformis* L. DC), Lab-Lab (*Lablab purpureus* (L.) Sweet), Mucuna ana (*Mucuna deeringiana* (Bort.) Merr.), Mucuna preta (*Mucuna aterrima* (Piper & Tracy) Holland), Cowpea (*Vigna unguiculata* (L.) Walp.), Black Pigeon Pea (*Cajanus cajan* (L.) Millsp.) and Mixed Pigeon Pea (*Cajanus cajan* (L.) Millsp.). Growth habits, strengths and limitations of the cover crops used are given in Table 1. Duke [19] states that the minimum and maximum temperatures required for perennial legume cover crops such as the ones adapted for this research are 18–28 °C. Based on such information we adapted the desired temperatures for growing these crops in a greenhouse. The range of temperatures listed in Duke's publication refer to a time when climatic changes in the tropics were minimal. However, climatic changes over time in the tropics have increased growing temperatures. Based on such changes in current regional temperatures, we selected day/night temperatures of 30/28 °C to evaluate these perennial legume cover crops.

Table 1. Common and scientific names, growth habits, strengths and limitations of legume cover crop species used in this experiment [1,2].

Common Name	Scientific Name	Growth Habit [3]	Strengths	Limitations
Calopo	*Calopogonium mucunoides* Desv.	P	Wide edaphic adaption, establishes rapidly, good tolerance to extreme conditions, erosion control, drought sensitive	Poor tolerance of heavy shade, some weed potential, susceptible to root-knot nematode and cowpea viruses
Ea-Ea	*Desmodium heterocarpon* (L.) DC	P	Well adapted to acid infertile soils, good for restoring degraded soil, good shade tolerance, good green manure crop	Poor drought tolerance, susceptible to false rust (*Synchytrium desmodii*), foliar blight (*Rhizoctonia solani*) and root-knot nematodes (*Meloidogyne javanica*)
Jack Bean	*Canavalia enisformis* (L.) DC.	P	Drought tolerant, good erosion control, good green manure crop, reduces nematode numbers	Host to many fungi and pests
Lab-lab	*Lablab purpureus* (L.) Sweet	A/P	Adapt to low soil fertility, tolerant to drought, good soil erosion control and N_2 fixation	Not tolerant to water logging
Mucuna ana	*Mucuna deeringiana* (Bort) Merr.	A, P	Fast growing, improved soil fertility, resistant to pests and diseases	Not tolerant of drought or high acidity, toxic to animals
Mucuna preta	*Mucuna aterrima* (Piper & Tracy) Holland	A/P	Fast growing, improved soil fertility, resistant to pests and diseases	Not tolerant of drought or low fertility, toxic to animals
Cowpea	*Vigna unguiculata* (L.) Walp.	A/P	Moderately tolerant to drought, easy to establish	Not tolerant to water logging, sensitive to salinity
Pigeon Pea (black)	*Cajanus cajan* (L.) Huth	A	Very heat tolerant, good N fixer, drought tolerant	Sensitive to waterlogging and salinity
Pigeon Pea (mixed color)	*Cajanus cajan* (L.)	A	Very heat tolerant, good N fixer, drought tolerant	Sensitive to waterlogging and salinity

[1] Seeds were obtained from Globo Rural Seed Company, Goania, Go Brazil by Dr. N. K. Fageria and Dr. Corival da Silva of EMBRAPA Rice and Bean Center, Goiania, GO, Brazil. [2] References: [1,19–21]. [3] A = Annual, C = Climbing, N = Non-climbing; P = Perennial.

2.2. Greenhouse and Mini-Chamber Parameters

A greenhouse (16.5 m^2) with day/night temperatures of 30/28 °C and ambient CO_2 (400 ± 50 µmol mol^{-1}) was used for the duration of the plant growth. CO_2 levels were monitored with a WMA4 infrared CO_2 analyzer (PP Systems, Amesbury, MA, USA).

Within the greenhouse, mini-chambers were constructed with a 2 cm diameter PVC pipe to accommodate three light intensities (PPFD, 180, 450 and 900 µmol m^{-2} s^{-1}). The dimensions of the mini-chambers were 114 cm W × 119 cm L × 81 cm D (45″ × 47″ × 32″). The desired light levels in each mini-chamber were achieved by the use of plastic shade cloths: a single-ply of 22% white shade cloth from National Tool Grinding, Inc. in Erie, PA, USA was used for adequate light (900 µmol m^{-2} s^{-1}); a double-ply of charcoal fiberglass window screen from New York Wire in Mt. Wolf, PA, USA was used for moderate light (450 µmol m^{-2} s^{-1}) and a single layer of 70% blue shade cloth from Easy Gardener, Waco, TX, USA was used for inadequate light (180 µmol m^{-2} s^{-1}). Light levels were measured at mid-day with a LI-190S quantum sensor (Li-Cor Inc., Lincoln, NE, USA). Six of these shade chambers were used with two of each light intensity to achieve the desired replication of treatments.

2.3. Growth Medium and Plant Growth Conditions

The growth medium was prepared by mixing sand, perlite and peat moss (2:2:1 on a volume basis) in a cement mixer along with the required macro- and micronutrients to provide supplemental nutrients. Each kg of growth medium received (mg kg^{-1}) 600 N, 331 P, 240 K, 1012 Ca, 288 Mg, 416 S, 100 Fe, 1.0 B, 5.0 Mn, 5 Cu, 4 Zn and 0.1 Mo. Nutrients were applied as Osmocote 18-6-12 (The Scotts Company, Marysville, OH, USA), triple super phosphate, urea, calcium sulfate, dolomitic lime, Sprint 330 and a micromix made of boric acid, copper sulfate pentahydrate, manganese sulfate monohydrate, zinc sulfate heptahydrate and ammonium molybdate.

Seeds of various species of cover crops (10 to 30 seeds each) were planted in 2.5 L pots (Classic-300 series from Nursery Supplies, Inc., Fairless Hills, PA, USA) containing 2 kg of sand, perlite and peat moss mixture and possessing adequate bottom drainage. Seeds were not inoculated with rhizobium strains, mainly because there are no commercially available rhizobial strains compatible with these legume cover crops; we therefore supplemented the crop N need by adding N fertilizer to the growth medium. The pots were watered as needed to maintain soil moisture at −33 kPa while keeping a record of how much water was added each day. One container of growth medium without plants was placed in each mini-chamber to monitor the evaporative water loss. After 14 days, plants were thinned and the removed plants were used as an initial harvest. All experimental units were replicated three times.

2.4. Determination of Growth Parameters

After a growth period of 35 days, plants were harvested and roots and shoots were separated. The fresh weights of shoots were recorded and the total leaf area (LA, cm^2 $plant^{-1}$) was measured using a LI-3100 leaf area meter (Li-Cor Inc., Lincoln, NE, USA). Plants were washed in deionized water, freeze-dried and leaf dry weight and shoot dry weight were recorded.

The roots were removed from the soil, washed in deionized water, blotted dry and weighed. Root lengths were determined with a Comair Root Length Scanner (Commonwealth Aircraft, Melbourne, Vic., Australia). The roots were then oven dried at 70 °C and weighed.

Plant growth parameters were determined as follows:

$$\text{Root/Shoot (R/S) Ratio} = \text{Wr/Ws} \tag{1}$$

where Wr is the root dry weight and Ws is the shoot dry weight (g $plant^{-1}$)

$$\text{Specific Leaf Area (SLA, cm}^2\text{/g)} = \text{LA/LDW} \tag{2}$$

where LA is the total leaf area (cm^2 $plant^{-1}$) and LDW is the total leaf dry weight (g $plant^{-1}$).

$$\text{Leaf Mass/Unit Leaf Area (LMA, g/cm}^2\text{)} = [1/\text{SLA}] \tag{3}$$

$$\text{Leaf Area Ratio (LAR, cm}^2\text{/g)} = \text{LA/Wt} \tag{4}$$

where Wt is the total plant dry weight (shoot + root, g $plant^{-1}$).

$$\text{Relative Growth Rate (RGR, g g}^{-1}\text{ day}^{-1}\text{)} = [\ln(\text{Wt}_2/\text{Wt}_1)/(\text{T}_2 - \text{T}_1)] \tag{5}$$

where Wt is the total plant dry weight (root + shoot, g $plant^{-1}$) and T is the time interval in days. Subscripts 1 and 2 refer to initial and final harvest times.

$$\text{Net Assimilation Rate (NAR, g cm}^{-2}\text{ day}^{-1}\text{)} = [\text{RGR/LAR}] \tag{6}$$

2.5. Determination of Physiological Parameters

One week before harvest, physiological parameters were determined. A Licor 6400 Portable Photosynthesis System (Licor Inc., Lincoln, NE, USA) was used to determine net photosynthesis (P_N, μmol (CO_2) m^{-2} s^{-1}) and its components, stomatal conductance (gs, mol H_2O m^{-2} s^{-1}), internal leaf CO_2 (Ci, cm^3 m^{-3}), transpiration (E, mmol m^{-2} s^{-1}) and vapor pressure deficit (VPD, kPa). The net photosynthetic rate (P_N) and the instantaneous transpiration rate (E) were measured in the second or third mature leaf from the apex of the plants. Measurements were conducted between 10 a.m. and

2 p.m. The artificial light source used was the Li-6400-40 Leaf Chamber Fluorometer, adjusted to the PPFD that the plants were grown under (180, 450, 900 µmol m^{-2} s^{-1}). The CO_2 concentration in the chamber was adjusted to maintain 400 µmol mol^{-1} and the leaf temperature was kept constant at 30 °C. A SPAD-502 Chlorophyll Meter (Konica Minolta, Osaka, Japan) was used to estimate the chlorophyll content.

2.6. Determination of Water Flux (Vo) and Water Use Efficiency (WUE)

The rate of water flux (Vo, H_2O influx/cm^2 of roots s^{-1}) over the growth of the crop was calculated with the formula:

$$\text{Water Flux (Vo)} = \{[\text{TRANS}/(T_2 - T_1)]\ [(\ln RL_2 - \ln RL_1)/(RL_2 - RL_1)]\}/(2\pi RR) \tag{7}$$

where TRANS is the amount of H_2O transpired over entire growth period, RL is the total root length at harvest and T is the time in seconds. Subscripts 1 and 2 refer to the initial and final harvests and RR is the Root Radius (cm) = $(RFW/RL * \pi)^{1/2}$ where RFW is the root fresh wt. (cm^3).

$$\text{Total Water Use Efficiency } (WUE_{TOTAL}) = \text{shoot dry wt (g plant}^{-1})/\text{g } H_2O \text{ transpired over the entire growth period} \tag{8}$$

where water transpired was calculated by subtracting the evaporation for the total water loss for the whole experiment.

$$\text{Instantaneous WUE } (WUE_{INST}) = P_N/E\ (\mu\text{mol } CO_2/\text{mmol } H_2O) \tag{9}$$

where P_N is the net photosynthesis and E is the transpiration measured by Licor 6400.

$$\text{Intrinsic WUE } (WUE_{INTR}) = P_N/gs,\ (\mu\text{mol } CO_2/\text{mol } H_2O) \tag{10}$$

where PN is net photosynthesis and gs is stomatal conductance measured by a Licor 6400 [22].

2.7. Determination of Nutrient Uptake Parameters

Dried stems and leaves were ground to pass through a 1 mm sieve and sent to the University of Florida, Indian River Research and Education Center (UF-IRREC), Fort Pierce, FL for elemental analysis. Macro-micronutrient concentrations were determined by digesting 0.4 g plant samples in 5 mL of concentrated nitric acid (14 N) and nutrient concentrations in the digested solutions were determined by using Inductively Coupled Plasma Atomic Emission Spectrometry (ICPAES, Ultima JY Horiba Inc. Edison, NJ, USA).

The nutrient parameters were determined as follows:

$$\text{Uptake (U, mg plant}^{-1}) = \text{concentration of any element (mg/g or µm/g)} \times \text{shoot dry weight (g plant}^{-1}). \tag{11}$$

$$\text{Nutrient Use Efficiency (NUE, mg of shoot/mg of nutrient)} = \text{mg of Ws/mg of any given element in the shoot} \tag{12}$$

2.8. Statistical Analysis

The experiment was a randomized block design with three replicates. The results were subjected to analysis of variance using a general linear model (GLM) with the procedures of SAS (Ver. 9.1, SAS Institute, Cary, NC, USA).

3. Results and Discussion

3.1. Light Intensity on Growth Parameters

Tropical cover crops are sensitive to reduced light and shading reduces the yields of most tropical legume cover crops [14,23,24]. Cover crop species that tolerate lower PPFD have better chances of growing and persisting as understory plants in agroforestry-based plantings. Light intensity had highly significant effects on leaf, shoot and root growth parameters and increasing the light intensity from 180 to 900 μmol m^{-2} s^{-1} increased the growth parameters (Table 2). The cover crop species studied in this experiment showed significant interspecific differences for growth parameters at inadequate to adequate light intensities.

Table 2. Effect of photosynthetic photon flux density (PPFD) on shoot and root growth in cover crops.

Species	PPFD (μmol m^{-2} s^{-1})	Total Dry Weight (g $plant^{-1}$)	Root Dry Weight (g $plant^{-1}$)	Root Length (cm $plant^{-1}$)	Root/Shoot Ratio	Leaf Area (cm^2 $plant^{-1}$)	Specific Leaf Area (cm^2 g^{-1})	Leaf Mass/Unit Area (g cm^{-2}) (*10^{-3})	Relative Growth Rate (g g^{-1} d^{-1}) ($\times 10^{-2}$)
Calopo	180	0.038	0.004	51	0.116	9.3	343.7	2.91	2.15
	450	0.062	0.007	105	0.120	17.5	383.5	2.67	3.26
	900	0.203	0.025	309	0.141	42.2	270.3	3.71	6.72
Ea-Ea	180	0.018	0.002	36	0.099	6.2	479.5	2.10	6.18
	450	0.069	0.007	152	0.118	21.7	475.3	2.39	10.28
	900	0.099	0.013	212	0.150	21.1	316.5	3.17	11.38
Jack Bean	180	1.341	0.161	1194	0.138	258.6	314.8	3.19	0.57
	450	2.631	0.230	1483	0.095	542.3	321.7	3.12	2.58
	900	2.607	0.348	1751	0.155	426.3	229.2	4.39	2.50
Lab-Lab	180	0.458	0.055	1078	0.128	154.2	491.2	2.04	2.80
	450	0.376	0.035	574	0.105	121.6	453.0	2.27	2.98
	900	0.823	0.094	1361	0.129	183.4	301.9	3.31	5.01
M. ana	180	0.354	0.037	276	0.113	121.5	501.8	2.00	2.76
	450	0.990	0.078	746	0.087	346.4	471.7	2.12	6.33
	900	0.568	0.065	616	0.154	171.4	381.2	2.64	3.79
M. preta	180	0.415	0.049	463	0.134	138.9	467.5	2.14	2.39
	450	0.815	0.075	807	0.110	269.1	456.0	2.21	4.14
	900	0.859	0.105	936	0.162	222.5	326.9	3.06	4.10
Cowpea	180	0.444	0.024	468	0.084	134.2	570.1	1.77	3.62
	450	0.313	0.094	199	0.089	83.1	480.1	2.11	2.62
	900	0.940	0.016	1000	0.108	156.2	271.4	3.74	5.90
PPB	180	0.157	0.016	228	0.113	56.6	573.5	1.75	2.47
	450	0.206	0.024	298	0.133	66.6	515.8	1.96	3.10
	900	0.474	0.081	968	0.205	93.1	320.7	3.13	6.07
PPM	180	0.235	0.025	357	0.120	66.0	415.8	2.41	3.96
	450	0.409	0.046	579	0.125	103.0	361.2	2.81	5.62
	900	1.200	0.145	1752	0.148	194.5	234.9	4.28	8.51
Significance									
Species (S)		**	**	**	**	**	**	**	**
PPFD (P)		**	**	**	**	**	**	**	**
S × P		*	*	NS	NS	*	NS	NS	NS
$LSD_{0.05}$		1.161	0.125	1356	0.067	260.4	176.8	1.05	5.13

*, ** Significant at 0.05 and 0.01 levels of probability, respectively. NS = Not significant. PPB = Pigeon Pea (black), PPM = Pigeon Pea (mixed).

Increasing the light intensity reduced the SLA and increased the leaf mass/unit area indicating the thickening of leaves at a higher light intensity. Many tropical legume cover crops are known to reduce their yield under low light intensities [13,14]. Baligar et. al. [16,25,26] reported that increasing light intensity from 100 to 450 μmol m^{-2} s^{-1} increased shoot, leaf and root growth parameters of tropical legume cover crops. However, in the current study, Lab-Lab had decreasing trends in all measured traits at a PPFD of 450 μmol m^{-2} s^{-1}. Overall, in other cover crop species, the root/shoot ratio decreased with decreases in light intensity indicating that low light intensity is detrimental, especially to shoot growth. Baligar et al. [16] observed the beneficial effects of increased light intensities with Jack Bean recording the highest and Ea-Ea recording the lowest shoot and root growth parameters. Fujita et al. [14] reported that low light intensity (shading) reduced root weight in Kudzu (*Pueraria* *lobata* Ohwi) but had no effect on shoot weight. In Calopo, an increased root/shoot ratio at low light intensity has been reported [27]. Interactions of species and PPFDs were only significant for the total dry weight and leaf area. In plantation crops such as cacao beneficial effects of cover crops have been observed during the first 3–4 years of establishment; as the cacao and the associated shade trees grow and a heavy canopy is formed, the effectiveness of the cover crop diminishes because of insufficient light [14,23,24].

3.2. Light Intensity on Physiological Parameters

In all the legume cover crops increasing the light intensity significantly increased the net assimilation rates (NAR). Among the nine cover crops Ea-Ea recorded the highest NAR followed by Pigeon Pea (mixed) at all three levels of PPFD (Table 3). Species, PPFD and their interactions had highly significant effects on NAR indicating that the cover crop species responded differently to the light levels. The overall SPAD index increased significantly with increases in the PPFD from 180 to 900 µmol m^{-2} s^{-1}. Species, PPFD and their interactions were significant for the SPAD index and Jack Bean, Cowpea and Pigeon Pea (mixed) recorded the highest SPAD index. Izaguirre-Mayoral et al. [24] reported significant reductions in leaf chlorophyll in 24 native legume species from Venezuela grown at a 75% reduction of the incident sunlight (480 µmol m^{-2} s^{-1}). The net photosynthesis (P_N) and its components, stomatal conductance (gs), transpiration (E) and vapor pressure deficit (VPD) increased and internal CO_2 (Ci) decreased with increasing the PPFD (Table 3). Species, PPFD and their interactions had significant effects on P_N and its components. Species differed in their photosynthetic response to changing the PPFD, as observed in all genotypes. Cowpea and Pigeon Peas (Black, Mixed) had the highest P_N and Mucunas (Ana, Preta) had the lowest P_N. Imbamba and Tieszen [28] reported that P_N in *Crotalaria brevidens* increased with increasing irradiance to 1000 µmol m^{-2} s^{-1} and increased gradually with further increases in irradiance to 2000 µmol m^{-2} s^{-1}. In the current study, the overall increases in PPFD from 180 to 900 µmol m^{-2} s^{-1} increased P_N, gs, E and VPD by 88, 25, 60 and 5 percent, respectively and clearly indicated that these legume cover crops are sensitive to low light intensity and would not survive once an associated tree canopy grew and reduced the amount of light reaching the cover crop canopy level. Baligar et al. [17,18] evaluated the independent short-term effects of PPFD ranging from 50 to 1500 µmol m^{-2} s^{-1} on P_N and its components. In Calopo, Jack Bean, Mucuna, Perennial Peanut and White Lead Tree decreasing the PPFD from 1000 to 50 µmol m^{-2} s^{-1} reduced the P_N by 90% and substantially reduced gs and E [17]. Baligar et al. [18] reported in Crotalaria species increasing the PPFD from 50 to 1500 µmol m^{-2} s^{-1} increased the P_N by 21-fold, increased the gs by 2.3-fold, decreased the Ci by 3.9 times and increased the E by 2.1 times.

Table 3. Effect of photosynthetic photon flux density (PPFD) on physiological parameters in cover crops.

Species	PPFD (μmol m^{-2} s^{-1})	NAR ($\times 10^{-4}$)	SPAD Values	P_N	gs	Ci	E	VPD
Calopo	180	0.893	28.90	5.46	0.113	291.7	1.58	1.45
	450	1.170	24.80	9.94	0.160	270.3	2.35	1.45
	900	3.238	33.43	18.50	0.257	231.0	3.89	1.53
Ea-Ea	180	1.814	27.07	9.01	0.190	295.0	2.61	1.35
	450	3.523	30.80	9.92	0.106	219.0	1.70	1.59
	900	5.336	27.63	8.16	0.078	206.0	1.38	1.72
Jack Bean	180	0.295	42.70	6.35	0.063	203.7	0.99	1.55
	450	1.266	45.10	10.08	0.098	195.7	1.50	1.57
	900	1.546	48.53	13.27	0.137	186.7	2.05	1.56
Lab-Lab	180	0.832	37.13	8.96	0.183	290.0	2.79	1.50
	450	0.938	30.45	12.10	0.122	206.3	2.02	1.63
	900	2.239	42.37	13.97	0.141	200.7	2.40	1.69
M. ana	180	0.827	30.40	6.34	0.043	137.1	0.68	1.54
	450	1.811	32.33	8.41	0.074	159.5	1.20	1.65
	900	1.253	29.53	7.58	0.060	155.2	1.02	1.69
M. preta	180	0.715	32.00	4.41	0.030	138.5	0.55	1.80
	450	1.247	34.57	7.83	0.066	175.0	1.17	1.74
	900	1.586	30.20	6.33	0.043	121.9	0.74	1.73
Cowpea	180	1.204	43.27	8.24	0.088	219.0	1.34	1.49
	450	0.986	40.23	12.20	0.132	214.0	1.89	1.42
	900	3.521	55.27	17.07	0.170	171.3	2.55	1.58
PPB	180	0.686	31.33	8.46	0.203	300.0	2.47	1.27
	450	0.962	31.63	13.60	0.193	245.0	2.70	1.41
	900	3.113	42.13	21.07	0.339	246.3	4.22	1.25
PPM	180	1.412	37.77	8.35	0.144	257.7	2.11	1.54
	450	2.294	38.83	15.10	0.171	216.7	2.41	1.40
	900	5.228	47.57	17.17	0.154	176.3	2.28	1.47
Significance								
Species (S)		**	**	**	**	**	**	**
PPFD (P)		**	**	**	*	**	**	NS
S × P		**	**	**	**	NS	**	NS
$LSD_{0.05}$		2.519	13.78	5.70	0.145	116.2	1.68	0.386

*, ** Significant at 0.05 and 0.01 levels of probability, respectively. NS = Not significant. PPB = Pigeon Pea (black), PPM = Pigeon Pea (mixed). NAR = Net Assimilation Rate= g cm^2 d^{-1}; P_N = Net Photosynthesis (μmol CO_2 m^{-2} s^{-1}); gs = stomatal conductance (mol H_2O m^{-2} s^{-1}); Ci = Internal CO_2 (cm^3 m^{-3}); E = Transpiration (mmol H_2O m^{-2} s^{-1}); VPD = Vapor Pressure Deficit (kPa).

3.3. Light Intensity on Water Flux and Water Use Efficiency

Water stress affects many physiological and metabolic processes in plants. Total water use efficiency (WUE_{TOTAL}) is considered an important component of drought tolerance in plants and such determination also helps to understand the ability of a genotype/cultivar to utilize available soil water efficiently in order to produce higher dry matter. In the current study, species and PPFD and their interactions significantly affected water flux (Vo) and various water use efficiency parameters (WUE_{TOTAL}, WUE_{INST} and WUE_{INTR}) (Table 4). Increasing the PPFD from 180 to 900 μmol m^{-2} s^{-1} significantly reduced the Vo but increased all three WUE parameters. This reflected increased P_N, E and gs due to increased PPFD (Table 3). In other crops, the relationships between the WUE_{TOTAL} and the WUE_{INST} were either positive or negative [29]. Interspecific differences were observed in water use efficiency parameters.

Table 4. Effect of photosynthetic photon flux density (PPFD) on water use efficiency in cover crops.

Species.	PPFD (μmol m^{-2} s^{-1})	V_O	WUE_{TOTAL}	WUE_{INST}	WUE_{INTR}
Calopo	180	10.31	0.495	3.65	54.55
	450	8.41	0.727	4.19	61.59
	900	6.43	1.383	4.82	74.56
Ea-Ea	180	35.59	0.368	3.47	47.47
	450	12.96	1.158	5.84	94.23
	900	8.56	1.570	5.94	104.49
Jack Bean	180	2.01	3.638	6.85	109.04
	450	2.07	6.059	6.79	108.70
	900	1.89	4.887	6.86	110.41
Lab-Lab	180	17.42	0.766	3.33	50.46
	450	6.34	3.791	5.99	99.23
	900	9.42	1.819	5.82	99.59
M. ana	180	6.96	1.170	9.60	151.71
	450	3.30	3.286	7.86	134.21
	900	2.00	2.905	7.89	138.20
M. preta	180	5.65	3.191	8.23	152.11
	450	5.49	3.929	6.98	124.74
	900	2.64	7.078	9.02	160.56
Cowpea	180	17.28	1.303	6.46	97.48
	450	31.40	1.552	6.57	95.01
	900	10.44	2.198	7.06	114.33
PPB	180	23.36	0.791	3.54	46.26
	450	12.79	1.368	5.10	72.92
	900	7.34	1.772	4.98	61.92
PPM	180	18.43	1.089	4.48	72.19
	450	10.73	1.776	6.34	89.28
	900	8.92	2.353	7.52	111.24
Significance					
Species (S)		**	**	**	**
PPFD (P)		**	**	**	**
S × P		*	**	*	NS
$LSD_{0.05}$		2.23	2.891	3.86	77.51

*, ** Significant at 0.05 and 0.01 levels of probability, respectively. NS = Not significant. PPB = Pigeon Pea (black), PPM = Pigeon Pea (mixed). Vo = Water Flux (cm^3 H_2O influx cm^{-2} of roots s^{-1} $\times 10^{-6}$); WUE_{TOTAL} = Total water use efficiency (g shoot/g transpired $\times 10^{-3}$); WUE_{INST} = Instantaneous water use efficiency (μmol CO_2/mmol H_2O); WUE_{INTR} = Intrinsic water use efficiency (μmol CO_2/mol H_2O).

3.4. Light Intensity on Nutrient Uptake Parameters

3.4.1. Concentration and Uptake of Nutrients

The effects of shading on nutritive value is often negative [11]. Concentrations of macro- and micronutrients were significantly influenced by species and PPFD (Table 5). Increasing the PPFD decreased the concentrations of N, P, K, B but increased the concentrations of Mg, Fe and Mn. Intraspecific differences were observed for variation in the nutrient concentrations of macro- and micronutrients. The differential effects of varying levels of PPFD on the nutrient uptake by perennial cover crop legumes have been reported [15,16,25,26]. Wong [30] reported changes in the mineral composition of Joint Vetch, Calopo, Centro, Ea-Ea, Tropical Kudzu and Brazilian Lucerne grown in varying levels of shade (18 to 100% of daylight) in greenhouse conditions. In this study, the mean concentrations of P, Ca, Mg and K in all the legumes increased significantly with increasing shade.

Table 5. Effect of photosynthetic photon flux density (PPFD) on plant nutrient concentrations in cover crops.

Species	PPFD (μmol m^{-2} s^{-1})	N	P	K	Ca	Mg	B	Cu	Fe	Mn	Zn
		mg g^{-1}					μg g^{-1}				
Calopo	180	66.68	8.09	22.83	13.28	1.86	88.79	46.32	317.5	60.3	52.67
	450	66.68	8.09	22.83	13.28	1.86	88.79	46.32	317.5	60.3	52.67
	900	59.73	7.58	22.72	13.18	1.91	65.01	31.33	173.6	45.0	49.02
Ea-Ea	180	47.81	6.80	21.76	13.54	3.16	47.40	32.32	356.9	55.5	62.77
	450	47.81	6.80	21.76	13.54	3.16	47.40	32.32	356.9	55.5	62.77
	900	44.59	7.37	17.40	14.42	3.47	46.28	33.45	391.8	63.4	60.88
Jack Bean	180	78.60	10.24	20.97	12.33	2.45	59.22	50.67	536.0	67.2	55.53
	450	66.23	9.28	18.74	12.39	3.00	57.65	43.57	424.6	64.4	52.13
	900	67.50	10.64	16.94	12.89	3.11	62.78	47.53	587.3	70.4	60.04
Lab-Lab	180	71.48	20.25	30.00	12.78	1.41	61.14	26.43	52.4	44.8	62.30
	450	71.48	20.25	30.00	12.78	1.41	61.14	26.43	52.4	44.8	62.30
	900	70.87	20.37	27.35	12.16	1.63	61.00	27.99	75.9	49.3	63.67
M. ana	180	69.51	20.24	20.31	10.37	1.73	68.36	47.65	74.3	73.6	85.12
	450	78.78	17.49	18.44	11.72	1.70	73.37	58.98	124.8	72.2	90.81
	900	67.07	19.15	21.20	9.96	1.64	67.37	46.85	70.6	60.2	77.14
M. preta	180	73.14	21.12	18.72	8.87	1.87	74.84	48.55	184.9	71.0	92.41
	450	72.06	21.54	19.64	10.25	1.65	73.69	47.96	208.5	79.3	95.20
	900	72.73	22.93	20.61	8.62	1.46	72.22	55.72	134.5	76.8	99.73
Cowpea	180	73.52	16.32	31.56	14.39	3.47	56.66	11.07	63.8	56.9	69.44
	450	77.47	16.78	31.03	12.22	3.59	51.96	10.64	52.1	67.9	65.83
	900	70.70	17.72	25.02	13.63	4.17	60.36	18.69	142.6	112.3	81.02
PPB	180	61.33	19.05	25.99	9.21	1.98	81.47	38.33	175.0	43.9	115.9
	450	61.33	19.05	25.99	9.21	1.98	81.47	38.33	175.0	43.9	115.9
	900	60.42	18.47	20.42	10.55	2.08	78.37	49.95	378.7	66.5	116.5
PPM	180	60.01	22.33	21.83	10.51	1.93	98.75	44.05	189.1	58.6	122.8
	450	54.93	21.32	23.81	10.21	1.68	91.15	31.00	70.2	58.8	104.8
	900	53.09	16.75	18.50	10.35	1.73	83.96	33.30	184.2	64.0	107.1
Significance											
Species (S)		**	**	**	**	**	**	**	**	**	**
PPFD (P)		**	**	**	NS	*	**	NS	NS	**	NS
S × P		**	**	**	**	**	**	**	**	**	**
$LSD_{0.05}$		6.38	1.54	3.65	1.55	0.64	8.41	13.86	186.7	14.9	14.7

*, ** Significant at 0.05 and 0.01 levels of probability, respectively. NS = Not significant. PPB = Pigeon Pea (black), PPM = Pigeon Pea (mixed).

Species and PPFD had highly significant effects on the uptake of all macro- and micronutrients (Table 6). In general, the uptake of nutrients increased with increasing the PPFD from 180 to 900 μmol m^{-2} s^{-1}. Wong [30] reported changes in the mineral composition of Calopo, Ea-Ea and Tropical Kudzu grown in varying levels of shade (18 to 100%) in greenhouse conditions. In all of these legumes, the mean uptake of P, Ca, Mg and K increased significantly with increasing shade. In a growth chamber experiment, cover crops (Sunnhemp, Crotalaria ochroleuca, Showy Crotalaria, Sesbania) responded significantly to growth and nutrient uptake parameters with increasing the PPFD from 200 to 400 μmol m^{-2} s^{-1} [16]. The variations in nutrient uptake were related to the differences in dry matter accumulation between species. Jack Bean produced the highest dry matter yield and had the highest nutrient uptake. Ea-Ea produced the lowest dry matter yield and had the lowest nutrient uptake. The differences in nutrient uptake and yield among species have been related to differences in absorption, translocation, shoot demand and dry matter production potentials per unit of nutrient absorbed [31–33]. Furthermore, across all crop species and light intensities, the uptake of nutrients was in the order of N > K > P > Ca > Mg for macro-nutrients and Fe > Zn > B > Mn > Cu for micronutrients. Fageria et al. [34] and Baligar et al. [15,16,26] have reported similar trends in nutrient uptake by legume crops.

Table 6. Effect of photosynthetic photon flux density (PPFD) on nutrient uptake (U) in leguminous cover crops.

Species	PPFD (μmol m^{-2} s^{-1})	N	P	K	Ca	Mg	B	Cu	Fe	Mn	Zn
		mg plant^{-1}					μg plant^{-1}				
Calopo	180	2.3	0.28	0.79	0.46	0.06	3.06	1.59	10.9	2.08	1.81
	450	3.7	0.45	1.27	0.74	0.10	4.95	2.58	17.7	3.37	2.94
	900	10.6	1.35	4.05	2.35	0.34	11.58	5.58	30.9	8.02	8.73
Ea-Ea	180	0.8	0.11	0.35	0.22	0.05	0.77	0.53	5.8	0.90	1.02
	450	2.9	0.42	1.35	0.84	0.20	2.93	2.00	22.1	3.43	3.89
	900	3.9	0.64	1.51	1.25	0.30	4.00	2.89	33.9	5.48	5.27
Jack Bean	180	92.6	12.18	24.80	14.80	2.97	72.31	59.71	644.3	81.10	66.39
	450	159.7	22.38	44.81	29.57	7.28	138.88	102.83	1016.5	157.19	124.85
	900	150.5	24.03	37.29	28.94	7.14	140.47	107.41	1312.4	158.84	134.90
Lab-Lab	180	28.8	8.17	12.10	5.16	0.57	24.66	10.66	21.1	18.06	25.13
	450	24.4	6.90	10.23	4.36	0.48	20.84	9.01	17.8	15.26	21.23
	900	51.6	14.78	19.58	8.92	1.24	45.32	20.81	57.1	39.11	48.97
M. ana	180	22.1	6.42	6.44	3.29	0.55	21.69	15.12	23.6	23.37	27.01
	450	71.9	15.96	16.83	10.70	1.55	66.96	53.83	113.9	65.86	82.88
	900	34.8	9.95	11.01	5.17	0.85	34.99	24.33	36.7	31.25	40.06
M. preta	180	31.2	9.00	7.98	3.78	0.80	31.90	20.70	78.8	30.28	39.39
	450	51.7	15.51	14.07	7.64	1.25	55.25	36.52	151.7	60.23	72.39
	900	54.1	17.46	15.99	6.76	1.05	52.33	37.95	74.5	56.87	70.14
Cowpea	180	30.2	6.71	12.98	5.91	1.43	23.30	4.55	26.2	23.43	28.55
	450	22.6	4.90	9.05	3.57	1.05	15.16	3.10	15.2	19.81	19.21
	900	58.6	14.47	19.72	11.57	3.72	51.86	18.26	165.8	91.93	72.72
PPB	180	8.7	2.69	3.68	1.30	0.28	11.52	5.42	24.7	6.21	16.40
	450	11.2	3.47	4.73	1.68	0.36	14.83	6.97	31.8	7.99	21.10
	900	23.7	7.25	8.02	4.14	0.82	30.74	19.60	146.5	26.24	45.75
PPM	180	12.6	4.69	4.58	2.21	0.40	20.73	9.25	39.7	12.29	25.78
	450	19.9	7.76	8.66	3.72	0.61	33.17	11.28	25.5	21.40	38.13
	900	55.9	17.65	19.56	10.90	1.81	88.41	34.88	182.9	67.29	112.46
Significance											
Species (S)		**	**	**	**	**	**	**	**	**	**
PPFD (P)		**	**	**	**	**	**	**	**	**	**
S × P		NS	NS	NS	*	**	NS	NS	**	*	NS
$LSD_{0.05}$		67.5	18.01	20.83	12.25	3.15	72.11	48.24	394.0	81.29	86.45

*, ** Significant at 0.05 and 0.01 levels of probability, respectively. NS = Not significant. PPB = Pigeon Pea (black), PPM = Pigeon Pea (mixed).

3.4.2. Nutrient Use Efficiency (NUE)

The interactions of plant genetic and physiological components and the nutrient status of the soil profoundly affect the ability of plants to acquire and transport nutrients to shoots [33]. Nutrient use efficiency in plants is profoundly influenced by the levels of available nutrients in the soil (supply) and the genetic and physiological components (demand) of plants [33,35]. At high shade (low PPFD), cover crops that have high nutrient use efficiency (NUE) might have a better chance of survival. NUE differences are related to the differences in absorption and translocation of nutrients [33,36]. However, information is lacking on the influence of various light levels on nutrient use efficiency in cover crops. In the current study with few exceptions over all nutrient use efficiency (NUE) of all the nutrients was significantly influenced by species, PPFD and their interactions (Table 7). With the exception of the NUE for Mn, increasing the PPFD from 180 to 900 μmol m ^{2} s^{-1} increased the NUE for all the nutrients. The NUE values are useful in assessing the ability of plants to use absorbed nutrients efficiently or non-efficiently. The NUE values observed in the current study for various nutrients were similar to the NUE values reported earlier for other cover crops [15,16,18,25].

Table 7. Effect of photosynthetic photon flux density (PPFD) on nutrient use efficiency (NUE) in cover crops.

Species	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} (x10^4)———				
Calopo	180	15.00	123.5	43.80	75.30	538.8	1.13	2.16	0.32	1.66	1.90
	450	15.00	123.5	43.80	75.30	538.8	1.13	2.16	0.32	1.66	1.90
	900	16.74	131.9	44.02	75.89	522.7	1.54	3.19	0.58	2.22	2.04
Ea-Ea	180	20.91	146.9	45.97	73.85	316.8	2.11	3.09	0.28	1.80	1.59
	450	20.91	146.9	45.97	73.85	316.8	2.11	3.09	0.28	1.80	1.59
	900	22.43	135.6	57.48	69.33	288.0	2.16	2.99	0.26	1.58	1.64
Jack Bean	180	12.75	97.7	47.70	81.63	413.8	1.72	1.98	0.19	1.50	1.80
	450	15.11	107.9	53.44	80.83	334.3	1.74	2.35	0.24	1.57	1.92
	900	14.90	94.2	59.73	78.85	330.7	1.60	2.27	0.18	1.47	1.68
Lab-Lab	180	13.99	49.4	33.33	78.23	710.0	1.64	3.78	1.91	2.23	1.61
	450	13.99	49.4	33.33	78.23	710.0	1.64	3.78	1.91	2.23	1.61
	900	14.11	49.1	36.61	82.28	616.1	1.64	3.58	1.32	2.07	1.58
M. ana	180	14.39	49.4	49.24	96.48	579.1	1.46	2.10	1.35	1.36	1.17
	450	12.69	57.2	54.23	85.31	589.8	1.36	1.70	0.80	1.39	1.10
	900	14.91	52.2	47.17	100.31	608.4	1.48	2.13	1.42	1.66	1.30
M. preta	180	13.67	47.3	53.41	112.62	535.4	1.34	2.06	0.54	1.41	1.08
	450	13.92	46.5	51.08	97.59	606.6	1.36	2.09	0.48	1.26	1.05
	900	13.75	43.6	48.62	116.41	687.8	1.39	1.84	0.95	1.30	1.01
Cowpea	180	13.60	61.3	31.68	69.51	288.0	1.76	9.03	1.57	1.76	1.44
	450	12.91	59.6	32.23	81.80	278.5	1.92	9.40	1.92	1.47	1.52
	900	14.17	56.8	41.11	73.35	243.1	1.66	5.97	1.32	0.90	1.26
PPB	180	16.30	52.5	38.48	108.51	504.8	1.23	2.61	0.57	2.28	0.86
	450	16.30	52.5	38.48	108.52	504.8	1.23	2.61	0.57	2.28	0.86
	900	16.55	54.2	49.01	94.98	480.8	1.28	2.00	0.27	1.50	0.86
PPM	180	16.66	44.8	45.81	95.17	518.4	1.01	2.27	0.53	1.71	0.81
	450	18.21	46.9	42.00	97.93	595.6	1.10	3.23	1.42	1.70	0.95
	900	18.84	59.7	54.16	96.63	582.0	1.19	3.03	1.03	1.57	0.94
Significance											
Species (S)		**	**	**	**	**	**	**	**	**	**
PPFD (P)		**	**	**	NS	NS	**	*	NS	**	NS
S × P		**	**	**	**	**	**	**	**	**	**
$LSD_{0.05}$		1.35	6.6	8.26	11.2	82.3	0.21	1.43	0.78	0.35	0.23

*, ** Significant at 0.05 and 0.01 levels of probability, respectively. NS = Not significant. PPB = Pigeon Pea (black), PPM = Pigeon Pea (mixed).

4. Conclusions

The cover crop species studied in this experiment showed significant interspecific differences for growth and physiological traits at inadequate to adequate light intensities. A PPFD of less than 450 µmol m^{-2} s^{-1} appeared to be detrimental for growth, nutrient and water use efficiency and photosynthesis of tropical perennial legume cover crops. The quantity of light reaching the cover crop canopy limited the cover crops' growth and development. Therefore, canopy management of upper story trees is vital for the longevity of cover crops in plantation crops.

Author Contributions: V.C.B. conceived the original idea and was in charge of the overall direction and planning; M.K.E. conducted the experiment, analyzed data and prepared the manuscript; Z.H. performed soil, plant analysis and writeup of early draft; Y.L. reviewed writeup of early drafts; A.d.Q.P. provided the genetic background of perennial legume cover crops and early drafts; A.A.F.A. provided experimental guidance and critical feedback in the manuscript writing; D.A. provided experimental guidance and writeup draft preparation. All authors have read and agreed to the published version of the manuscript.

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References

1. Fageria, N.K.; Baligar, V.C.; Jones, C.A. *Growth and Mineral. Nutrition of Field Crops*, 3rd ed.; CRC Press: Boca Raton, FL, USA, 2011.
2. Jordan, D.; Opoku, A.A. The effect of selected soil covers on the establishment of cacao. *Trop. Agric. (Trinidad)* **1966**, *43*, 155–166.
3. Opoku, A.A. *Combined Annual Report for Academic Years 1968/69 and 1969/70*; Agricultural Research Station, Kade. University of Ghana: Legos, Ghana, 1970.
4. Cunningham, R.K.; Smith, R.W. Comparison of soil covers during cocoa establishment on clear-felled land. *Trop. Agric. (Trinidad)* **1961**, *38*, 13–22.
5. Willson, K.C. *Coffee, Cocoa and Tea*; CABI Publishing: New York, NY, USA, 1999.
6. Wood, G.A.R.; Lass, R.A. *Cocoa*, 4th ed.; Blackwell Science: Oxford, UK, 2001; p. 619.
7. Grace, J.; Lloyd, J.; Miranda, A.C.; Miranda, H.; Gash, J.H.C. Fluxes of carbon dioxide and water vapour over a C4 pasture in southwestern Amazonia (Brazil). *Aust. J. Plant Physiol.* **1998**, *25*, 519–530. [CrossRef]
8. Miyaji, K.I.; Da Silva, W.S.; Paulo, T.A. Longevity of leaves of a tropical tree, *Theobroma cacao*, grown under shading, in relation to position within the canopy and time of emergence. *New Phytol.* **1997**, *135*, 445–454. [CrossRef]
9. Liang, N.; Tang, Y.; Okuda, T. Is elevation of carbon dioxide concentration beneficial to seedling photosynthesis in the understory of tropical rain forests? *Tree Physiol.* **2001**, *21*, 1047–1055. [CrossRef] [PubMed]
10. Wong, C.C.; Sharudin, M.A.M.; Rahim, H. Shade tolerance potential of some tropical forages for integration with plantations. 2. Legumes. *MARDI (Malays. Agric. Res. Dev. Inst.) Res. Bull.* **1985**, *13*, 249–269.
11. Shelton, H.M.; Humphreys, L.R.; Batello, C. Pastures in the plantations of Asia and the Pacific: Performance and prospect. *Trop. Grassl.* **1987**, *21*, 159–168. Available online: http://www.tropicalgrasslands.info/Vol_21_04_87_pp159_168.pdf (accessed on 29 July 2020).
12. Stur, W.W. Screening forage species for shade tolerance-a preliminary report. In *Forages for Plantation Crops*; Shelton, H.M., Stür, W.W., Eds.; ACIAR (Australian Center for International Agricultural Research) Proceedings, No 32; ACIAR: Canberra, Australia, 1991; pp. 58–63.
13. Eriksen, F.I.; Whitney, A.S. Growth and N fixation of some tropical forage legumes as influenced by solar radiation regimes. *Agron. J.* **1982**, *74*, 703–708. [CrossRef]
14. Fujita, K.; Matsumoto, K.; Ofosu-Budu, G.K.; Ogata, S. Effects of shading on growth and dinitrogen fixation of kudzu and tropical pasture legumes. *Soil Sci. Plant. Nutr.* **1993**, *39*, 43–54. [CrossRef]
15. Baligar, V.C.; Fageria, N.K.; Paiva, A.Q.; Silveira, A.; Pomella, A.W.V.; Machado, R.C.R. Light intensity effects on growth and micronutrient uptake by tropical legume cover crops. *J. Plant Nutr.* **2006**, *29*, 1959–1974. [CrossRef]
16. Baligar, V.C.; Fageria, N.K.; Paiva, A.; Silveira, A.; Souza, J.O., Jr.; Lucena, E.; Faria, J.C., Jr.; Cabral, R.; Pomella, A.W.V.; Jorda, J. Light intensity effects on growth and nutrient use efficiency of tropical legume cover crops. In *Toward Agroforestry Design: An Ecological Approach*; Jose, S., Gordon, A., Eds.; Advances in Agroforestry; Springer: Dordrecht, The Netherlands, 2008; Volume 4, pp. 67–79. [CrossRef]
17. Baligar, V.C.; Bunce, J.A.; Elson, M.K.; Fageria, N.K. Irradiance, external carbon dioxide concentration and temperature influence photosynthesis in tropical cover crop legumes. *Trop. Grassl.* **2010**, *44*, 24–32. Available online: https://pubag.nal.usda.gov/download/58978/PDF (accessed on 29 July 2020).
18. Baligar, V.C.; Bunce, J.A.; Elson, M.K.; Fageria, N.K. Photosynthetic photon flux density, carbon dioxide concentration and temperature influence photosynthesis in Crotalaria species. *Open Plant. Sci. J.* **2012**, *6*, 1–7. [CrossRef]
19. Duke, J.A. *Handbook of Legumes of World Economic Importance*; Plenum Press: New York, NY, USA, 1981.
20. Cook, B.G.; Pengelly, B.C.; Brown, S.D.; Donnelly, J.L.; Eagles, D.A.; Franco, M.A.; Hanson, J.; Mullen, B.F.; Partridge, I.J.; Peters, M.; et al. Tropical Forages: An Interactive Selection Tool. CSIRO Sustainable Ecosystems (CSIRO), Department of Primary Industries and Fisheries (DPI&F Queensland), Centro Internacional de Agricultura Tropical (CIAT) and International Livestock Research Institute (ILRI). 2005. Available online: http://www.tropicalforages.info/ (accessed on 29 July 2020).

21. Faridah Hanum, I.; van der Maesen, L.J.G. *Plant. Resources of South.-East. Asia (PROSEA), No. 11, Auxiliary Plants*; Backhuys Publishers: Leiden, The Netherlands, 1997.
22. Tanner, C.B.; Sinclair, T.R. Efficient water use in crop production: Research or re-search. In *Limitations to Efficient Water Use in Crop. Production*; Taylor, H.M., Jordan, W.A., Sinclair, T.R., Eds.; American Society of Agronomy: Madison, WI, USA, 1983; pp. 1–27. Available online: https://dl.sciencesocieties.org/publications/books/pdfs/acsesspublicati/limitationstoef/1 (accessed on 29 July 2020).
23. Wong, C.C. Shade tolerance of tropical forages: A review. In *Forages for Plantation Crops*; Shelton, H.M., Stur, W.W., Eds.; ACIAR (Australian Center for International Agricultural Research) Proceedings No 32; ACIAR: Canberra, Australia, 1991; pp. 64–69.
24. Izaguirre-Mayoral, M.L.; Vivas, A.V.; Oropeza, T. New insights into the symbiotic performance of native tropical legumes: I. Analysis of the response of thirty-seven native legume species to artificial shade in a neotropical savanna. *Symbiosis* **1995**, *19*, 111–129.
25. Baligar, V.C.; Elson, M.; He, Z.L.; Li, Y.; Paiva, A.Q.; Ahnert, D.; Almeida, A.-A.F.; Fageria, N.K. Ambient and elevated carbon dioxide on growth, physiological and nutrient uptake parameters of perennial leguminous cover crops under low light intensities. *Int. J. Plant Soil Sci.* **2017**, *15*, 1–16. [CrossRef]
26. Baligar, V.C.; Elson, M.K.; He, Z.L.; Li, Y.; Paiva, A.Q.; Ahnert, D.; Almeida, A.-A.F. Growth, physiological and nutrient uptake traits of Crotalaria cover crops influenced by levels of carbon dioxide under low light intensities. *Int. J. Plant Soil Sci.* **2018**, *23*, 1–14. [CrossRef]
27. Ludlow, M.M.; Wilson, G.L.; Heslehurst, M.R. Studies on the productivity of tropical pasture plants. V. Effects of shading on growth, photosynthesis and respiration in two grasses and two legumes. *Aust. J. Agric. Res.* **1974**, *25*, 425–433. Available online: http://www.publish.csiro.au/AR/pdf/AR9740425 (accessed on 29 July 2020). [CrossRef]
28. Imbamba, S.K.; Tieszen, L.L. Influence of light and temperature on photosynthesis and transpiration in some C3 and C4 vegetable plants from Kenya. *Physiol. Plant.* **1977**, *39*, 311–316. [CrossRef]
29. Medrano, H.; Magdalena, T.; Martorell, S.; Flexas, J.; Hernandez, E.; Rossello, J.; Pou, A.; Bota, J.M.E.J. From leaf to whole-plant water use efficiency (WUE) in complex canopies: Limitations of leaf WUE as a selection target. *Crop. J.* **2015**, *3*, 220–228. [CrossRef]
30. Wong, C.C. Mineral composition and nutritive value of tropical forage legumes as affected by shade. *MARDI (Malays. Agric. Res. Dev. Inst.) Res. J.* **1990**, *18*, 135–144.
31. Gerloff, G.C.; Gabelman, W.H. Genetic basis of inorganic plant nutrition. In *Inorganic Plant. Nutrition: Encyclopedia and Plant. Physiology*; Lauchli, A., Bieleski, R.L., Eds.; New Series, 15B; Springer: New York, NY, USA, 1983; pp. 453–480.
32. Vose, P.B. Effects of genetic factors on nutritional requirements of plants. In *Crop. Breeding: A Contemporary Basis*; Vose, P.B., Blixt, S.G., Eds.; Pergamon Press: Oxford, UK, 1984; pp. 67–114.
33. Baligar, V.C.; Fageria, N.K.; He, Z.L. Nutrient use efficiency in plants. *Commun. Soil Sci. Plant.* **2001**, *32*, 921–950. [CrossRef]
34. Fageria, N.K.; Baligar, V.C.; Li, Y.C. Nutrient uptake and use efficiency by tropical legume cover crops at varying pH of an Oxisol. *J. Plant Nutr.* **2014**, *37*, 294–311. [CrossRef]
35. Baligar, V.C.; Duncan, R.R. *Crops as Enhancers of Nutrient Use*; Academic Press Inc.: San Diego, CA, USA, 1990. [CrossRef]
36. Clark, R.B.; Duncan, R.R. Improvement of plant mineral nutrition through breeding. *Field Crops Res.* **1991**, *27*, 219–240. [CrossRef]