Received: 22 August 2022 | Accepted: 2 January 2023

DOI: 10.1002/agg2.20343

Agrosystems, Geosciences & Environment OPEN ACCESS

ORIGINAL ARTICLE

Agrosystems

Growth, nodulation, production, and physiology of leguminous plants in integrated production systems

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Assigned to Associate Editor Anil Somenahally.

Funding information
Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Grant/Award Number: FinanceCode001; Bayer S.A.; Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant/Award Number: 316873/2021-7; Fundação de Amparo à Pesquisa do Estado de Minas Gerais, Grant/Award Number: PPM-00617-18

Abstract

Choosing the species suitable for cultivation in intercropped systems is a critical limiting factor for grain and forage production. This study aimed to evaluate the growth, nodulation, production, and physiology of cowpea (*Vigna unguiculata*) and guandu bean (*Cajanus cajan*) intercropped with buffel grass (*Cenchrus ciliaris*) and *Eucalyptus urograndis* (*Eucalyptus grandis* × *Eucalyptus urophylla*) in an integrated production system. Two experiments were conducted simultaneously, arranged in a 2 × 4 factorial scheme. The first factor consisted of the use of two integrated production systems. The second factor consisted of four distances (2, 4, 6, and 8 m) from the eucalyptus planting rows. In the first experiment, crop–forest systems (CFIS: eucalyptus trees and cowpea) and crop–livestock–forest systems (CLFIS: eucalyptus trees, cowpea, and buffel grass) were evaluated. In the second, livestock–forest systems LFIS1 (eucalyptus trees and guandu bean) and LFIS2 (eucalyptus trees, guandu bean, and buffel grass) were evaluated. In cowpea, nodulation was not influenced by production systems and distances, whereas in guandu bean, there was a reduction in the nodules' number and dry mass with the distance from the trees. Higher growth, biomass production, and photosynthetic activity of cowpea and guandu bean were observed with the increasing distance from the eucalyptus planting rows. However, cowpea increased the 100-seed weight at 2 and 4 m distances from the eucalyptus rows, improving the grains' quality close to the trees. The simultaneous cultivation with buffel grass did not affect the leguminous plant yield, promoting greater diversification and increasing the total biomass production of the intercropping.

Abbreviations: CFIS, eucalyptus, cowpea intercropping; CLFIS, cowpea, buffel grass, eucalyptus intercropping; LFIS1, guandu bean and eucalyptus intercropping; LFIS2, guandu bean, buffel grass, and eucalyptus intercropping.

1 | INTRODUCTION

Intercropping systems' benefits include stability of production, higher yield per unit area, reduction of pests' incidence, and the need to use agrochemicals, which provides an increase in biodiversity, characteristics that favor the sustainability of production systems (Jensen et al., 2020). In addition, Namatsheve et al. (2021) reported that the leguminous plants and grass integration make it possible to significantly add nitrogen to the system through the biological N_2 fixation, helping to increase the soil productivity. Silva et al. (2022) reported that adding leguminous plants to grass in integrated systems may increase crop and livestock productivity.

The decrease in light intensity in intercropping systems due to shading is a critical factor in crop growth and productivity as light regulates the photosynthetic efficiency of plants (Jumrani & Bhatia, 2020). Reduction in photosynthetic rate due to shading decreases the production of photo assimilates necessary for plant growth and productivity (Mwamlima et al., 2020). Thus, shorter species may impair development by competing for light with higher plants (Fan et al., 2019). However, despite reducing light and usually decreasing crop productivity, productivity loss varies according to the species used (Angadi et al., 2022).

Reducing photosynthetically active radiation in agroforestry systems is the most critical limitation to crops under the understory, which can be managed by increasing the tree planting distance (Surki et al., 2020). Honnayya et al. (2020) observed a gradual reduction in the growth and productivity of guandu bean by reducing the distance from tree planting rows in agroforestry systems. Nevertheless, Manoj et al. (2021) reported the guandu bean as a leguminous plant with the potential to be used in intercropped systems. Angadi et al. (2022) also determined cowpea, among different leguminous plants under shading, as one of the most tolerant species to light reduction.

Cowpea can be grown in different types of soil and is tolerant to conditions of low water availability (Osipitan et al., 2021). Different studies show that cowpea is a crop adapted to different edaphoclimatic conditions (Boukar et al., 2019; Carvalho et al., 2017; Sindhu et al., 2019) and has multiple uses. It can be used in animal feed (Iqbal et al., 2018) and human food (Kebed & Bekeko, 2020), as well as for green manure (Abera & Gerkabo, 2021) and is an important source of protein. Dakora and Belane (2019) observed leaf protein values in cowpea ranging from 23% to 40% between different genotypes and, in seeds, up to 40% protein content. The species has a high ability to fix atmospheric nitrogen from the formation of root nodules in association with rhizobia (Kebede & Bekeko, 2020). Freitas et al. (2012) observed that 79% of the nitrogen accumulated in cowpea cultivated in the Brazilian semiarid region came from biological fixation, which corresponded to 45 kg ha^{-1} of N. Additionally, Kebede and Bekeko (2020) placed cowpea as a crop option that presents very early maturity, which provides a reduction in the production period in relation to many other cultivated species.

Guandu bean is an important crop worldwide, especially in semiarid regions, due to its tolerance to drought (Varshney et al., 2010). Studies also show that guandu bean is adapted to different edaphoclimatic conditions (Lobato et al., 2020; Musokwa & Mafongoya, 2020). The species is used in animal (Neres et al., 2012) and human (Miano et al., 2020) food and also as a strategy to maintain soil sustainability (Teodoro et al., 2018). Abebe (2022) highlighted the potential use of guandu bean for human and animal food due to its high protein content and low production cost. In general, the protein content in guandu bean grains is 20%–22% (Venkata et al., 2019) and 23%–33% in dry matter (leaves and tender portions of stems) (Neres et al., 2012). The process of biological nitrogen fixation resulting from the symbiosis between guandu bean and rhizobia and the production of litter by the crop increases soil fertility (Varshney et al., 2010). Adu-Gyamfi et al. (2007) observed values between 37.5 and 117.2 kg ha^{-1} year^{-1} of nitrogen from biological N_2 fixation in pigeon pea varieties cultivated in a semiarid region of Africa.

Despite the different uses and production possibilities under different edaphoclimatic conditions, there are still few studies evaluating the performance of leguminous plants in integrated production systems, resulting in a lack of information to optimize the productive capacity in these systems. Therefore, this study aimed to evaluate the growth, nodulation, production, and physiology variables of cowpea and guandu bean intercropped with buffel grass and eucalyptus in an integrated production system.

Core Ideas

- Higher nodulation was observed in the guandu bean close to eucalyptus trees.
- The leguminous plants' photosynthetic activity increased with distance from the trees.
- The leguminous plants' biomass growth and production decreased close to the trees.
- Cowpea increased 27% in 100 seed weight close to eucalyptus trees.
- The leguminous plants' productivity does not decrease intercropped with buffel grass.

2 | MATERIAL AND METHODS

The experiments were carried out from December 2019 to May 2020 at Hamilton de Abreu Navarro Experimental Farm (16°40′03″S, 43°50′41″W, 598 m altitude), located at the Institute of Agricultural Sciences of the Federal University of Minas Gerais, in the municipality of Montes Claros, Minas Gerais, Brazil. The area is located in the Cerrado biome (*stricto sensu*) with slightly wavy relief. The climate is tropical savanna (AW according to Köppen classification (Köppen & Geiger, 1928)), with rainy summers and dry winters. The average annual rainfall along the evaluations of this study was

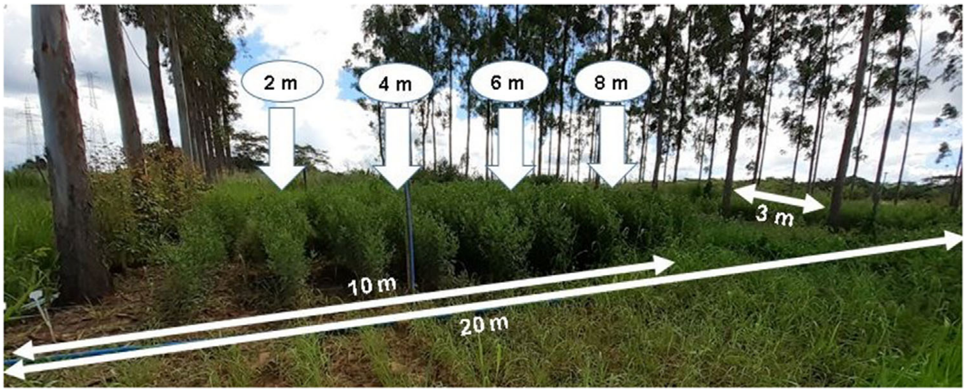

FIGURE 1 Spacing between planting lines (20 m) and eucalyptus trees (3 m) area occupied by plot (10 × 3 m) and distances (2, 4, 6, and 8 m) of the eucalyptus planting lines used in the evaluation of the cowpea and pigeon pea

923 mm, with an average temperature of 24.9˚C (Instituto Nacional de Meteorologia, 2022).

The soil was classified as eutrophic red-yellow latosol (Oxisol) with the following chemical and physical characteristics in the 0–20 cm depth layer: pH in water = 6.50; P Mehlich = 17.52 mg dm^{-3}; solution equilibrium P = 39.00 mg L^{-1}; K = 217 mg dm^{-3}; Ca = 7.60 $cmol_c$ dm^{-3}; mg = 3.28 $cmol_c$ dm^{-3}; Al = 0.00 $cmol_c$ dm^{-3}; H + Al = 2.08 $cmol_c$ dm^{-3}; sum of bases = 11.44 $cmol_c$ dm^{-3}; effective cation exchange capacity = 11.44 $cmol_c$ dm^{-3}; aluminum saturation = 0.00%; potential cation exchange capacity = 13.52 $cmol_c$ dm^{-3}; base saturation = 84.61%; organic carbon = 20.37 g kg^{-1}; sand = 38.00 g kg^{-1}; silt = 32.00 g kg^{-1}; clay = 30.00 g kg^{-1}; and density = 1.41 g cm^{-3}.

The experimental area has 6900 m^2 and was introduced in 2009 over a low-productivity pasture. The tree component is the hybrid *Eucalyptus urograndis* (*Eucalyptus grandis* × *Eucalyptus urophylla*), with an arrangement of 20 × 3 m (166 trees ha^{-1}) and planting rows in the east–west direction (Figure 1). In the inventory, the trees presented a total height of 44.74 m and a diameter at breast height of 118.6 cm. The mean photosynthetically active radiation was obtained from the AccuPAR LP-80 model at 2, 4, 6, and 8 m distances from the tree planting rows, which presented 324.92, 369.20, 378.08, and 395.98 μmol m^2 s^{-1}, respectively, whereas unshaded was 647 μmol m^2 s^{-1}.

Two experiments were simultaneously conducted in the same experimental area. A randomized block design with four replications was used in both cases, with treatments arranged in a 2 × 4 factorial scheme. The first factor consisted of the use of two integrated production systems. The second factor consisted of four distances from the eucalyptus trees planting rows (Figure 1). The crop–forest systems (CFIS) and crop–livestock–forest systems (CLFIS) were evaluated for the first experiment, whereas the LFIS1 and LFIS2 livestock–forest systems were evaluated for the second experiment.

The CFIS system was composed of eucalyptus and cowpea, whereas the CLFIS system was formed by eucalyptus, cowpea, and buffel grass intercropping. The LFIS1 system was composed of eucalyptus and guandu bean, whereas the LFIS2 system was formed by eucalyptus, guandu bean, and buffel grass intercropping. In both experiments, the distances of the eucalyptus rows used to determine the attributes studied were 2, 4, 6, and 8 m (Figure 1). The experimental units, allocated between tree rows, had a dimension of 10 × 3 m (30 m^2), keeping a 1-m distance from the eucalyptus rows (Figure 1).

The planting of guandu bean, cowpea, and buffel grass was carried out between the eucalyptus rows in December 2019, when the integrated system was 11-year old. The desiccation of remaining species from previous crops (mainly *Brachiaria* sp.) with glyphosate athanor (4.0 L ha^{-1}) was performed to implement the integrated systems. The soil was prepared for planting by harrowing, and then planting fertilization was performed by applying 20 kg ha^{-1} of P_2O_5 and then 25 days after sowing (DAS), 20 kg ha^{-1} of K_2O and 20 kg ha^{-1} of N. During crop implantation, a sprinkler irrigation system was set up to prevent plants loss due to water stress during periods without rain, allowing a 16 mm irrigation depth per week.

Guandu bean (*Cajanus cajan* (L.) Millsp., cv. IAPAR 43/Aratã) was sown, aiming for a final stand of 10 plants m^{-1} with 1 m row spacing, and cowpea (*Vigna unguiculata* (L.) Walp., cv. BRS Potengi) with 8 plants m^{-1} and 0.5 m row spacing. For the buffel grass sowing (*Cenchrus ciliaris* L., cv. Aridus), 35 kg ha^{-1} of seeds (2 kg ha^{-1} of viable pure seeds) were used in rows spaced 0.5 m apart. The sowing of the plots with an intercropping with leguminous plants and buffel grass followed the same spacing and plant population used in the plots with no intercropping. Cowpea was managed for grain production, whereas guandu bean and buffel grass were used for forage production. Buffel grass remained in the area after the leguminous plants' crop.

Two plants were randomly selected at full bloom (50% of plants with open flowers) at each distance, totaling 62 plants in each experiment. Each plant was collected with a soil volume of 25 cm long × 25 cm wide × 30 cm deep using a straight shovel to open the pit. The soil was taken apart from the root system by a water jet over a 2 × 2 mm mesh sieve. The variables evaluated in the cowpea flowering were as follows: plant main stem length, number of leaves, number of nodules, nodules dry mass, main stem dry mass, leaf dry mass, and root dry mass. In addition to the variables mentioned for cowpea, the main stem diameter, stems diameters, and the number of stems were also measured for the guandu bean.

The main stem length was determined by a measuring tape, measuring from the ground level to the last trifoliate leaf insertion and recording the number of leaves and stems in the whole plant. The main stem and stems diameters were obtained using a digital vernier. The aboveground part was separated from the roots with a cutoff at the cotyledonary insertion point. The nodules were removed from the roots, counted, and dried on absorbent paper. The aboveground parts of the plants (stem and leaves), roots, and nodules were packed in paper bags and left in a forced air circulation oven at 65˚C until reaching a constant weight (±72 h) to evaluate the nodules, main stem, leaf, and root dry mass.

The variables CO_2 consumed, internal carbon, transpiration rate, stomatal conductance, and photosynthetic rate were evaluated using the infrared gas analyzer LCpro—SD model in the leguminous plants at 50% flowering. The evaluations were carried out in the morning (between 7 and 9 h) on days without clouds. Three plants were randomly sampled in each experimental unit, using fully expanded leaves located near the apex of the plants.

The cowpea harvest was performed manually at approximately 75 DAS, when the pods were ripe, in an area of 0.5 m^2 at each evaluated distance. The number of pods, pod total weight, 100-seeds weight, and effective grain yield were analyzed. The humidity was corrected to 13% using the greenhouse method at 105˚C to determine the weight of 100 seeds and yield. The guandu bean was cut at 150 DAS to determine the effective dry mass yield. The effective dry mass yield of the guandu bean was obtained from the collection of all plants along a 1-m linear at each distance. Then, the plants collected were taken to a forced circulation oven (65˚C) until a constant weight check (approximately 72 h).

The values of effective grain and dry mass yield were determined according to the following equation: effective yield of green (MV) or dry (MS) mass (kg ha^{-1}) = {MV or MS yield (kg ha^{-1}) × [(10,000 − area occupied by trees (m^2))/10,000)}. The area occupied by the trees corresponded to 10%, whereas the area available for agricultural production was equivalent to 90%.

The Shapiro–Wilk test was applied to verify the occurrence of normal distributions and the Bartlett test to verify the homogeneity of variances. The values obtained from counting (number of stems, leaves, nodules, and pods) were transformed into $\sqrt{x+0.5}$ to meet the presuppositions of normality for the analysis of variance, and the results presented the non-transformed values. Based on the presuppositions of normality validation and homogeneity of variances, the analysis of variance was performed, and the Tukey test was applied at a level of 5% significance. The statistical analysis was carried out using R software, version 3.6.2 (R Core Team, 2019).

3 | RESULTS AND DISCUSSION

The effect of the interaction between the systems and distance factors was observed for the CO_2 consumed and transpiration rate variables in cowpea (Table 1), and the other variables were independently evaluated (Figures 2–4).

For guandu bean, the effect of the interaction between the systems and distance factors was observed for the number of stems, transpiration rate, and photosynthetic rate variables (Table 2), and the other variables were independently evaluated (Figures 5 and 6).

3.1 | Growth, production, nodulation, and physiological characteristics of cowpea

The main stem length, number of leaves, number of nodules, nodules dry mass, main stem dry mass, leaf dry mass, and root dry mass variables for cowpea were similar between CFIS and CLFIS systems (Figure 2). There was an increase in the main stem length, main stem dry mass, leaf dry mass, root dry mass, and total dry mass with the distance from the eucalyptus plantation rows. In general, a higher main stem length and a higher biomass production were observed at an 8 m distance. The number of leaves and nodules and nodules dry mass variables did not show any difference between the evaluated systems and the distances studied (Figure 2).

Variation in biomass production of cowpea plants in the understory was also observed in a study carried out by Alam et al. (2018). The authors reported a reduction in the production of cowpea biomass in the understory of *Dalbergia sissoo* genotypes that presented a higher leaf area index, providing a higher light interception by the canopy and, therefore, a higher shading on the agricultural crop. According to Alam et al. (2018), the different shading intensities influenced the cowpea growth in the agro-silvicultural systems evaluated.

The distance from the eucalyptus planting rows in the present study contributed to an increase in the production of cowpea biomass due to the lower level of shading and higher light interception by the culture in the understory, showing the need for proper planning when choosing the tree component

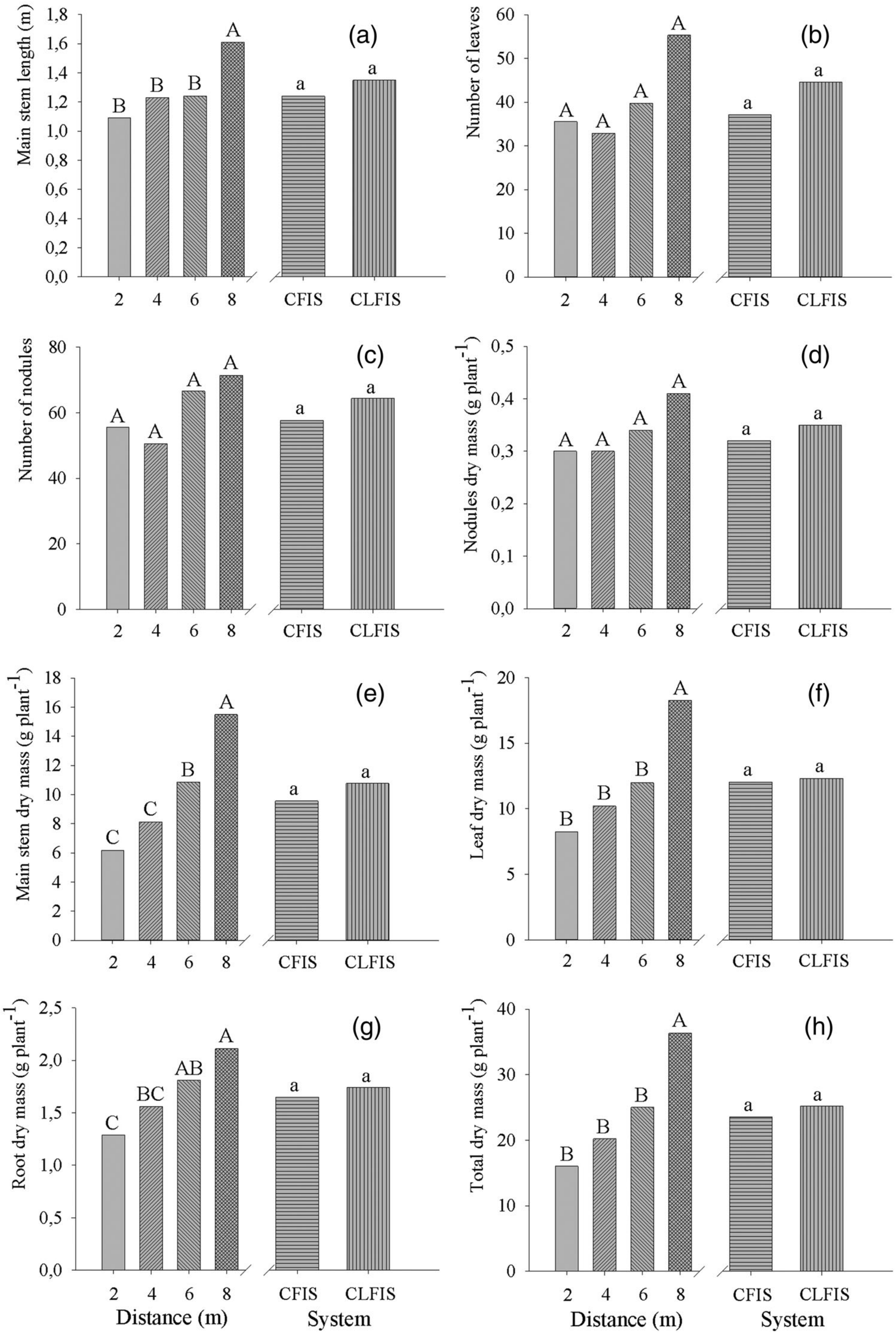

FIGURE 2 Main stem length (A), number of leaves (B) and nodules (C), nodules dry mass (D), main stem (E), leaf (F), root (G), and total dry mass (H) of cowpea in crop–forest systems (CFIS) and crop–livestock–forest systems (CLFIS) at different distances from the eucalyptus planting rows. CFIS: intercropping between eucalyptus and cowpea. CLFIS: intercropping among cowpea, buffel grass, and eucalyptus. Means followed by the same letter, upper case for distances and lower case for systems, do not present statistical difference between themselves by the Tukey test at a 5% probability level

TABLE 1 Summary of the analysis of variance for growth, production, nodulation, and physiological characteristics of cowpea in different systems with eucalyptus (*S*) and at different distances (*D*) from eucalyptus planting rows

	Variables																
SV	***msl***	***nl***	***nn***	***dmn***	***dms***	***dml***	***dmr***	***dmt***	***np***	***pw***	***ws***	***prod***	**Δ*C***	***Ci***	***E***	***Gs***	***A***
S	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	*	*	ns
D	*	ns	ns	ns	*	*	*	*	ns	ns	*	ns	*	*	*	*	*
S × *D*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	*	ns	ns
CV%	19.8	25.4	21.3	43.6	31.9	30.3	21.1	26.9	12.7	29.3	11.6	33.0	13.9	2.2	6.0	27.9	14.8

Note: SV: source of variation; *S*: system; *D*: distance; variables: main stem length (*msl*), number of leaves (*nl*) and nodules (*nn*), dry mass of nodules (*dmn*), main stem (*dms*), leaf (*dml*), root (*dmr*) and total dry mass (*dmt*), number of pods (*np*), pods weight (*pw*), weight of 100 seeds (*ws*), effective grain yield (*prod*), CO_2 consumed (Δ*C*), internal carbon (*Ci*), transpiration rate (*E*), stomatal conductance (*Gs*), and photosynthetic rate (*A*); ns: not significant.

*Significant at the level of 0.05 by the *F* test.

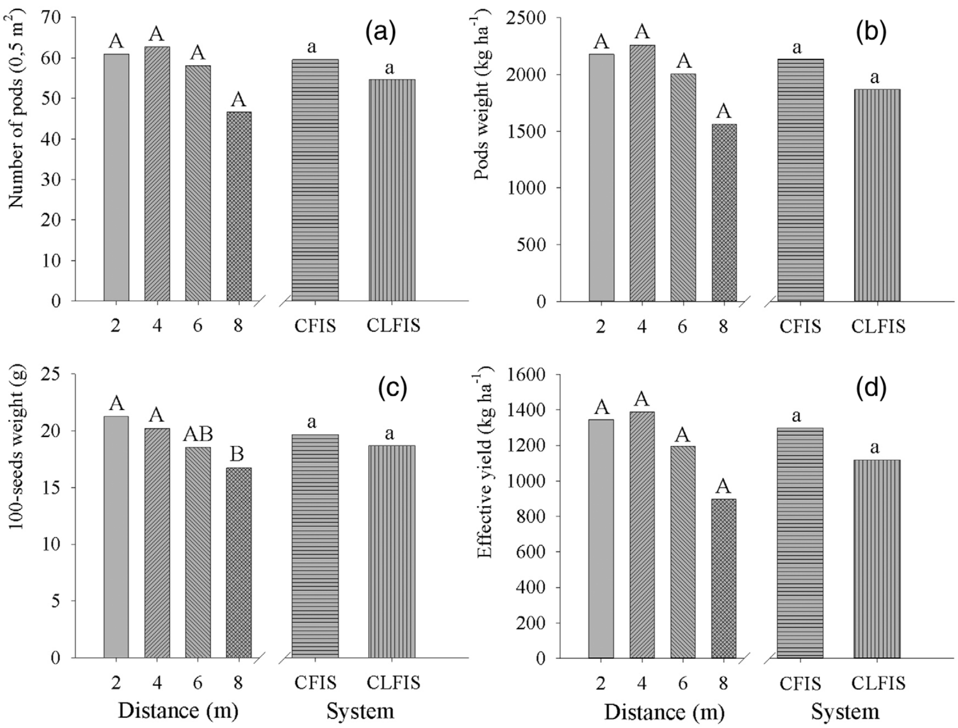

FIGURE 3 Number of pods (a), pods weight (b), 100-seeds weight (c), and effective grain yield (d) of cowpea in crop–forest systems (CFIS) and crop–livestock–forest systems (CLFIS) at different distances from eucalyptus planting rows. CFIS: intercropping between eucalyptus and cowpea. CLFIS: intercropping among cowpea, buffel grass, and eucalyptus. Means followed by the same letter, upper case for distances and lower case for systems, do not present statistical difference between themselves by the Tukey test at a 5% probability level

TABLE 2 Summary of the analysis of variance for growth, production, nodulation, and physiological characteristics of guandu bean in different systems with eucalyptus (*S*) and at different distances (*D*) from eucalyptus planting rows

	Variables															
SV	***msl***	***msd***	***sd***	***nh***	***nl***	***nn***	***dms***	***dml***	***dmr***	***dmn***	***prod***	**Δ*C***	***Ci***	***E***	***Gs***	***A***
S	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	*	ns	ns	*
D	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
S × *D*	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	*
CV%	3.8	4.5	9.0	4.2	17.5	18.6	30.8	30.1	26.9	45.2	35.1	22.5	3.5	5.2	32.7	12.6

Note: SV: source of variation; *S*: system; *D*: distance; variables: main stem length (*msl*), main stem (*msd*) and stems (*sd*) diameters, number of stems (*nh*), leaves (*nl*) and nodules (*nn*), dry mass of main stem (*dms*), leaf (*msl*), root (*msr*) and nodules (*msn*), effective dry mass yield (*prod*), CO_2 consumed (Δ*C*), internal carbon (*Gs*), static conductance (*Ci*), transpiration rate (*E*), and photosynthetic rate (*A*); ns: not significant.

*Significant at the level of 0.05 by the *F* test.

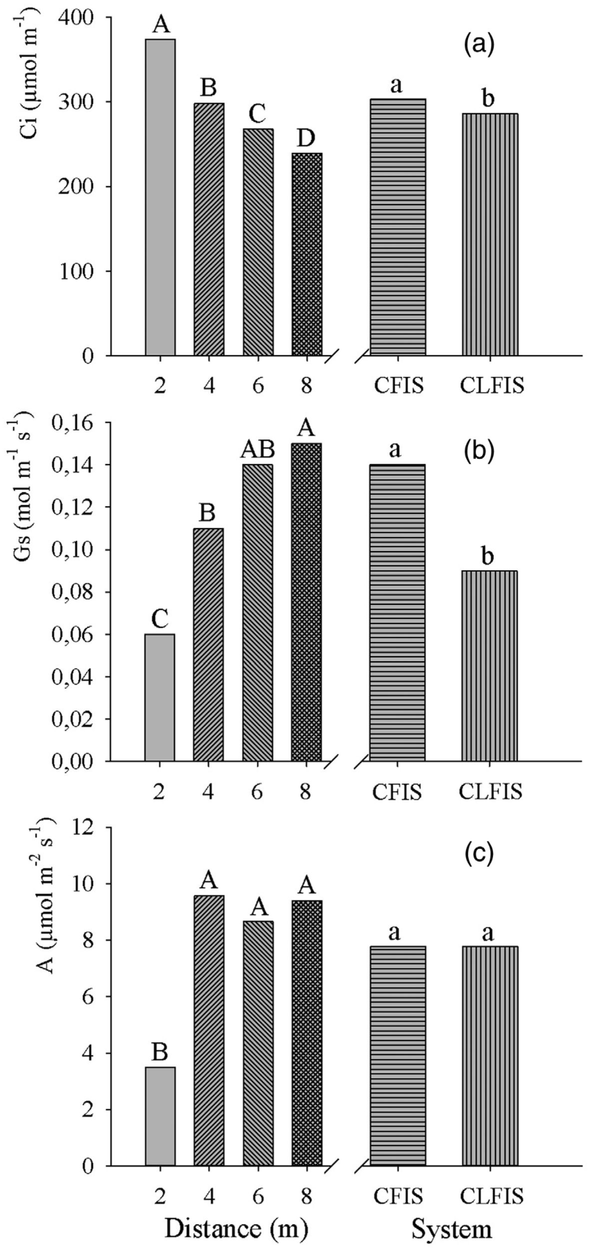

FIGURE 4 (a) Internal carbon (*Ci*), (b) stomatal conductance (*Gs*), and (c) photosynthetic rate (*A*) of cowpea in crop–forest systems (CFIS) and crop–livestock–forest systems (CLFIS) at different distances from eucalyptus planting rows. CFIS: intercropping between eucalyptus and cowpea. CLFIS: intercropping among cowpea, buffel grass, and eucalyptus. Means followed by the same letter, upper case for distances and lower case for systems, do not present statistical difference between themselves by the Tukey test at a 5% probability level

and in the arrangement of the integrated system to produce greater biomass. Similar results were also observed by Correia et al. (2021) when evaluating the growth of cowpea and by Jumrani and Bhatia (2020) in a soybean study under different light conditions.

Correia et al. (2021) determined that artificial shading with a shade screen did not influence the number of nodules and nodules dry mass variables in flowering cowpea compared to unshaded cultivation, corroborating the results of the present study as the distance from the eucalyptus planting rows did not change the nodulation variables. Oliveira et al. (2017) observed that cowpea in consortium with millet presented a nodule dry mass equal to the monoculture system, showing that the millet-generated shading did not affect nodulation, similar to the tree component and buffel grass in the present study. Similar results were also observed by Silva et al. (2016) in single cowpea cultivation and cowpea intercropped with *Brachiaria decumbens*.

In this respect, Namatsheve et al. (2020) did not observe any difference in nitrogen content from biological fixation between single and intercropped cowpea cultivation. In the present study, all treatments provided nodulation apparently enough to ensure an efficient biological nitrogen fixation for cowpea (Zilli et al., 2011), with a minimum of 44 nodules plant.$^{-1}$

The number of pods, pod weight, 100-seed weight, and effective grain yield variables were also similar between CFIS and CLFIS systems (Figure 3). A reduction in the weight of 100 seeds was observed with the distance from the eucalyptus plantation rows (Figure 3), whereas the number of pods, pods weight, and effective grain yield variables did not show a significant difference ($p \leq 0.05$) between the studied distances.

The higher weight of 100 grains ($p \leq 0.05$) at 2 and 4 m distances from the eucalyptus rows improved the grains' quality close to the trees. When evaluating the mass of 1000 grains, Silva et al. (2016) found 219.11 g in cowpea intercropped with *B. decumbens*, a mass higher than observed in the single cowpea cultivation (187.02 g). Silva et al. (2016) also reported similar grain yields between cowpea cultivation intercropped with *B. decumbens* and monocrop cowpea cultivation, with 845 and 782.25 kg ha^{-1}, respectively. It showed that the competition from intercropping under the conditions studied did not influence grain yield, similar to that observed in the present study.

Angadi et al. (2022) observed cowpea as one of the most tolerant species to luminosity reduction in a study that evaluated different leguminous species under shading (30%). Although the seed production generally presents a positive correlation with the accumulation of total dry mass, Jumrani and Bhatia (2020) and Khalid et al. (2019) identified that shading levels between 20% and 30% allow higher soybean seed yield under intercropping planting. Additionally, Wen et al. (2020) also reported that, although the soybean plants' dry mass reduced with shading, the weight of seeds did not show the same trend. In light shading, soybean plants use more photosynthetic compounds for seed development than for vegetative growth (Wen et al., 2020), which may explain the increase in the weight of cowpea seeds near the trees in the present study.

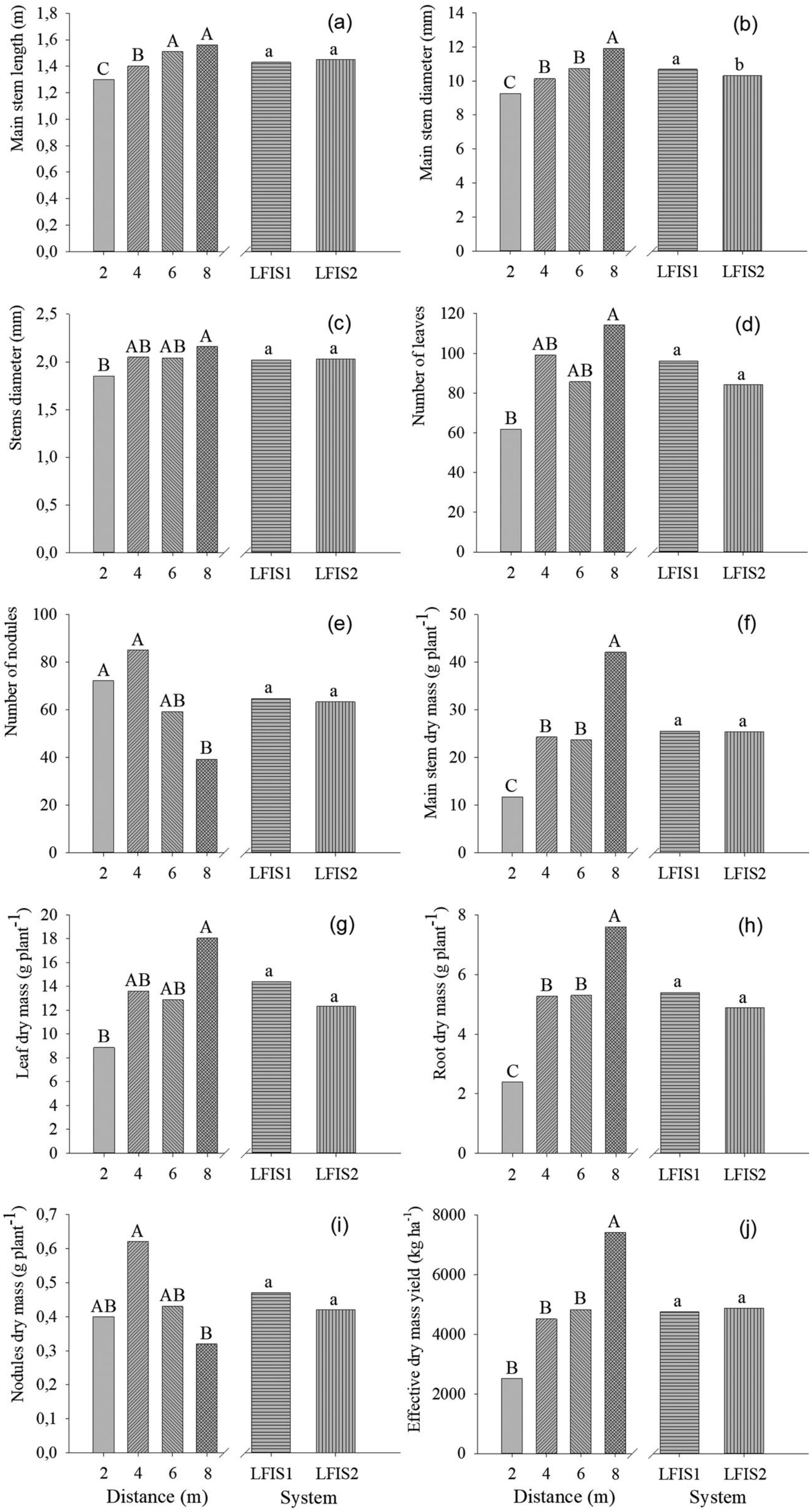

FIGURE 5 Main stem length (a), main stem diameter (b), stems diameter (c), number of leaves (d) and nodules (e), main stem (f), leaf (g), root (h), and nodules (i) dry mass, as well as effective dry mass yield (j) of guandu beans in LFIS1 and LFIS2 livestock–forest systems at different distances from eucalyptus planting rows. LFIS1: intercropping between guandu bean and eucalyptus. LFIS2: intercropping among cowpea, buffel grass, and eucalyptus. Means followed by the same letter, upper case for distances and lower case for systems, do not present statistical difference between themselves by the Tukey test at a 5% probability level

The CO_2 consumed showed a lower value at a distance of 2 m (higher proximity of trees) in both integrated systems (CFIS and CLFIS) (Table 3). A reduction in CO_2 consumed in the CLFIS system (4 m) was also observed compared to the CFIS system. The internal carbon and stomatal conductance variables were higher in the CFIS system (Figure 4). A reduction in internal carbon was observed with the distance from the eucalyptus plantation rows, whereas the stomatal conductance increased with the distancing.

The transpiration rate showed a lower value at a distance of 2 m (higher proximity of trees) in both integrated systems (CFIS and CLFIS) (Table 3). A reduction in transpiration rate

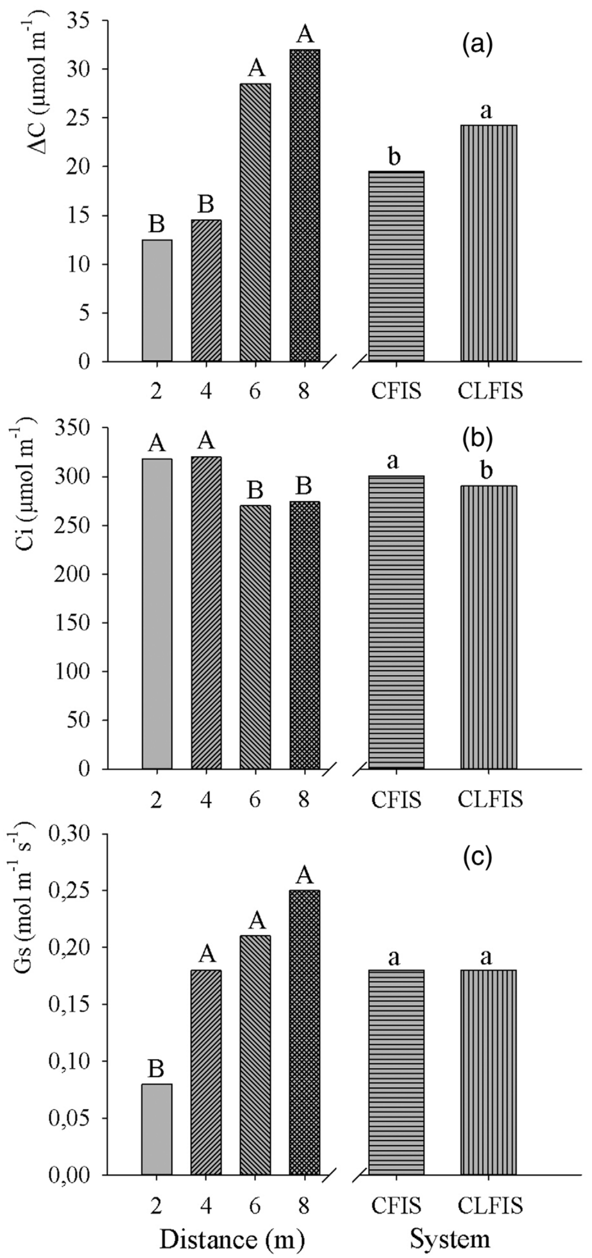

FIGURE 6 (a) CO_2 consumed (Δ*C*), (b) internal carbon (*Ci*), and (c) stomatal conductance (*Gs*) of guandu bean in LFIS1 and LFIS2 livestock–forest systems at different distances from eucalyptus planting rows. LFIS1: intercropping between guandu bean and eucalyptus. LFIS2: intercropping among cowpea, buffel grass, and eucalyptus. Means followed by the same letter, upper case for distances and lower case for systems, do not present statistical difference between themselves by the Tukey test at a 5% probability level. CFIS, crop–forest systems; CLFIS, crop–livestock–forest systems

was observed in the CLFIS system at all distances evaluated (2, 4, 6, and 8 m). As for the photosynthetic rate, similar values were observed between CFIS and CLFIS systems and lower values close to eucalyptus planting rows (2 m) (Figure 4).

The present study's findings agree with Alam et al. (2018), who observed a decrease in photosynthetic rate, stomatal conductance, and transpiration in cowpea due to a reduction

TABLE 3 CO_2 consumed (Δ*C*) and transpiration rate (*E*) of cowpea in crop–forest systems (CFIS) and crop–livestock–forest systems (CLFIS) at different distances from eucalyptus planting rows

	Distance			
System	**2 m**	**4 m**	**6 m**	**8 m**
	Δ*C*			
	μmol m^{-1}			
CFIS	8.00 aC	32.25 aA	25.00 aB	29.00 aAB
CLFIS	12.00 aB	27.00 bA	28.00 aA	25.00 aA
	E			
	mol H_2O m^2 s^{-1}			
CFIS	0.97 aD	1.42 aC	1.89 aB	2.04 aA
CLFIS	0.52 bB	1.03 bA	0.99 bA	1.13 bA

Note: CFIS: intercropping between eucalyptus and cowpea. CLFIS: intercropping among cowpea, buffel grass, and eucalyptus. Means followed by the same letter, upper case in line and lower case in a column, do not present statistical difference between themselves by the Tukey test at a 5% probability level.

in the light under the trees canopy in agroforestry systems with higher light restriction. According to the authors, microclimatic variables such as increased relative humidity and a reduced air and soil temperature between tree rows also contribute to the physiological changes observed in cowpea under understory cultivation and reduce the interception of photosynthetic photon flow by the shaded culture. Reduced temperature in less-luminosity places leads to decreased transpiration due to a lower need for leaf cooling.

In the present study, it was observed that the photosynthetic rate did not show variation from 4 m tree distance, indicating that only the reduction of solar radiation at a 2 m distance could change the photosynthetic efficiency. Reducing photosynthetically active radiation in agroforestry systems is the most critical limitation to crops in the understory, which can be managed by increasing the tree planting distance (Surki et al., 2020). In addition, competition for water and nutrients can also interfere with the development of intercropped species, which also requires planning for choosing the distance from which trees are grown (Razouk et al., 2016). There is higher shading and competition near the tree rows (Honnayya et al., 2020). However, the present study results showed that a reduced photosynthetic rate was insufficient to reduce grain yield, as discussed earlier. However, it may have contributed to decreased dry matter production.

Coelho et al. (2014) also observed that stomatal conductance and transpiration rate followed a trend similar to the photosynthetic rate in cowpea submitted to different shading levels, and this result was attributed to a high correlation between these variables. With the increase in transpiration rate, stomatal conductance, and photosynthetic rate, there was an increase in CO_2 consumed and a reduction of internal carbon, similar to that observed in the literature (Ayalew et al., 2022; Cotrim et al., 2021). Under ideal conditions, there is an increase in the photosynthetic rate and consequent increase

TABLE 4 Number of guandu bean stems in livestock–forest systems (LFIS1 and LFIS2) at different distances from eucalyptus planting rows

System	Distance 2 m	4 m	6 m	8 m
	Number of stems			
LFIS1	21.75 aB	23.15 aB	24.15 aAB	27.45 aA
LFIS2	20.75 aC	19.65 bC	24.75 aB	30.00 aA

Note: LFIS1: intercropping between guandu bean and eucalyptus. LFIS2: intercropping between cowpea, buffel grass, and eucalyptus. Means followed by the same letter, upper case in line and lower case in the column, do not present statistical difference between themselves by the Tukey test ($p \leq 0.05$).

in CO_2 processing, which results in internal carbon reduction (Cotrim et al., 2021). Coelho et al. (2014) also noticed that the photosynthetic efficiency under light restriction differs between cowpea varieties, and there may be greater efficiency in no shading (BRS Pujante variety) or shading under 30% (BRS Acauã) cultivation, which indicates the need to select varieties suitable for cultivation in integrated systems.

The evaluation of cowpea's growth, production, nodulation, and physiological variables along with the trees transect in 11-year old integrated systems allowed the identification of cowpea as a strategic species for crop–livestock–forest systems. Nodulation and grain yield were not reduced, despite changes in biomass production and physiological variables. In addition, improvement in grain quality (weight of 100 seeds) was observed close to the trees planting rows. New studies for identifying and validating cowpea varieties and different arrangements are necessary to optimize production and benefit from intercropping different production components.

3.2 | Growth, production, nodulation, and physiological characteristics of guandu bean

The main stem length, stems diameter, number of leaves, number of nodules, main stem dry mass, leaf dry mass, root dry mass, nodules dry mass, and effective dry mass yield variables were similar between LFIS1 and LFIS2 systems, whereas the main stem diameter was lower in the LFIS2 system (Figure 4). A reduction in the number of stems in the LFIS2 system (4 m) was observed compared to the LFIS1 system (Table 4).

Higher main stem length, main stem diameter, diameter, and the number of stems and higher main stem, leaf, and root biomass production and effective dry mass yield were generally obtained by distancing the trees planting rows. The number of nodules and nodules dry mass production decreased with distancing (Figure 5; Table 4).

Honnayya et al. (2020) observed higher growth and productivity of guandu bean by increasing the distance from trees (*Azadirachta indica*) in agroforestry systems. The authors considered the lower growth and yield of guandu beans close to the tree rows (2–7.4 m) possibly due to the higher shading and competition. From 12.8 m, Honnayya et al. (2020) did not observe interference of trees in the guandu bean development in the integrated system evaluated. In the present study, the dry mass yield of guandu bean showed variations of 195.08%, 64.26%, and 53.62% when comparing the 2, 4, and 6 m distances with the effective dry mass yield obtained in the distance of 8 m, respectively, considering the LFIS1 and LFIS2 systems averages. The higher productivity obtained at an 8 m distance from the eucalyptus planting rows occurred, possibly due to reducing tree shading and competition at this distance.

According to Khalid et al. (2019), adequate sunlight availability on the plant canopy increases the production of carbohydrates for use in biochemical and physiological processes, resulting in a higher biomass accumulation. Manoj et al. (2021) reported that the interception of photosynthetically active radiation by leaves is a critical process for biomass production and that the luminosity reduction led to lower biomass yield in guandu beans.

It was observed that the introduction of buffel grass into the system (LFIS2) influenced only the guandu bean main stem diameter and number of stems, not changing the growth and production variables. This result showed a low competition for moisture, light, space, and nutrients in the guandu bean and grass intercropping as there was no interference in the leguminous plant yield (Rajashree et al., 2022). Using guandu bean in integrated systems is a possible strategy for increasing total biomass production. Manoj et al. (2021) reported the guandu bean as a leguminous plant with potential for intercropped systems. In addition, Neres et al. (2012) suggested replacing the nitrogen fertilization with guandu bean intercropped in grass cultivation (Piatan and Tifton 85), because, despite not having a total dry mass production increase, the guandu bean helped to reduce costs with nitrogen fertilizers and to produce forage with higher nutritional value (higher crude protein content and lower neutral detergent fiber).

The guandu bean can establish a symbiotic association with rhizobia for biological nitrogen fixation, supplying its nitrogen demand for growth and production (Araújo et al., 2020). Rufini et al. (2016), Degefu et al. (2018), and Rufini et al. (2016) observed 29 and 38 nodules and 0.36 and 0.35 g of nodules dry mass in guandu bean inoculated with BR2003 and BR 2801 strains of *Bradyrhizobium* (reference strains approved as an inoculant for guandu beans), respectively, in vessels containing Oxisol. The number and dry mass of maximum nodules observed by Rufini et al. (2016) were 71 nodules (UFLA 04-212 strain inoculation) and 0.439 g of nodules dry mass (UFLA 03-320 strain inoculation). In a field experiment, the authors concluded that the strains tested, except BR 2003, effectively fixed N_2 and allowed the guandu bean growth.

TABLE 5 Transpiration rate (E) and photosynthetic rate (A) of guandu bean in livestock–forest systems (LFIS1 and LFIS2) at different distances from eucalyptus planting rows

System	Distance 2 m	4 m	6 m	8 m
	E			
	mol H_2O m^2 s^{-1}			
LFIS1	0.84 aB	1.97 aA	1.89 bA	1.95 bA
LFIS2	0.85 aC	1.21 aB	2.34 aA	2.21 aA
	A			
	μmol m^{-2} s^{-1}			
LFIS1	3.37 aC	7.84 aB	6.56 bB	11.23 aA
LFIS2	4.53 aB	5.01 bB	11.96 aA	10.81 aA

Note: LFIS1: Intercropping between guandu bean and eucalyptus. LFIS2: intercropping between cowpea, buffel grass, and eucalyptus. Means followed by the same letter, upper case in line and lower case in the column, do not present statistical difference between themselves by the Tukey test at a 5% probability level.

In the present study, 72.25, 85.13, 59.25, and 39.25 nodules plant^{-1} and 0.40, 0.62, 0.43, and 0.32 g plant^{-1} of nodules dry mass were obtained at distances of 2, 4, 6, and 8 m from the tree rows, respectively, indicating that native rhizobia may have promoted an efficient fixation in the different treatments evaluated as no inoculation was performed, In addition, the study area may contain rhizobia with potential for future inoculant development. Araujo et al. (2020) observed that the rhizobia native population had shown performance similar to that obtained with a 210 kg ha^{-1} fertilization. Degefu et al. (2018) reported phenotypically diverse and symbiotically effective guandu bean-nodulating rhizobia.

In general, a reduction was observed in nodulation variables at a distance of 8 m from the tree planting rows, which may be associated with less nitrogen competition due to less influence of the trees on this site. Jensen et al. (2020) reported that nitrogen competition in intercropping stimulates a higher biological nitrogen fixation in leguminous plants. Additionally, Morgado and Willey (2003) reported an increase in nodules with the increase in corn (*Zea mays*) and beans (*Phaseolus vulgaris*) intercropping plant population.

The carbon consumed variable was lower in the LFIS1 system, whereas the internal carbon was higher in this system. However, the stomatal conductance did not differ between the LFIS1 and LFIS2 systems (Figure 6). As for the distances evaluated, an increase in the carbon consumed was observed with tree distance (6 and 8 m), whereas the internal carbon reduced with distance. The stomatal conductance also increased in the most distant places from the trees (4, 6, and 8 m), showing a lower value at a 2 m distance from eucalyptus planting rows.

Reductions in transpiration rate (6 and 8 m) and photosynthetic rate (6 m) were observed in the LFIS1 system (Table 5) compared to the LFIS2 system. As for distances, the transpiration rate and photosynthetic rate were generally lower in the places closest to the trees, increasing in the most distant points from the planting rows.

Reduced transpiration rate (6 and 8 m) and photosynthetic rate (6 m) in the LFIS1 system (Table 5), when compared with the LFIS2 system, as well as the reduced carbon consumed and increased internal carbon in the LFIS1 system, occurred possibly due to the presence of weeds remaining after weeding, causing shading. The reduced photosynthetic rate in the 4 m distance was also observed in the LFIS2 system compared to the LFIS1 system, which may also have been caused by shading generated by the presence of remaining weeds, as buffel grass has a lower height than the guandu bean.

Physiological variables results evaluated in guandu bean in the different treatments studied agree with other leguminous plants studies found in the literature, which showed reduced internal carbon and increased CO_2 consumed, transpiration rate, stomatal conductance, and photosynthetic rate variables with tree spacing (lower shading). Fan et al. (2019) determined a reduction in the photosynthetic rate in shaded soybean plants (maize and soybean intercropping), whereas the internal carbon increased. Khalid et al. (2019) observed that the optimal light availability (no shading or shading up to 25%) provided a higher photosynthetic and transpiration rate in soybean. Mwamlima et al. (2020) reported a reduced photosynthetic rate, transpiration, and stomatal conductance in soybean intercropped with maize. According to Mwamlima et al. (2020), the reduction of luminosity over the canopy and the consequent reduction of the photosynthetically active radiation interception contributed to the reduced stomatal conductance, limiting the capture of carbon dioxide for photosynthesis.

In the present study, the higher light availability favored the guandu bean photosynthetic activity, contributing to the growth and production of leguminous plants, as the higher growth and productivity rates generally coincided with the photosynthetic activity improvement. Khalid et al. (2019) also confirmed that the higher light availability favored the photosynthetic rate and improved the soybean development and production. Reduction in photosynthetic rate due to shading decreases the production of photoassimilates necessary for plant growth and productivity (Mwamlima et al., 2020).

Buffel grass (LFIS2) did not change the physiological variables of the guandu bean as the grass has a lower height than the guandu bean. Physiological variables were influenced only by the tree component. In intercropping, shorter plants have their development impaired by competing for light with higher plants (Fan et al., 2019). Thus, the guandu bean and tree intercropping should be planned to reduce shading and competition as the results showed that the leguminous plant evaluated may have its growth, production, and physiology impaired when intercropped with eucalyptus. However, even though the guandu bean productivity is reduced in intercropping, the main crop and the other intercropped species' yield are generally significantly high and justify the production system (Rajashree et al., 2022). In addition, the east–west trees planting direction, the distance between trees and crops, trees pruning, and the proper time to plant between rows are essential factors for increasing luminosity and performance of guandu bean in the agroforestry system (Honnayya et al., 2020). Practices, such as tree pruning and east–west direction planting, were adopted in the present study, which may have contributed to the growth and development of crops in the understory.

Despite reducing the effective dry mass yield of guandu beans close to the eucalyptus planting rows, the average yield was 4756.63 and 4873.51 kg ha^{-1} in LFIS1 and LFIS2 systems, respectively. Calvo et al. (2010) obtained a 3278 kg ha^{-1} yield of guandu bean dry phytomass 90 DAS in monoculture in Oxisol in southeastern Brazil. Cavalcante et al. (2012) found a yield of 4000 kg ha^{-1} of single guandu bean dry matter at 92 days from planting in Oxisol, in the northeast region of Brazil. In the southern region of Brazil, Neres et al. (2012) reported 3412 kg ha^{-1} of guandu bean dry mass (cv. Super N) in Oxisol, also in monoculture. Thus, it was possible to infer that, even in an integrated system, high effective productivity of guandu bean dry matter was obtained in the present study, indicating the high viability of leguminous dry matter production in the different integrated systems evaluated.

4 | CONCLUSIONS

Nodulation in cowpea was not influenced by the systems and distances, whereas in guandu bean, there was a reduction in the nodules' number and dry mass with the distance from the trees.

Higher growth, biomass production, and photosynthetic activity of cowpea and guandu bean were observed with increasing distance from the eucalyptus trees. However, cowpea increased the 100-seed weight at 2 and 4 m distances from eucalyptus rows, improving the grains' quality close to the trees.

The simultaneous cultivation with buffel grass did not affect the leguminous plant yield, promoted greater diversification, and increased the total biomass production of the intercropping system.

ACKNOWLEDGMENTS

We thank the Hamilton de Abreu Navarro Experimental Farm (FEHAN) for logistical support provided during this study. We also thank the Pró-Reitoria de Pesquisa of Universidade Federal de Minas Gerais (UFMG) for providing funding assistance to publication. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; Finance Code 001), the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG; Grant number: PPM-00617-18), and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Grant number: 316873/2021-7) and Bayer S.A.

AUTHOR CONTRIBUTIONS

Igor C. de Freitas: Data curation; investigation; methodology; writing - original draft; writing - review and editing. **Evander A. Ferreira**: Data curation; methodology; writing - review and editing. **Matheus A. Alves**: Methodology; writing - review and editing. **Jaqueline C. de Oliveira**: Methodology; writing - review and editing. **Leidivan A. Frazão**: Data curation; methodology; supervision; writing - review and editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data presented in this study are available upon request from the corresponding author.

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How to cite this article: de Freitas, I. C., Ferreira, E. A., Alves, M. A., de Oliveira, J. C., & Frazão, L. A. (2023). Growth, nodulation, production, and physiology of leguminous plants in integrated production systems. *Agrosystems, Geosciences & Environment*, *6*, 1–14. https://doi.org/10.1002/agg2.20343