

ISSN: 1809-4430 (on-line)

www.engenhariaagricola.org.br



Doi: http://dx.doi.org/10.1590/1809-4430-Eng.Agric.v40n4p420-432/2020

## CHICKEN MANURE AND LUMINOUS AVAILABILITY INFLUENCE GAS EXCHANGE AND PHOTOCHEMICAL PROCESSES IN *Alibertia edulis* (Rich.) A. Rich SEEDLINGS

# Cleberton C. Santos<sup>1\*</sup>, Rodrigo da S. Bernardes<sup>1</sup>, Ademir Goelzer<sup>2</sup>, Silvana de P. Q. Scalon<sup>1</sup>, Maria do C. Vieira<sup>1</sup>

<sup>1\*</sup>Corresponding author. Universidade Federal da Grande Dourados/ Dourados - MS, Brasil. E-mail: cleber frs@yahoo.com.br | ORCID ID: https://orcid.org/0000-0001-6741-2622

## **KEYWORDS**

## ABSTRACT

marmelo-do-cerrado, chlorophyll-*a* fluorescence, physiological adjustments, organic residue, shaded. Information on the physiological responses of native plants from the Brazilian Cerrado regarding the use of chicken manure and luminosities are important for their silvicultural management. Thus, aimed to evaluate the effect of chicken manure and luminous availability on the photosynthetic metabolism of *Alibertia edulis* (Rich) A. Rich. The experiment was carried out in pots, testing five doses of chicken manure – CM (0.00, 2.08, 4.16, 6.24 and 8.32 g kg<sup>-1</sup>) incorporated at Dystroferric Red Latosol, and two luminous environmental (full sun and 50% shaded). The higher CO<sub>2</sub> assimilation rate, stomatal conductance and carboxylation efficiency of Rubisco occurred in seedlings grown in full sun. The seedlings showed higher photochemical indicators in photosystem II in the shaded and with addition of 8.32 g kg<sup>-1</sup> of CM. The intercellular CO<sub>2</sub> concentration in leaves was lower with addition of 5.55 g kg<sup>-1</sup> of CM. The leaves presented larger stomatal dimensions under shaded. The cultivation of *A. edulis* in full sun and the addition of about 4.16 g kg<sup>-1</sup> of CM favored greater stomatal regulation and CO<sub>2</sub> assimilation.

### **INTRODUCTION**

Among the native species of the Cerrado, *Alibertia* edulis (Rich) A. Rich. (marmelo-do-cerrado, Rubiaceae), is a fruiting dioecious semideciduous plant, subarboreal in size, with large leaves, white flowers, globose-like fruits, and a large number of seeds (Paiva et al., 2017). The pulp is brown, fleshy, and sweetish, and may be consumed *in* natura or processed in the form of liqueurs, jellies, and sweets (Lorenzi et al., 2006). The aqueous extract of the leaves of the species has antidiuretic and antihypertensive activities (Aquino et al., 2017).

*A. edulis* continues to be collected for food and medicinal purposes in native areas. There is no record of its cultivation *ex situ*, which means that there is little information about the species' physiological responses to abiotic factors as well as to luminosity and the use of organic residues. Therefore, studies are necessary to establish silvicultural management practices.

The luminosity in the environment where a species is introduced, planted, and cultivated is a determining factor in the success of silvicultural practices and in the recovery

Area Editor: Edilson Costa Received in: 2-24-2020 Accepted in: 6-2-2020 of degraded areas as this depends on the resilience capacity of the species to ensure its survival and stability of growth. High or low light availability can trigger physiological changes such as the photosynthetic metabolism of plants through leaf changes (Bunce, 2016; Souza et al., 2017), involving differences in the magnitude of electron transport and the proton gradient that potentiates phosphorylation (Jim et al., 2016).

Therefore, knowledge regarding the acclimatization strategies of plants through physiological adjustments to contrasting conditions of irradiance are necessary (Gonçalves et al., 2010) because it assists in the selection of the area that will be used for seedling transplantation. It also allows us to understand if the species can be planted in areas that are under full sun and highly anthropized aiming at the restoration of the ecosystem, the enrichment of native forests, or enhancement of the composition of biodiverse agroforestry systems, with a lower incidence of luminous radiation.

In addition, the use of organic residues is an established agronomic practice for the production of tree species seedlings as it contributes to the maintenance of

<sup>&</sup>lt;sup>1</sup> Universidade Federal da Grande Dourados/ Dourados - MS, Brasil. <sup>2</sup> Universidade Federal de Lavras/ Lavras - MG, Brasil.

humidity and temperature, and releases nutrients gradually, contributing to plant nutrition and metabolism. A vast majority plants native to the Cerrado, show slow growth, and the use of organic residues is a sustainable alternative that favors the establishment of plants in the introduced areas because of the higher quality of seedlings.

Chicken manure (CM) is among the solid organic residues with potential for addition in the formulation of substrates, which in turn, is easily available in broiler chicken producing areas. The addition of chicken manure to the soil improves physical attributes, and during the decomposition/mineralization stage, it contributes to the chemical attributes by contributing to the composition of nutrient content, mainly in terms of nitrogen, phosphorus, and magnesium (Gonzaga et al., 2016; Soremi et al., 2017). The use of organic residue also increases the microbiological activity of the soil (Devi et al., 2012; Malik et al., 2013), which accelerates biogeochemical cycling, favoring the availability of nutrients associated with photosynthetic metabolism and plant biomass production.

However, there are few studies associating ecophysiology and the adequate amount of CM to the photochemical and biochemical reactions within the photosynthetic process of the species. Considering the initial secondary classification of *A. edulis* (Leles et al., 2011), i.e., occurring in locations with moderate shading (Gandolfi et al., 1995), in this study, we hypothesized that its seedlings would reduce photosynthetic metabolism if grown in full sun; however, a greater availability of organic matter would favor metabolism and responses that adjust to contrasting conditions of light. The aim of this study was to evaluate the effect of CM and luminous availability on gas exchange and chlorophyll-*a* fluorescence in *Alibertia edulis* seedlings.

#### MATERIAL AND METHODS

The fruits of *A. edulis* were collected (Access Registration No. A9CDAAE – CGEN-MMA, of 15/10/2018) randomly from natural populations in the Cerrado area ( $18^{\circ}07'03''$  S and  $54^{\circ}25'07''$  W, 452 m), Dourados – State Mato Grosso do Sul, Brazil. The species was identified and an exsiccate was deposited at the Herbarium DDMS, of the *Universidade Federal da Grande Dourados*, under No.4649. To obtain the seedlings, mature fruits were processed, and the seeds were immersed in 2% sodium hypochlorite for 5 min. After this, sowing was performed in 128-cell expanded polystyrene trays, filled with Bioplant<sup>®</sup> substrate (pine bark, peat, expanded

vermiculite, potassium nitrate, and simple superphosphate) and maintained under nursery conditions with 50% Sombrite<sup>®</sup> and daily irrigation.

The factors under study comprised five doses of CM with a rice husk base (0.00, 2.08, 4.16, 6.24, and 8.32 g kg<sup>-1</sup> soil) incorporated into the substrate formulation, and two levels of luminous availability (full sun and 50% shaded) (22°11'43.7"S and 54°56'08.5"W, 452 m). The treatments were arranged in a  $5 \times 2$  factorial scheme, in a randomized block design with four replications. The experimental unit comprised four plastic pots with a capacity of 4.2 dm<sup>3</sup>, with one plant each. The doses of CM were established based on the study by Santos et al. (2020) wherein, among the organic residues tested, the CM with rice husk base in a single dose of 4.16 g kg<sup>-1</sup>, contributed to the production of seedlings of *A. edulis*.

The shaded ambience was achieved by means of a simulation using a black screen with 50% luminosity retention (Sombrite<sup>®</sup>). The ambience under full sun and shading presented the following conditions on average at the end of the cultivation cycle: temperature, 31.62 and 28.42 °C; relative humidity, 65% and 78%; water vapor pressure deficit (Sadler & Evans, 1989), 4.36 and 2.33 KPa; and photosynthetically active radiation (PAR) 1245.25 and 560.12 µmol m<sup>-2</sup> s<sup>-1</sup>; respectively.

The substrate used to fill the pots was Dystroferric Red Latosol (Santos et al., 2018), with clay texture, which had the following chemical properties determined according to the methodology described by Silva (2009): pH in CaCl<sub>2</sub> = 6.2; P = 1.7 mg dm<sup>-3</sup>; Ca = 3.0 mmol<sub>c</sub> dm<sup>-3</sup>, K = 0.30 mmol<sub>c</sub> dm<sup>-3</sup>; Mg = 1.4 mmol<sub>c</sub> dm<sup>-3</sup>; Al = 0.12 mmol<sub>c</sub> dm<sup>-3</sup>; H+Al = 29.9 mmol<sub>c</sub> dm<sup>-3</sup>; Sum of bases (SB) = 4.9 mmol<sub>c</sub> dm<sup>-3</sup>; Cation exchange capacity (CEC) = 42.4 mmol<sub>c</sub> dm<sup>-3</sup> and Bases per saturation (V%)= 60.5. The semi-composted CM came from an aviary after the production of five batches of broiler chicken from Dourados, having the following chemical attributes: pH =7.50; N, P, K, Ca, Mg, S, C, and organic matter (g kg<sup>-1</sup>) = 23.90, 15.36, 20.00, 19.15, 6.95, 18.65, 260, and 447.00, respectively; C/N ratio = 10.87; and humidity of 11%.

When the seedlings in the expanded polystyrene trays had a mean height of 7.0 cm, which occurred at 60 days after sowing, transplanting to the pots (Days after transplanting – DAT) was performed. The cultural treatments included daily irrigation with the aim of maintaining 70% of the water retention capacity in the substrate according to method described by Souza et al. (2000). The chemical compositions of the substrates in the different treatments are shown in Table 1.

TABLE 1. Chemical composition of substrates for cultivation of A. edulis grown with chicken manure, in full sun or shaded, at
210 DAT.

Chicken manure –	pН	Р	Κ	Ca	Mg	Al	H+A1	SB	CEC	OM	V
Chicken manure –	H <sub>2</sub> O	mg dm <sup>-3</sup>				mmol <sub>c</sub> d	lm <sup>-3</sup>				%
(g kg <sup>-1</sup> )					F	ull sun					
0.00	6.24	1.82	0.32	3.89	1.46	0.00	2.30	5.74	8.04	0.82	70.13
2.08	6.75	2.58	0.58	4.81	2.10	0.00	2.20	7.45	9.69	2.41	77.35
4.16	6.66	4.62	0.71	3.84	1.83	0.00	1.98	6.38	8.36	1.25	76.31
6.24	6.66	5.63	0.59	4.67	1.82	0.00	2.35	6.81	9.44	0.60	75.10
8.32	6.54	5.12	0.66	4.37	2.06	0.00	2.55	6.74	9.64	0.57	73.38
					Sh	aded					
0.00	6.28	4.11	0.27	4.57	2.06	0.08	2.44	7.01	9.45	0.74	74.23
2.08	6.78	3.85	0.55	4.92	2.08	0.00	2.10	7.55	9.65	2.19	78.13
4.16	6.81	5.63	0.55	4.02	2.04	0.00	2.05	6.61	8.66	0.74	76.36
6.24	6.82	4.62	0.54	4.02	1.86	0.00	1.97	6.43	8.39	1.11	75.96
8.32	6.42	5.38	0.67	4.44	1.83	0.00	2.34	6.82	9.29	1.34	74.20

SB: sum of bases; CEC: cationic exchange capacity; OM: organic matter; V (%): bases per saturation.

At 210 DAT, when the seedlings were ready for transplanting in the field and presented an average height of 40 cm, gas exchange was quantified in the morning (8:00 to 11:00 am) in fully expanded leaves located in the middle third of the branch. CO<sub>2</sub> assimilation rate – photosynthesis (*A*), intercellular CO<sub>2</sub> concentration (*C*<sub>i</sub>), stomatal conductance ( $g_s$ ), transpiration (*E*) and leaf temperature (LT) were recorded using a portable photosynthesis meter (LCIPro- SD ADC BioScientific Ltd.; IRGA – *Infra Red Gas Analyzer*). From the obtained data, the water-use efficiency (*WUE* = *A*/*E*), intrinsic water-use efficiency (*iWUE* = *A*/*g*<sub>s</sub>), carboxylation of Rubisco (*A*/*C*<sub>i</sub>), and *Ci*/*C*<sub>a</sub> ratio (atmospheric CO<sub>2</sub> concentration) were calculated.

The chlorophyll index was determined with a portable SPAD chlorophyll meter (Soil Plant Analyzer Development; Konica Minolta SPAD 502), on the same leaves as those on which gas exchange was quantified. Subsequently, the leaves were adapted to the dark condition for 30 min, using leaf clips, and soon after, the initial (F<sub>0</sub>), variable (F<sub>v</sub>), and maximum (F<sub>m</sub>) fluorescence emission of chlorophyll-a was measured under a 1500 µmol m<sup>-2</sup> s<sup>-1</sup> flash using a portable fluorometer OS30p (Opti-Sciences Chlorophyll Fluorometer, Hudson, USA). The photochemical efficiency of photosystem II (PS II)  $[F_v/F_m =$  $(F_m - F_0/F_m)$ ], absorbed energy conversion efficiency  $(F_v/F_0)$ , maximum non-photochemical performance  $(F_0/F_v)$ , and the electron transport rate (ETR) were calculated (Baker, 2008).

To determine the morpho-stomatic characteristics, paradermic sections were made on the leaves in the morning (8:00 to 11:00 am), using the "Super Bonder<sup>®</sup>" printing technique. Samples from the medial limbus region of the adaxial and abaxial surfaces of fully expanded leaves in the middle third of the branch were used. The material was photographed with a Moticam 2000 digital camera attached to an optical microscope. Subsequently, the polar (PD) and equatorial (ED) diameter, and the ostiolar opening (OO) were measured using the Motic Image 2000 program, and the stomata functionality was calculated using the DP/DE ratio.

Data were subjected to analysis of variance and when significant according to the F test (p < 0.05), the means were compared using the Student's *t*-tests for luminous availability, and regression for CM doses ( $p \le$ 0.05), using SISVAR 5.6. Multivariate principal component analysis (PCA) was also performed using variance and covariance matrices, as a complementary analysis, with PAST 3.21.

## **RESULTS AND DISCUSSION**

The gas exchange characteristics of *A. edulis* seedlings during the initial growth phase were influenced by the factors under study. The *A*,  $g_s$ ,  $A/C_i$ , and leaf temperature were influenced by the luminous ambience, with higher values in seedlings grown in full sun (Figure 1). *iWUE* was not influenced by the factors under study (p > 0.05), with average of 106.75 µmol CO<sub>2</sub>/mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>.

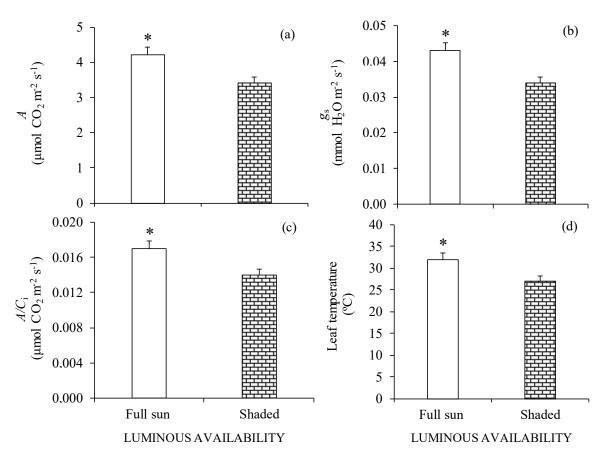


FIGURE 1. CO<sub>2</sub> assimilation rate -A (a), stomatal conductance  $-g_s$  (b), intrinsic efficiency carboxylation of Rubisco  $-A/C_i$  (c) and leaf temperature (d) in A. edulis leaves grown in full sun or shaded. \*Student's t-test (p < 0.05).

The higher  $g_s$  in full sun contributed to the greater entry of atmospheric CO<sub>2</sub>, and  $A/C_i$  being more efficient under these conditions, as evidenced by the greater A of A. *edulis* seedlings. Similarly, young plants of *Bertholletia excelsa* Bonpl. (Souza et al., 2017), Anadenanthera falcata (Benth.) Speg., and *Stryphnodendron adstringens* (Mart.) (Ronquim et al., 2018) showed greater photosynthetic activity under high irradiance, i.e., in full sun.

We observed that *E* and *WUE* were influenced by the interaction between the factors under study; however, the data did not adjust to the mathematical models tested. The highest values of *E* and *WUE* were observed in full sun and shading, with values of 1.43 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> and 5.34 µmol CO<sub>2</sub>/mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>, respectively. With the lowest amount of gas in the shaded leaves, seedlings tend to lose less water through transpiration, resulting in greater *WUE* 

and maintenance of metabolic processes. These changes demonstrate the physiological plasticity of seedlings through adjustments when exposed to limiting conditions (Rosa et al., 2017), as has been reported in the literature for other species in order to optimize environmental resources.

The  $C_i$  and  $C_i/C_a$  ratio were influenced only by the doses of CM, with both characteristics presenting a minimum of 240.09 µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> and 0.51, respectively, with the addition of 5.55 g kg<sup>-1</sup> of CM (Figures 2a and 2b). The reduction in  $C_i$  indicates that with the addition of CM, *A. edulis* seedlings were more efficient in CO<sub>2</sub> carboxylation and assimilation, potentiating the photosynthetic rate, possibly owing to the maintenance of moisture and availability of nutrients in the solution and physical quality of the substrate, ensuring physiological processes.

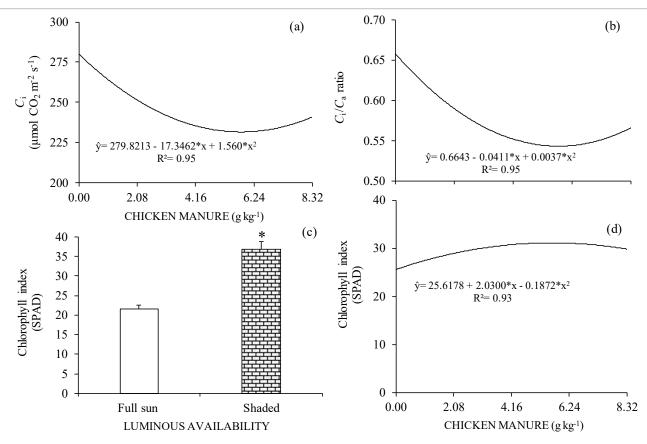


FIGURE 2. Intercellular CO<sub>2</sub> concentration  $-C_i$  (a),  $C_i/C_a$  ratio - atmospheric CO<sub>2</sub> concentration (b) and chlorophyll index (c-d) in *A. edulis* leaves grown with chicken manure (a–b–d) or in full sun or shaded (c). \*Student's *t*-test for luminous availability, and regression for CM doses (p < 0.05).

The chlorophyll index was influenced by the isolated factors, showing that the highest index (37 SPAD) occurred in the leaves of shaded seedlings (Figure 2c). This increase in leaves under shade condition has been considered a strategy to compensate the species for the lowest amount of luminous radiation available (Lima et al., 2010), resulting in the maximization of photosynthesis processes. Other native species have shown the same mechanism, as in *Bertholletia excelsa* Bonpl. in a shaded environment (Albuquerque et al., 2015).

As for organic residue, the maximum SPAD index (31) was obtained with the addition of 5.42 g kg<sup>-1</sup> of CM (Figure 2d). The addition of CM to the soil may have favored the availability/solubilization of nutrients as well as that of N and Mg, which favors an increase in the synthesis of chlorophylls, as these nutrients participate in the structuring of the molecule of this pigment (Guo et al., 2016; Taiz et al., 2017).

The  $F_0$  of chlorophyll-*a*, photochemical efficiency of PS II ( $F_v/F_m$ ), and electron transport were influenced by the luminous availability. The highest  $F_0$  (0.345 electrons

quantum<sup>-1</sup>) and ETR (253.0 µmol m<sup>-2</sup> s<sup>-1</sup> electrons) occurred in the leaves of seedlings in full sun (Figure 3a and 3c, respectively,), and the higher  $F_v/F_m$  ratio (0.711 electrons quantum<sup>-1</sup>) in seedlings under shade (Figure 3b). With the lowest  $F_v/F_m$  in full sun, it can be inferred that this growing ambience is stressful for this species and that the highest ETR is associated with the highest PAR in this environment, which favored the greatest levels of gas exchange (*A*, g<sub>s</sub>, and *A*/*C*<sub>i</sub>) (Figure 1).

In contrast, the increase in  $F_0$  and decrease in  $F_m$  cause damage to the reaction center  $P_{680}$  or a reduction in the capacity to transfer the excitation energy from the antenna to the reaction center (Baker, 2008), owing to the blocking of electron transfer from plastoquinone A to B, as a result of partial inactivation of PS II (Gilmore et al., 1996; Biswal et al., 2011). This is because when plants are exposed to high irradiance, they can generate the degradation of protein  $D_1$ , and consequently photoinhibition (Telfer, 2014) if the energy dissipation through the chlorophyll-*a* fluorescence does not occur synergistically.

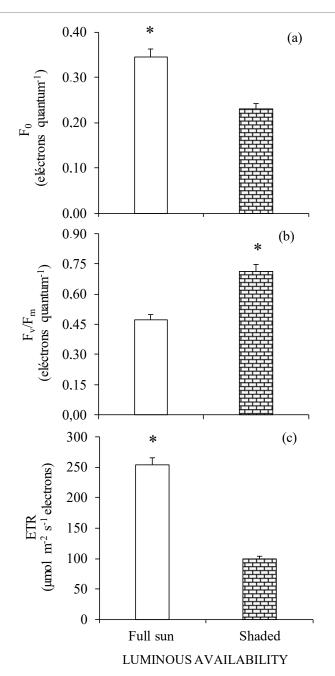


FIGURE 3. Initial fluorescence –  $F_0$  (a), photochemical efficiency of photosystem II –  $F_v/F_m$  (b), and electron transfer rate – ETR (c) in *A. edulis* leaves grown in full sun or shaded. \*Student's *t*-test (p < 0.05).

The maximum  $(F_m)$  and variable  $(F_v)$  chlorophyll-*a* fluorescence were influenced by the isolated factors, with lower values in the leaves in full sun (Figure 4a and 4b). As for the CM, for  $F_m$ , the data did not adjust to the tested

mathematical models, with an average of 0.646 electrons quantum<sup>-1</sup>. For  $F_v$ , there was linear growth with a higher value (0.560 electrons quantum<sup>-1</sup>) with the addition of 8.32 g kg<sup>-1</sup> of CM (Figure 4c).

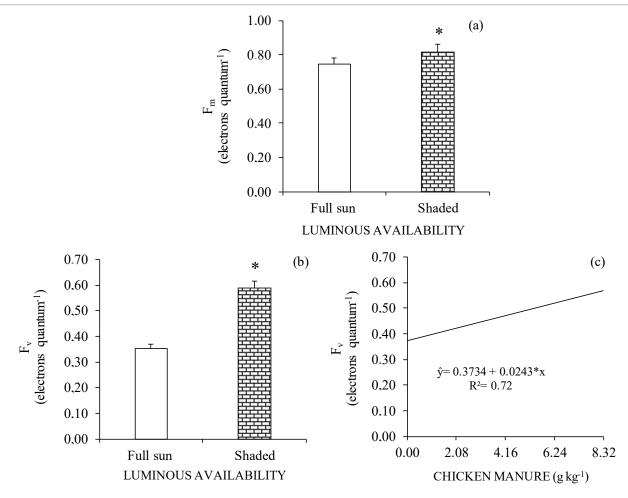


FIGURE 4. Maximum fluorescence ( $F_m$ ) (a) and variable ( $F_v$ ) (b–c) in *A. edulis* leaves grown in full sun or shaded (a–b) or with chicken manure (c). \*Student's *t*-test for luminous availability, and regression for CM doses (p < 0.05).

The absorbed energy conversion efficiency  $(F_v/F_0)$ and the maximum non-photochemical performance  $(F_0/F_v)$ were influenced by the factors under study (Figure 5). There was an increase in  $F_v/F_0$  and a reduction in  $F_0/F_v$ , with increasing doses of CM; the highest (2.18 electrons quantum<sup>-1</sup>) and the lowest (0.582 electrons quantum<sup>-1</sup>) ratio were observed with the addition of 8.32 g kg<sup>-1</sup> of CM, respectively (Figure 5b and 5d). As for the ambience, the highest  $F_v/F_0$  was 2.58 electrons quantum<sup>-1</sup> in the leaves of the shaded seedlings (Figure 5a), and the  $F_0/F_v$  was 1.16 electrons quantum<sup>-1</sup> at full sun (Figure 5c), demonstrating the stability of electron transfer in the reaction center because the  $F_v/F_0$  ratio is considered as an indicator of the maximum efficiency in the photochemical process in PS II and/or of the potential photosynthetic activity (maximum quantum production reason of photochemical competing processes in PS II) (Silva et al., 2015), demonstrating the beneficial effect of CM on the photochemical reactions of photosynthesis.

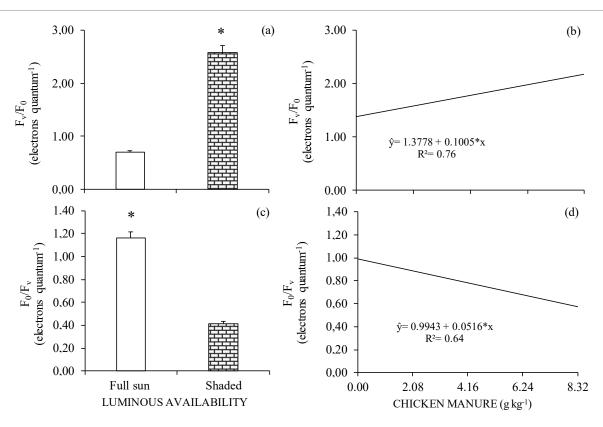


FIGURE 5. Absorbed energy conversion efficiency  $(F_v/F_0)$  (a–b) and maximum non-photochemical performance  $(F_0/F_v)$  (c–d) in *A. edulis* leaves grown in full sun or shaded (a–b) or with chicken manure (c–d). \*Student's *t*-test for luminous availability, and regression for CM doses (p < 0.05).

The results of the chlorophyll-*a* fluorescence parameters indicate that *A. edulis* seedlings presented adjustment mechanisms under adverse conditions. They are more efficient in harnessing luminosity because they present a greater photochemical efficiency of photosystem II  $F_v/F_m$  in the shaded environment, and a greater dissipation when subjected to full sun exposure. Similarly, young plants of *Dipteryx odorata* showed accentuated changes in the values of  $F_0$  and  $F_m$  in leaves exposed to high irradiance (Gonçalves et al., 2010).

Thus, plants under high irradiance tend to have less demand for energy from photosynthetic metabolism, i.e., excess light becomes a saturating factor, requiring plants to be able to dissipate excess energy (Gu et al., 2017), making it possible to maintain PS II integrity by mitigating damage to the photosynthetic apparatus and biochemical reactions later. The ability to use and dissipate excess energy efficiently is crucial for the net assimilation of  $CO_2$  and the establishment of tree species in contrasting conditions of light (Souza et al., 2017), which is important for silvicultural activities.

Physiological adjustments are desirable characteristics, as they contribute substantially so that plants can perform their metabolic processes and ensure stability of growth even under unfavorable conditions, mainly the abiotic factors that are directly related to the habitat and ecological system, allowing them to adapt to different luminous environments. The best photochemical responses of seedlings under higher doses of CM are owing to the fact that their addition to the soil favored an increase in organic matter; consequently, a greater availability of nutrients (Soremi et al., 2017), such as N and P for seedlings. These nutrients participate effectively in the formation of adenosine triphosphate and nicotinamide adenine dinucleotide phosphate (Carstensen et al., 2018) and the chloroplastid content (Agbor et al., 2018), favoring the stability of the electron transfer process in PS II.

Regarding the morpho-stomatal aspects, it was found that the leaves of A. edulis seedlings are hypostomatic, with the presence of trichomes tectors on both epidermal sides, under both luminous availability conditions. The ostiolar opening (OO) and polar diameter (PD) were influenced by the factors under study in isolation, in which the largest OO and PD of stomata occurred in the leaves of the shaded seedlings (3.55 and 11.50 µm, respectively) (Figure 6). Under certain ambient conditions, plants alter their leaf structures as adaptive skills in mitigating water loss through transpiration and stomatal regulation (Taiz et al., 2017), such as having smaller dimensions in full sun. As for organic residue, the OO data did not adjust to the tested models, with an average of 2.4  $\mu$ m. The maximum PD (11.92  $\mu$ m) was obtained with 3.06 g of CM per kg of soil (Figure 6c).

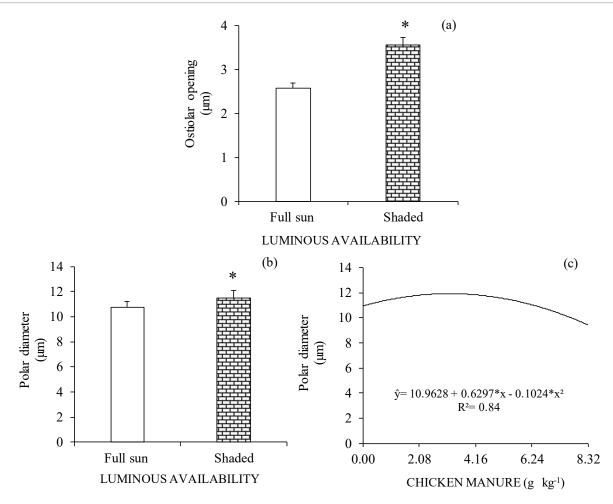


FIGURE 6. Ostiolar opening (a) and polar diameter (b–c) of stomata in *A. edulis* leaves grown in full sun or shaded (a – b) or with chicken manure (c). \*Student's *t*-test for luminous availability, and regression for CM doses (p < 0.05).

The equatorial diameter (ED) and functionality of the stomata were influenced by the interaction between CM and luminosity. The data for both characteristics in full sun did not adjust to the mathematical models tested, presenting an average of 7.82 and 0.71  $\mu$ m, respectively (Figure 7). It was observed that the maximum ED and minimum functionality were 14.32 and 0.78  $\mu$ m, respectively, in seedlings grown with the addition of 3.91 and 4.33 g of CM per kg soil, respectively, when subjected to shading (Figure 5). Similar results regarding light were described by Aragão et al. (2014), wherein young plants of *Carapa guianensis* Aubl. presented larger stomatal dimensions under shaded conditions.

Considering that *A. edulis* is an initial secondary species, its stomata would have a larger size and functionality under shade; thus, the data from the present study are consistent with the information in the literature, according to which reductions in OO, and PD and ED, under stress, favored greater *WUE* and lower *E*. However, our data are contrary to this information because under conditions of full sun, the seedlings presented higher *A*,  $g_s$ , and  $A/C_i$  (Figure 7), suggesting therefore, its plasticity even with high *E* being able to produce a high rate of photoassimilates.

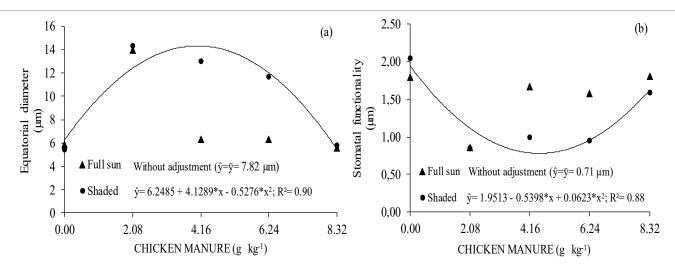


FIGURE 7. Equatorial diameter (a) and stomatal functionality (b) in *A. edulis* leaves grown with chicken manure under full sun and shaded. \*Student's t test for luminous availability, and regression for CM doses (p < 0.05).

It was found that 78.71% of the remaining variability of the data was explained through the PCA, with 59.42% corresponding to principal component – PC 1 and 19.29% corresponding to PC 2 (Figure 8). The characteristics of  $F_m$ ,  $A/C_i$ , and ostiolar opening were removed from the PCA, considering factor scores < 0.20, in both axes, indicating low representativeness. The use of PCA allows observation of qualitative standards without loss of data information from the difference and similarities represented by two components (dimensions), denoted as PC 1 and PC 2 (Sabharwal & Anjum, 2016). From this multivariate analysis it was identified which characteristics evaluated presented the highest constitution weight in each dimension (axis), considering the loads of factor scores.

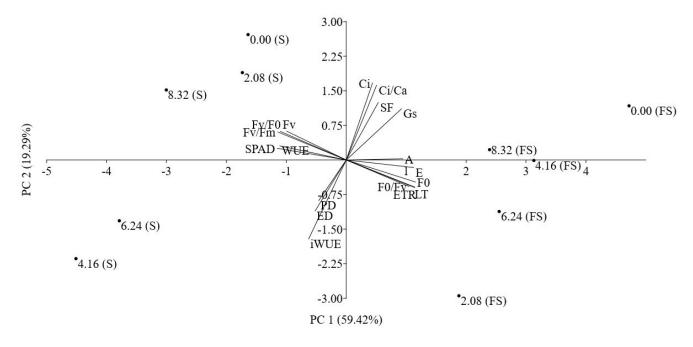


FIGURE 8. Principal components analysis of characteristics evaluated in plants of *A. edulis* grown with chicken manure (0.00, 2.08, 4.16, 6.24 and 8.32) and in full sun – FS or shaded – S.

The fluorescence characteristics in PC 1 had more weight in their constitution and were in the decreasing order of  $F_0$ ,  $F_v/F_m$ , chlorophyll index (SPAD),  $F_v/F_0$ , ETR, and *WUE*, being more responsive positively regarding the addition of CM to the soil, under the shaded environment (Table 2). Stomatal characteristics in PC 2 had more weight

in their constitution and were represented in decreasing order by  $C_i$ , SF,  $g_s$ , ED, *iWUE* and PD under intermediate doses of CM. Thus, it was found that the increasing addition of CM contributed to greater photochemical indicators in the leaves of *A. edulis* in the shaded environment, whereas gas exchange was more responsive to full sun and lower doses of this organic residue.

	PC 1	PC 2	PC 3	PC 4	PC 5
SPAD	-0.2915	0.0655	-0.0031	0.0973	-0.3879
A	0.2381	0.0098	-0.1031	0.5366	-0.0274
Ε	0.2854	-0.0370	0.0315	0.2790	0.0582
$g_{ m s}$	0.2083	0.3065	0.1755	0.3087	-0.2340
$C_{\mathrm{i}}$	0.1110	0.4227	0.3165	-0.1645	0.0386
WUE	-0.2805	0.0740	-0.1209	-0.2737	-0.0714
i <i>WUE</i>	-0.1584	-0.4320	-0.1908	-0.0790	0.0228
$C_{\rm i}/{\rm C_a}$	0.1315	0.4073	0.3133	-0.1603	0.0292
LT	0.2901	-0.1485	-0.0539	0.0100	0.2020
$F_0$	0.2924	-0.1300	-0.0504	-0.0421	0.0707
$F_v/F_m$	-0.2905	0.1530	0.0443	0.0726	-0.0388
$F_{\mathbf{v}}$	-0.2547	0.1493	-0.1492	0.3774	0.2861
$F_v/F_0$	-0.2798	0.1517	-0.0807	0.2491	0.0952
$F_0/F_v$	0.2664	-0.1390	0.0745	-0.2436	-0.4918
ETR	0.2884	-0.1507	-0.0683	0.0556	0.1407
ED	-0.1304	-0.2697	0.4976	0.1576	-0.0403
PD	-0.1163	-0.2202	0.5037	-0.1091	0.5209
SF	0.1346	0.3031	-0.4060	-0.2927	0.3284

TABLE 2. Eigenvectors and their respective scores for physiological and morpho-stomatic characteristics in *A. edulis* seedlings grown with chicken manure and in full sun or shaded.

Although the seedlings of *A. edulis* are considered an initial secondary species, which could be proven by the effect of full sun on photosynthesis photochemical, the damage in the reaction centers of PS II does not harm the photosynthetic performance of seedlings considering the high *A*,  $g_s$ , and  $A/C_i$ . Therefore, it suggests that this species has plasticity to contrasting conditions of light, and it can be planted in environments with different gradients of incident radiation, i.e., full sun conditions (e.g., anthropized open areas) or shade (e.g., understory in agroforestry systems or enrichment of native forest).

Based on our results, we disagree with our hypothesis that seedlings presented less leaf metabolism in full sun; however, we agree that adding organic matter to the substrate, evaluated here by the use of chicken manure, favors the metabolic responses that guarantee the quality of the seedlings under contrasting conditions of light.

#### CONCLUSIONS

Cultivation in full sun and the addition of 4.32 g CM kg<sup>-1</sup> soil favored the functioning of the photosynthetic apparatus in the seedlings of *Alibertia edulis* Rich. and provided greater production of photoassimilates. In a shaded environment, the seedlings present more viable photochemical indicators with a maximum dose (8.32 g) of CM.

## **ACKNOWLEDGEMENTS**

To CNPq and CAPES, for granting scholarships and to FUNDECT, for financial support.

## REFERENCES

Agbor J, Etim T, Obi U (2018) A study of the effects of cassava peels and poultry manure based compost on soil properties, growth and yield of water leaf. African Journal of Poultry Farming 6(4):282-289.

Albuquerque TCS, Evangelista TC, Albuquerque Neto AAR (2015) Níveis de sombreamento no crescimento de mudas de castanheira do Brasil. Revista Agroambiente 9(4):440-445. DOI: https://dx.doi.org/10.18227/1982-8470ragro.v9i4.3025

Aragão DS, Lunz AMP, Oliveira LC, Raposo A, Fermino Júnior PCP (2014) Efeito do sombreamento na anatomia foliar de plantas jovens de andiroba (*Carapa guianensis* Aubl.). Revista Árvore 38(4):631-639. DOI: https://doi.org/10.1590/S0100-67622014000400006

Aquino DFDES, Tirloni CAS, Menegatti SELT, Cardoso CAL, Vieira SCH, Vieira MC, Simonet AM, Macías FA, Gasparoto A (2017) *Alibertia edulis* (L.C. Rich.) AC Rich – A potencial diuretic arising from Brazilian indigenous species. Ethnopharmacology 9(6):193-200. DOI: https://doi.org/10.1016/j.jep.2016.12.024

Baker B (2008) Chlorophyll Fluorescence: A Probe of Photosynthesis *In Vivo*. Annual Review of Plant Biology 59:89-113. DOI:

https://doi.org/10.1146/annurev.arplant.59.032607.092759

Biswal B, Joshi PN, Raval MK, Biswal UC (2011) Photosynthesis, a global sensor of environmental stress in green plants: stress signaling and adaptation. Current Science 101(1):47-56. Bunce JA (2016) Light dependence of carboxylation capacity for  $C_3$  photosynthesis models. Photosynthetica 54(4):484-490. DOI: https://doi.org/10.1007/s11099-016-0215-9

Carstensen A, Herdean A, Schmidt SB, Sharma A, Spetea C, Pribil M, Husted DS (2018) The impacts of phosphorus deficiency on the photosynthetic electron transport chain. Plant Physiology 3:1-38. DOI: https://doi.org/10.1104/pp.17.01624

Devi S, Sharma CR, Sing K (2012) Microbiological biodiversity in poultry and paddy straw wastes in composting systems. Brazilian Journal of Microbiology 43(1):288-296. DOI: https://doi.org/10.1590/S1517-83822012000100034

Gandolfi S, Leitão Filho HF, Bezerra CL (1995) Levantamento florístico e caráter sucessional das espécies arbustivo-arbóreas de uma floresta mesófila semidecídua no município de Guarulhos, SP. Revista Brasileira de Biologia 55(4):753-767.

Gilmore AM, Hazlett TL, Debrunner PG (1996) Comparative time-resolved photosystem II chlorophyll *a* fluorescence analyses reveal distinctive differences between photoinhibitory reaction center damage and xanthophyll cycle-dependent energy dissipation. Photochemistry and Photobiology 64:552-563. DOI: https://doi.org/10.1111/j.1751-1097.1996.tb03105.x

Gonçalves JF, Silva DE, Guimarães DG, Bernardes RS (2010) Análise dos transientes da fluorescência da clorofila *a* de plantas jovens de *Carapa guianensis* e de *Dipteryx adorata* submetidas a dois ambientes de luz. Acta Amazonica 40(1):89-98. DOI:

https://doi.org/10.1590/S0044-59672010000100012

Gonzaga LM, Silva SS, Campos SA, Ferreira RP, Campos ANR, Cunha ACMCM (2016) Evaluation of substrates and AMF sporulation in the production of seedlings of native forest species. Revista Árvore 40(2):245-254. DOI: https://doi.org/10.1590/0100-67622016000200007

Gu J, Zhou Z, Li Z, Chen Y, Wang Z, Zhang H, Yang J (2017) Photosynthetic properties and potentials for improvement of photosynthesis in pale green leaf rice under high light conditions. Frontiers in Plant Science 8(1082):1-14. DOI:

https://doi.org/10.3389/fpls.2017.01082

Guo W, Nazim H, Liang Z, Yang D (2016) Magnesium deficiency in plants: an urgent problem. The Crop Journal 4:83-91. DOI: https://doi.org/10.1016/j.cj.2015.11.003

Jim H, Li M, Duan S, Fu M, Dong X, Liu B, Feng D, Wang J, Wang HB (2016) Optimization of light-harvesting pigment improves photosynthetic efficiency. Plant Physiology 172:1720-1731. DOI: https://dx.doi.org/10.1104/pp.16.00698

Leles PSS, Abaurre GW, Alonso JM, Nascimento DF, Lisboa AC (2011) Crescimento de espécies arbóreas sob diferentes espaçamentos em plantio de recomposição vegetal. Scientia Forestalis 39(90):231-239. Lima ALS, Zanella F, Castro LDM (2010) Crescimento de *Hymenaea courbaril* L. var. *stilbocarpa* (Hayne) Lee et Lang. e *Enterolobium contortisiliquum* (Vell.) Morong (Leguminosae) sob diferentes níveis de sombreamento. Acta Amazônica 10(1):43-48. DOI: https://doi.org/10.1590/S0044-59672010000100006

Lorenzi H, Bacher L, Lacerda M, Sartori S (2006) Frutas brasileiras e exóticas cultivadas (de consumo *in natura*). São Paulo, Instituto Plantarum de Estudos da Flora, 640p.

Malik MA, Khan KS, Marschner P, Hassan FU (2013) Microbial biomass, nutrient availability and nutrient uptake by wheat in two soils with organic amendments. Journal of Soil and Plant Nutrition 13(4):955-966. DOI: https://dx.doi.org/10.4067/S0718-95162013005000075

Paiva SS, Albuquerque MCF, Luz PB, Camili EC (2017) Caracterização física de frutos e sementes de *Lafoensia pacari, Alibertia edulis* e *Genipa americana*. Revista de Ciências Agrárias 40(2):382-389. DOI: https://dx.doi.org/10.19084/RCA16034

Ronquim CC, Prado CHBA, Souza JP (2018) Irradiance availability and growth of leguminous trees of Cerrado. Scientia Forestalis 46(117):115-126. DOI: https://dx.doi.org/10.18671/scifor.v46n117.11

Rosa DBCJ, Scalon SPQ, Cremon T, Ceccon F, Dresch DM (2017) Gas exchange and antioxidant activity in seedlings of *Copaifera langsdorffii* Desf. Under different water conditions. Anais da Academia Brasileira de Ciências 89(4):3039-3050. DOI: https://doi.org/10.1590/0001-3765201720170499

Sabharwal CL, Anjum B (2016) Data reduction and regression using principal component analysis in qualitative spatial reasoning and health informatics. Polibits 1(5):1-13. DOI: https://dx.doi.org/10.17562/PB-53-3

Sadler EJ, Evans DE (1989) Vapor pressure deficit calculations and their effect on the equation combination equation. Agricultural and Forest Meteorology 49(1):55-80. DOI: https://doi.org/10.1016/0168-1923(89)90062-2

Santos CC, Vieira MC, Heredia Zárate NA, Carnevali TO, Gonçalves WV (2020) Organic residues and bokashi influence in the growth of *Alibertia edulis*. Floresta e Ambiente 27(1):1-8. DOI: https://doi.org/10.1590/2179-8087.103417

Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Lumbreras JF, Coelho MR, Almeida JA, Araujo Filho JC, Oliveira JB, Cunha TJF (2018) Sistema brasileiro de classificação de solos. Brasília, DF, Embrapa, 5 ed. ver. ampl. 356p.

Silva FC (2009) Manual de análises químicas do solo, plantas e fertilizantes. Brasília, DF, Embrapa Informação Tecnológica, 2 ed. rev. ampl. 627p.

Silva FGS, Dutra WF, Dutra AF, Oliveira IM, Filgueira LMB, Melo AS (2015) Trocas gasosas e fluorescência da clorofila em plantas de berinjela sob lâminas de irrigação. Revista Brasileira Engenharia Agrícola e Ambiental 19(10):946-952. DOI: https://doi.org/10.1590/1807-1929/agriambi.v19n10p946-952 Soremi AO, Adetunji MT, Adejuyigbe CO, Bodunge JG, Azeez JO (2017) Effects of poultry manure on some soil chemical properties and nutrient bioavailability to soybean. Journal of Agriculture and Ecology Research 11(3):1-10. DOI: https://doi.org/10.9734/JAERI/2017/32419

Souza CC, Oliveira FA, Silva IF, Amorin Neto MS (2000) Avaliação de métodos de determinação de água disponível e manejo da irrigação em terra roxa sob cultivo de algodoeiro herbáceo. Revista Brasileira de Engenharia Agrícola e Ambiental 4(3):338-342. DOI: https://doi.org/10.1590/S1415-43662000000300006 Souza CSCR, Santos VAHF, Ferreira MJ, Gonçalves JFC (2017) Biomassa, crescimento e respostas ecofisiológicas de plantas jovens de *Bertholletia excelsa* Bonpl. Submetidas a diferentes níveis de irradiância. Ciência Florestal 27(2):557-569. DOI: https://doi.org/10.5902/1980509827736

Taiz L, Zeiger E, Moller I, Murphy A (2017) Fisiologia e desenvolvimento vegetal. Porto Alegre, Artmed, 6 ed. 888 p.

Telfer A (2014) Singlet oxygen production by PS II under light stress: mechanism, detection and the protective role of  $\beta$ -carotene. Plant & Cell Physiology 55(7):1216-1223. DOI: https://doi.org/10.1093/pcp/pcu040