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Research article

Organic residues and *Parachlorella* microalgae favor the growth and gas exchange of cedar

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ABSTRACT: Using organic residues and bioinputs is a promising and sustainable practice to produce seedlings with forest essences, such as *Cedrela fissilis* Vell. (cedar), a vulnerable species close to extinction due to intensive exploitation in native areas. Thus, we aimed to evaluate the effect of different substrates based on organic residues associated with or without the application of *Parachlorella* sp. microalgae in the emergence and morphophysiology of *C. fissilis* seedlings. Sowing was carried out on six substrates: Oxisol with a clayey texture; Oxisol + *Parachlorella* sp.; Oxisol with sheep manure (3:1, v v^{-1}); Oxisol with sheep manure + *Parachlorella* sp.; Oxisol with cattle manure (3:1, v v^{-1}); and Oxisol with cattle manure + *Parachlorella* sp. The addition of organic residues to the soil, especially cattle manure, contributes to increasing the percentage of emergence, plant height, chlorophyll index, CO_2 assimilation rate, and instantaneous carboxylation efficiency of Rubisco due to the superior chemical attributes in the substrate, which promote greater physiological efficiency. Organic residues increased the water use efficiency of seedlings. The application of *Parachlorella* sp. microalgae contributes to increases in the CO_2 assimilation rate and stomatal conductance when seedlings are grown only in Oxisol. *C. fissilis* seedlings produced in the substrate with sheep and cattle manure showed better growth and gas exchange characteristics.

Keywords: *Cedrela fissilis* Vell., bioinputs, sheep manure, cattle manure, stomatal limitation

Introduction

Cedar (*Cedrela fissilis* Vell., Meliaceae) is a native tree species, classified as secondary initial according to the ecological succession group (Lorenzi, 2000). This species presents medicinal and silvicultural interest, with potential for inclusion in integrated production systems (Carminate et al., 2014; Borges et al., 2019). Additionally, it can recover degraded environments and ecological corridors (Siqueira et al., 2019). Due to all these potential applications, it is vital to obtain quality seedlings.

Considering the possible extinction risk of *C. fissilis* due to its vulnerability in natural environments, seedling production becomes an ex situ conservation strategy. Generally, the substrate for propagation in the nursery is a factor of significant influence (Santos et al., 2020a). For this reason, using organic residues and bioinputs in the nursery has been a growing practice in sustainable agriculture.

The addition of organic residues from animal origin, such as sheep and cattle manure, for the formulation of alternative substrates, changes the chemical, physical, and microbiological attributes (Santos et al., 2020b, c), especially when added to soils with characteristics of low natural fertility, such as the Oxisols found extensively in the tropical region of the Cerrado (Santos et al., 2019; Silverio et al., 2020; 2021).

Another sustainable management practice that has taken hold over the last few years is the application of microalgae, especially *Parachlorella* sp. (Chlorellaceae), close to the genus *Chlorella*, a photosynthetic organism that contributes to plant germination, nutrition and physiology, as well as promoting tolerance to multiple stresses (Puglisi et al., 2020; Kusvuran, 2021; Coronado-Reyes et al., 2022). The microalgae is rich in macro and micronutrients, polyamines, enzymes, carbohydrates, carotenoids, proteins, and vitamins, and several studies have reported the identification of phytohormones, such as auxins, cytokinins, gibberellins and brassinosteroids (Levasseur et al., 2020; Alvarez et al., 2021). Due to the production capacity of these phytohormones, microalgae extracts act as plant growth and soil regeneration stimulants that promote or modify the microbiota (Lee and Ryu, 2021).

Given that under this scenario, we hypothesized that using sheep and cattle manure associated with applying microalgae must be a promising practice in the production of cedar seedlings. This study aimed to evaluate the effect of different substrates with organic residues, associated or not with the application of the *Parachlorella* sp. Microalgae, on the emergence and morphophysiology of *C. fissilis* seedlings.

Materials and Methods

Fruit collection and seed processing

The fruit of *C. fissilis* was collected, still closed, from mother plants located in an area near the forest fragment in the municipality of Glória de Dourados (22°22′39.8″ S, 54°16′06.9″ W, altitude 390 m), Mato Grosso do Sul – Brazil. It was stored at room temperature (± 25 °C) until the onset of the natural opening process. Seeds were selected according to their integrity and visual absence of damage. They were immersed in 2 % sodium hypochlorite for 5 min for sanitization and then washed in a sieve under running water.

Microalgae production and characterization

Parachlorella sp. microalgae (Figure 1) used in this work was produced in a low-cost synthetic culture media named "Blue Green Nitrogen Mix" (BGNIM) (Ribeiro et al., 2020), grown in intermediate bulk containers (IBC) with a useful volume of 500 L in natural light with constant aeration for 30 days. Using a sequencing analysis of the 18S region, all identified markers were 100 % compatible with the genus. The concentration obtained for microalgae application was 2 × 10^7 viable cells per mL at pH of 6.3, productivity of 1.1 g L^{-1} of algae biomass and organic carbon, N, P, K, Ca, S and Mg (results expressed in %): 29.3; 0.2; 0.9; 0.1; 0.1; 0.1 and 0.1, and Zn, Bo, Fe, Cu and Mn (results expressed in mg kg^{-1}): 10.19; 0.001; 113.7; 11.13 and 4.57, respectively.

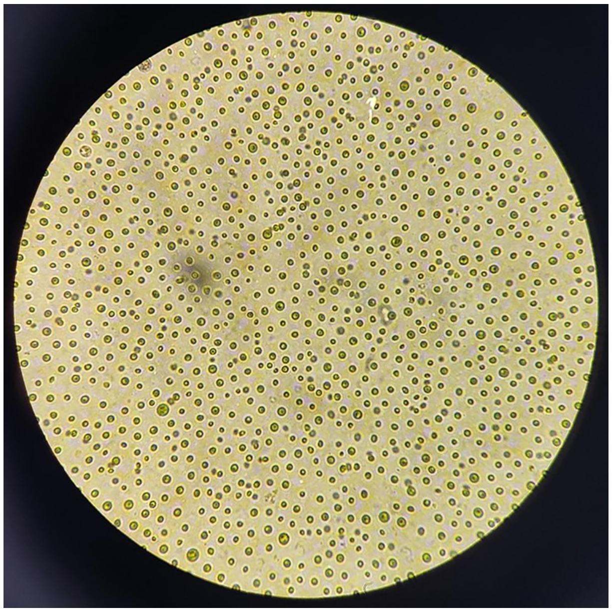

Figure 1 – Microscopic photo of *Parachlorella* sp. microalgae used in the experiment with *Cedrela fissilis* Vell. seedlings.

Management and experimental design

The experiment was carried out under shading with upper and lateral coverage of nylon screen with black color and 30 % shading (22°11′ S, 54°56′ W, altitude 446 m), Dourados – Mato Grosso do Sul, Brazil. Sowing was carried out in black polypropylene tubes with a volume of 290 cm^3.

Six substrates were formulated: Oxisols with a clayey texture in the USDA classification corresponding to the Dystropheric Red Latosol (Brazilian Classification), Oxisol + *Parachlorella* sp.; Oxisol with sheep manure (3:1, v v^{-1}); Oxisol with sheep manure + *Parachlorella* sp.; Oxisol with cattle manure (3:1, v v^{-1}); and Oxisol with cattle manure + *Parachlorella* sp. The experimental design used was randomized blocks with six replications, and each experimental unit consisted of six tubes with one seed per tube.

The sheep manure came from sheep farming on the Fazenda Experimental de Ciências Agrárias (FAECA) – Universidade Federal de Grande Dourados (UFGD) (22°13′52″ S, 54°59′10″ W, altitude 411.75 m). Cattle manure was purchased from a rural property (21°11′29.0″ S, 54°53′34.1″ W, altitude 484 m) in the municipality of Sidrolândia, Mato Grosso do Sul. The organic residues were tanned for 75 days. These materials and the soil were then sieved for the formulation of the substrates (Figure 2) to facilitate the homogeneity of mixtures. A composite sample of each substrate mixture was collected before starting the application of *Parachlorella* sp. microalgae for the characterization of chemical attributes (Table 1).

Every fifteen days, counting from time zero (day of sowing) until 60 days after sowing, we made the application of *Parachlorella* sp. microalgae at 10.0 mL L^{-1} per dose, which was diluted in distilled water and

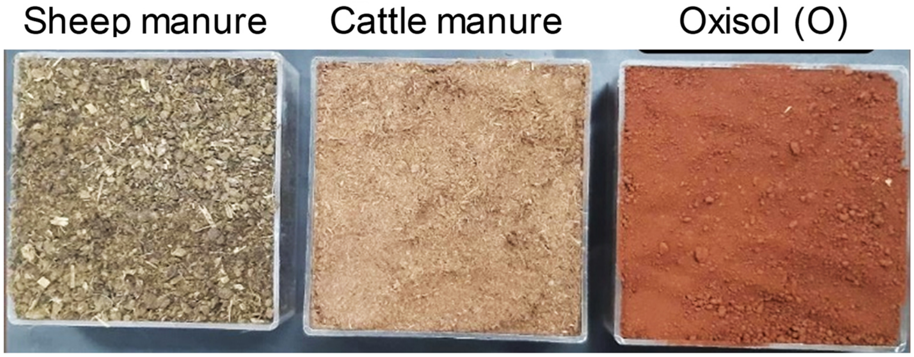

Figure 2 – Materials used in the formulation of substrates to produce *Cedrela fissilis* Vell. seedlings.

Table 1 – Chemical attributes of the substrates used in the experiment without the application of the *Parachlorella* sp. microalgae to produce *Cedrela fissilis* Vell. seedlings.

Substrates	pH $CaCl_2$	P	K	Ca	Mg	Al	H + Al
		mg dm^3	----------------- $cmol_c$ dm^3 -----------------				
Oxisol	4.47	0.04	0.03	0.76	0.45	0.60	3.23
OSM	5.56	16.32	2.27	2.39	2.53	0.00	2.20
OCM	6.22	20.10	3.97	3.43	3.17	0.00	1.69
Substrates	SB	CEC	V %	Cu	Mn	Fe	Zn
	---- $cmol_c$ dm^3 -----			------------------- mg dm^3 -------------------			
Oxisol	1.24	4.47	27.80	4.86	10.74	71.34	0.19
OSM	7.19	9.39	76.57	2.75	26.69	39.65	3.23
OCM	10.57	12.26	86.23	1.46	42.23	34.56	3.68

SB = Sum of bases; CEC = Cation exchange capacity; V (%) = Base saturation; Oxisol with a clayey texture; OSM = Oxisol with sheep manure (3:1, v v^{-1}); OCM = Oxisol with cattle manure (3:1, v v^{-1}).

showed electrical conductivity of 0.08 µS cm^{-1}. Four mL of solution was added to the substrate surface of each tube using a hypodermic syringe. In contrast, while the same amount of solution containing only water was added in plots without microalgae. Seedlings were irrigated daily with two watering shifts, and weeding was provided where needed.

Evaluations

At 20 days after sowing, with the seedling emission stable, the percentage of emergence was calculated according to Nakagawa (1994), considering the number of seedlings that formed fully expanded leaves. Sixty days after final emergence (80 days after sowing), the following characteristics were evaluated:

Growth and chlorophyll index: the height of seedlings was measured (distance from the collar to the apical bud), using a graduated ruler in cm; the steam diameter was measured with a digital caliper inserted 1.0 cm above the substrate level, and the number of leaves was counted. The chlorophyll index was determined by using a SPAD 502 portable chlorophyll meter (Soil Plant Analyzer Development), and measurements were taken on fully expanded leaves from 8h00 to 10h00.

Gas exchanges: carried out with an LCIPro-SD portable photosynthesis meter (IRGA – Infra Red Gas Analyzer) (Model ADC BioScientific Ltd.) on fully expanded leaves in the middle third of seedlings. We determined: the CO_2 assimilation rate (photosynthesis) (A; µmol CO_2 m^{-2} s^{-1}); intercellular CO_2 concentration (C_i; mmol CO_2 m^{-2} air^{-1}); stomatal conductance (g_s; mol H_2O m^{-2} s^{-1}) and transpiration (E; mmol H_2O m^{-2} s^{-1}). After this, we calculated the water use efficiency ($WUE = A/E$; µmol CO_2 m^{-2} s^{-1} / mmol H_2O m^{-2} s^{-1}) and the instantaneous carboxylation efficiency of Rubisco (A/C_i; µmol CO_2 m^{-2} s^{-1}/ mmol CO_2 m^{-2} air^{-1}). The evaluations were carried out from 8h00 and 11h00, with mean values for photosynthetically active radiation of 1,191.60 µmol photons m^{-2} s^{-1}, atmospheric CO_2 concentration of 428.13 mmol CO_2 m^{-2} s^{-1} and leaf temperature of 35.0 °C.

Stomatal limitation value (SLV) calculated according to Xingyang et al. (2020) through the following Eq. (1):

$$SLV = 1 - \frac{C_i}{C_a} \tag{1}$$

where: C_a = external concentration of CO_2 (mmol CO_2 m^{-2} s^{-1}) and C_i = intercellular CO_2 concentration (mmol CO_2 m^{-2} s^{-1}) registered by the IRGA, Model ADC BioScientific Ltd.

Data analysis

The data were submitted to the Shapiro-Wilk normality test, and subsequently submitted to analysis of variance (ANOVA) and, where significant, by the F test ($p \leq 0.05$). The means were compared by the Tukey test for substrates from the combination of organic residues, all with and without *Parachlorella* sp. ± standard deviation (SD) ($p \leq 0.05$), using the SISVAR software, version 5.6.

Results

Characteristics of emergence and growth of *C. fissilis* seedlings were influenced by alternative substrates, except stem diameter. The best values for emergence (80.5 and 90.0 %) were recorded when using Oxisol with sheep manure + *Parachlorella* sp. and Oxisol + with cattle manure, respectively, while for the other substrates, the values were lower and did not differ between themselves (Figure 3A). We verified growth using the best plant heights and number of leaves in *C. fissilis* seedlings that were produced in Oxisol with sheep manure and Oxisol + with cattle manure, regardless of the application of the *Parachlorella* sp. microalgae (Figure 3B and C). Seedlings produced with organic residues, regardless of the application of *Parachlorella* sp. microalgae, showed higher values (> 33.00 Soil Plant Analyzer Development) for the chlorophyll index than those seedlings presenting only Oxisol and Oxisol with *Parachlorella* sp. (Figure 3D).

The alternative substrates influenced the gas exchanges of seedlings. We observed higher values for the CO_2 assimilation rate (A) (3.08, 3.31, and 3.91 µmol CO_2 m^{-2} s^{-1}) in seedlings produced in the substrates Oxisol with sheep manure, both with and without *Parachlorella* sp., and Oxisol with cattle manure, respectively (Figure 4A). The instantaneous carboxylation efficiency of Rubisco (A/C_i) had the same tendency as A, showing higher value (0.0255 µmol mmol CO_2 m^{-2} s^{-1}) when produced in the Oxisol with cattle manure substrate, while those in the Oxisol and Oxisol + *Parachlorella* sp. recorded the lowest values (0.0018 and 0.0045 µmol mmol CO_2 m^{-2} s^{-1}) (Figure 4B).

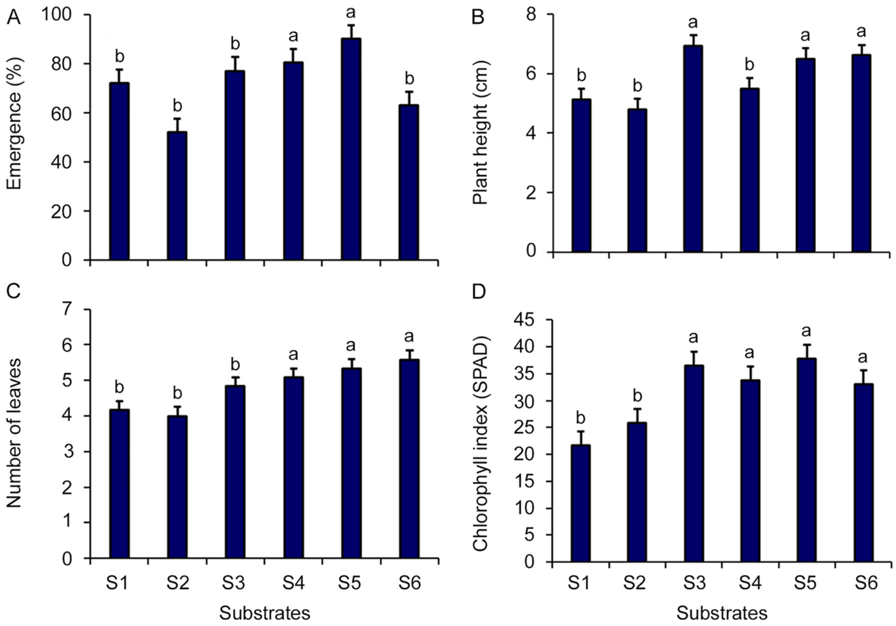

Figure 3 – Emergence (A), plant height (B), number of leaves (C) and chlorophyll index (D) in *Cedrela fissilis* Vell. seedlings produced with different substrates prepared with organic residues, with or without application of *Parachlorella* sp. microalgae. Different letters among columns differ statistically by the Tukey test ± SD ($p \leq 0.05$). S1 = Oxisol with a clayey texture; S2 = Oxisol + *Parachlorella* sp.; S3 = Oxisol with sheep manure (3:1, v v^{-1}); S4 = Oxisol with sheep manure + *Parachlorella* sp.; S5 = Oxisol with cattle manure (3:1, v v^{-1}); and S6 = Oxisol with cattle manure + *Parachlorella* sp. SPAD = Soil Plant Analyzer Development

The highest values for E were found in seedlings produced in the Oxisol substrates both with and without *Parachlorella* sp. and Oxisol with sheep manure (Figure 4C). With regard to stomatal conductance, only seedlings in the Oxisol with *Parachlorella* sp. and in the Oxisol with sheep manure showed the highest values (0.056 and 0.048 mol H_2O m^{-2} s^{-1}, respectively) (Figure 4D) compared to those observed in the other substrates. *C. fissilis* seedlings had higher values for *WUE* (2.97 and 3.95 µmol CO_2 mmol H_2O m^{-2} s^{-1}) and lower values for intercellular CO_2 concentration (157.66 and 179.00 mmol CO_2 m^{-2} air^{-1}) when produced in Oxisol with sheep manure, respectively, regardless of the application of *Parachlorella* sp. microalgae (Figure 4E and F).

The stomatal limitation value (SLV) was lower in seedlings produced in the Oxisol both with and without *Parachlorella* sp. (0.21 and 0.17, respectively) compared to the other substrates (Figure 5). *C. fissilis* seedlings presented different visual aspects depending on the substrates we used, where seedlings produced in the Oxisols were smaller and leaves showed chlorosis, different from those in the substrates with organic residues, which had better vigor (Figure 6).

Discussion

The lowest values for *C. fissilis* seedlings emergence in substrates containing Oxisol only are because they contain high aluminum content (0.60 $cmol_c$ dm^3). The direct contact of this element with the seed reduces its physiological and germination potential by increasing membrane permeability and solute efflux (Mota et al., 2020; Silverio et al., 2021), making it difficult for the seedling to emerge. Another essential aspect to consider is the physical characteristics of the substrate. In our study, the Oxisol had a very clayey texture, which impaired the emergence of *C. fissilis* seedlings. Adding organic residues to the Oxisol changed the physical attributes of the substrate, especially porosity, which favored imbibition, root protrusion, and seedling formation.

In general, the highest values for growth and gas exchange characteristics when using organic residues are because substrates with sheep and cattle manures had better chemical attributes, especially phosphorus, potassium, calcium, and magnesium (Table 1). These nutrients participate in the production of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate hydrogen (NADPH), the structure of the chlorophyll molecule and plant tissue, as well as being involved with the production of biomass (Santos et al., 2019; Lima et al., 2022).

In addition, we highlight that with these same substrates, the values of iron and aluminum were lower compared to the Oxisol (Table 1), which favored the foliar metabolism of seedlings. The excess of these mineral elements impairs the transport of electrons among the acceptors in the reaction centers of photosystems. It affects gas exchange negatively, especially A, in response to decreased stomatal conductance (Mota et al., 2020),

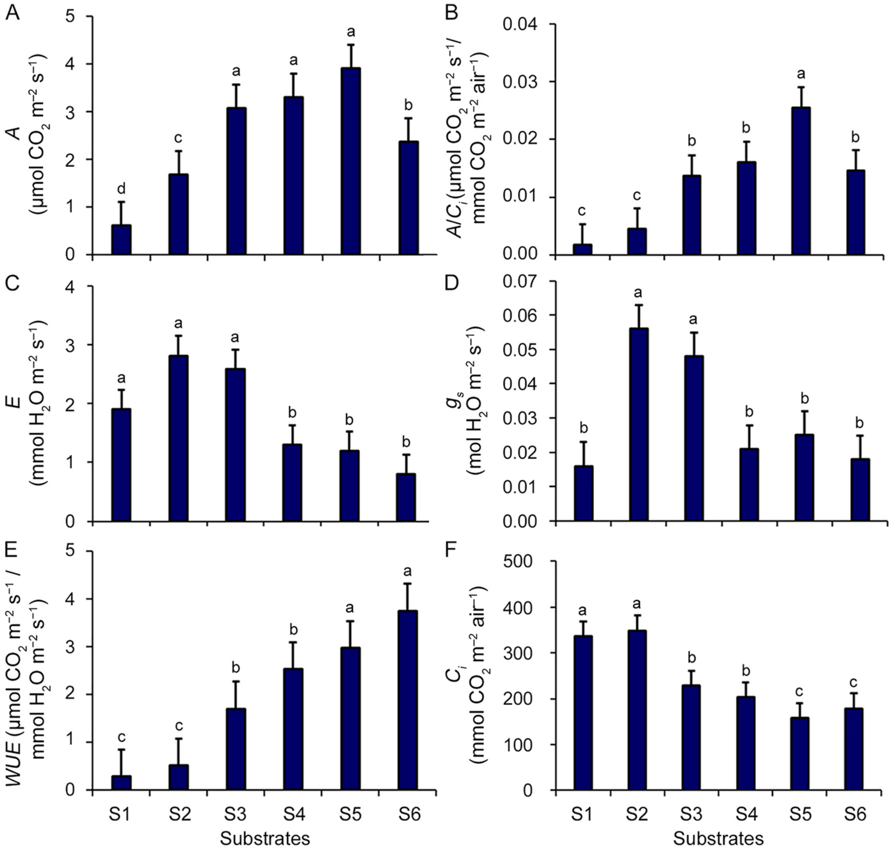

Figure 4 – A = the CO_2 assimilation rate (photosynthesis) (A); A/C_i = instantaneous carboxylation efficiency of Rubisco (B); E = transpiration (C); g_s = stomatal conductance (D); *WUE* = water use efficiency (E); and C_i = intercellular CO_2 concentration (F) in *Cedrela fissilis* Vell. seedlings produced with different substrates prepared with organic residues, without or with application of *Parachlorella* sp. microalgae. Different letters above the columns differ statistically by the Tukey test ± SD ($p \leq 0.05$). S1 = Oxisol with a clayey texture; S2 = Oxisol + *Parachlorella* sp.; S3 = Oxisol with sheep manure (3:1, v v^{-1}); S4 = Oxisol with sheep manure + *Parachlorella* sp.; S5 = Oxisol with cattle manure (3:1, v v^{-1}); and S6 = Oxisol with cattle manure + *Parachlorella* sp.

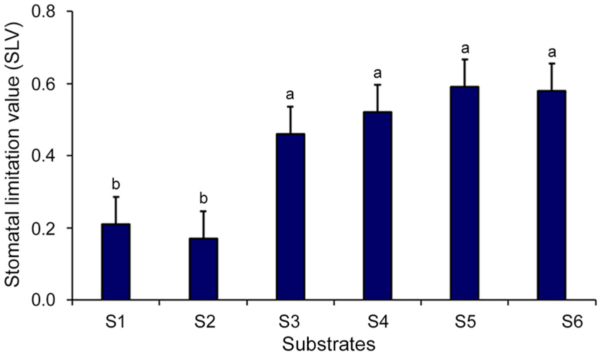

Figure 5 – Stomatal limitation value (SVL) in *Cedrela fissilis* Vell. seedlings produced with different substrates prepared with organic residues, with or without application of *Parachlorella* sp. microalgae. Different letters above the columns differ statistically by the Tukey test ± SD ($p \leq 0.05$). S1 = Oxisol with a clayey texture; S2 = Oxisol + *Parachlorella* sp.; S3 = Oxisol with sheep manure (3:1, v v^{-1}); S4 = Oxisol with sheep manure + *Parachlorella* sp.; S5 = Oxisol with cattle manure (3:1, v v^{-1}); and S6 = Oxisol with cattle manure + *Parachlorella* sp.

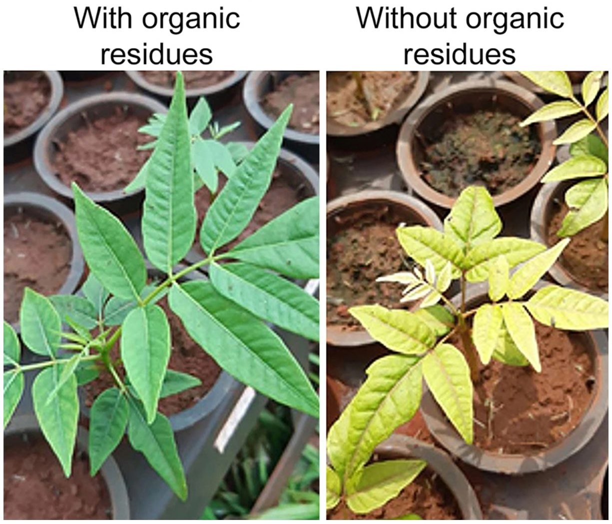

Figure 6 –Visual appearance of *Cedrela fissilis* seedlings with organic residues (left) and produced only in the Oxisols (right).

as verified in *C. fissilis* seedlings. We also emphasize that exposure to Al inhibits plant growth (Silverio et al., 2021).

Similarly, seedlings of *Alibertia edulis* Rich. (Santos et al., 2020b, c; Santos et al., 2023) and *Campomanesia xanthocarpa* O. Berg. (Goelzer et al., 2021), native species from Cerrado, also showed better morphophysiological characteristics when cultivated with organic residues. These authors attributed the results to the improvement of the substrate's chemical and physical attributes by adding manure.

On the other hand, the lowest CO_2 assimilation rate (0.61 µmol CO_2 m^{-2} s^{-1}) was recorded in seedlings produced in the Oxisol substrate without microalgae, even lower than that verified in the Oxisol + *Parachlorella* sp., demonstrating that for this characteristic, the application of microalgae when using only soil for the propagation of this species is a promising and sustainable practice. Compared to the microalgae, its beneficial effect is associated with its composition containing essential nutrients in metabolism, including 0.2 % nitrogen, other macro and micronutrients, and phytohormones, which may have favored the nutritional and hormonal balances of the seedlings. In addition, microalgae stimulate the microbiological activity of the soil, and improve the conditions of the microbiota and rhizosphere (Lee and Ryu, 2021), contributing to the soil-plant relationship.

The presence of high levels of aluminum, iron and copper when using only Oxisol represents a stressful cultivation condition for *C. fissilis*. This produced results like those observed by Mota et al. (2020) in *Jatropha curcas* L. plants, which also had lower A and A/C_i values when exposed to higher levels of Al in the Oxisol substrate. We noticed that adding organic residues in the substrate formulation reduces the Al and Fe content.

The *C. fissilis* tolerates stress conditions welldue to higher *WUE*, indicative of physiological plasticity (Griebeler et al., 2021). The *WUE* is defined as the amount of carbon assimilated as biomass per unit of water used by the plant, with a lower transpiration coefficient (Hatfield and Dold, 2019), as was found in seedlings produced in substrates with organic residues. However, seedlings produced in the Oxisol without organic residues, regardless of the application of *Parachlorella* sp., presented values for *WUE* and C_i that indicate unfavorable cultivation conditions. Furthermore, we observed that substrates containing organic residues are associated with the application of *Parachlorella* sp., the *WUE* results were higher, indicating that microalgae favor the optimization of water resources.

The lower SLV indicated lower physiological efficiency, which means these plants presented higher accumulated C_i due to lower A/C_i, which negatively affected the photosynthetic efficiency of seedlings and the production of photoassimilates.

In connection with this, substrates with higher levels of nutrients favor the mineral and photosynthetic metabolism of *C. fissilis* seedlings. Substrates containing cattle manure have a high capacity for cationic exchange, manganese, and zinc, in addition to macronutrients (Table 1). Generally, these residues are also rich in nitrogen, which stabilizes the metabolic processes. Furthermore, N participates in the composition of chlorophyll molecules, acting directly on the photosynthetic rate and other important processes such as the production of photoassimilates (Sampaio et al., 2021; Romero et al., 2022). Manganese is active in the photolysis of water and is a component of the energy bonds by ATP and the enzymatic complex. Zinc is involved in the metabolism of carbohydrates, which maintains the homeostasis of the activity of carbonic anhydrase, chlorophylls, and photochemical activities in PSII (Sadeghzadeh, 2013; Baroni and Vieira, 2020).

Although *C. fissilis* is found in areas with naturally low fertility, in different physiognomies in the Cerrado, we verified in our study that in the initial phase, the substrate with better chemical and physical characteristics favors the production of seedlings with maximum potential for the expression of metabolism and growth in the nursery, and possibly under field conditions.

However, the responses regarding the substrate for cultivation vary among species. For example, Santos et al. (2020a) evaluating the effect of two substrates (100 % Dystropheric Red Latosol = DRL (Oxisols) and 50 % DRL + 50 % commercial) in *Anadenanthera peregrina* L. Speg. seedlings, verified that this species presented the best growth when cultivated in 100 % DRL, with low organic matter content and CEC, which is different from what we observed in our work with *C. fissilis* seedlings.

From our work, we were able to verify that the use of organic residues to produce *C. fissilis* seedlings promotes several benefits. The nurseryman can adopted this practice even in the planting of these seedlings in degraded areas, native forests, forestry, or agroforestry system. Moreover, suppose the producer has any of these tanned materials available to use. In that case, it is possible to add them when transplanting seedlings, which will contribute to faster growth of the species.

Although we have not analyzed the production costs and profits, the use of organic residues available on the property or in the region favors the reduction of external mineral inputs, which are more expensive, and subsequently contributes to the profitability indexes in the commercialization of seedlings.

We emphasize that the little influence of microalgae, except for A and g_s, on the production of these seedlings is because the residues contribute with enough nutrients to the nutritional needs of the species. However, when using only Oxisol, applying *Parachlorella* sp. for long-term or higher doses can contribute to other characteristics in producing *C. fissilis* seedlings, a sustainable practice in nursery production.

We emphasize that the use of bioinputs, herein represented by *Parachlorella* sp., in agriculture and forestry is a market trend that is in use worldwide. In 2021, the National Bioinputs Program was developed by the Ministério da Agricultura, Pecuária e Abastecimento, which defined bioinputs as products, processes, or technologies of plant or animal origin and microorganisms applicable to agriculture, with the aim of boosting the use of biological resources (MAPA, 2021), thereby ensuring the objectives of sustainable development and bioeconomy.

In this context, in future perspectives, new works testing doses of microalgae should be developed to add information to the production of *C. fissilis* seedlings with organic residues and *Parachlorella* sp. microalgae such as evaluation of the nutritional status and subsequent transplanting under field conditions, aimed at conservation of this species.

Adding organic residues in the substrate formulation contributes to superior chemical attributes of the substrate and the emergence of *Cedrela fissilis* Vell. seedlings. Seedlings produced in the Oxisol with sheep or cattle manure show greater growth, gas exchanges, and physiological efficiency. The application of *Parachlorella* sp. microalgae contributes to the increase of photosynthesis and stomatal conductance in *C. fissilis* seedlings produced in the substrate with Oxisol alone.

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Authors' Contributions

Conceptualization: Santos CC, Oliveira ML, Ribeiro DM, Scalon SPQ, Linné JA, Silverio JM, Figueiredo VMA, Silva OHM. **Data curation:** Santos CC, Oliveira ML, Linné JA, Silverio JM, Figueiredo VMA, Silva OHM. **Formal analysis:** Santos CC. **Funding acquisition:** Santos CC, Scalon SPQ. **Project administration:** Santos CC. **Supervision:** Santos CC. **Methodology:** Santos CC, Oliveira ML, Scalon SPQ. **Writing – original draft:** Santos CC, Scalon SPQ. **Writing – review and editing:** Santos CC, Ribeiro DM, Scalon SPQ.

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