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Growth of *Campomanesia adamantium* (Cambess.) O. Berg, native to Brazilian Cerrado, with green manure in agroecological system contributes to the preservation of the species

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Abstract - *Campomanesia adamantium* (guavira) is a native plant of the Brazilian Cerrado used both as food and as medicine. The plant has undergone indiscriminate over-exploitation in its habitat, which, in association with fires and deforestation, puts the species at risk of extinction. To preserve the species, *in situ* and *ex situ* management actions are required and agroecological practices associated with green manuring is the recommended system. In this study, we investigated the development of *C. adamantium* grown with the green manures *Stylosanthes macrocephala*, *Pueraria phaseoloides*, *Calopogonium mucunoides*, and *Cajanus cajan*, as well as the chemical and microbiological properties of the soil. The green manures had the highest production of fresh and dry masses at the second cut and *C. mucunoides*, *S. macrocephala*, and *P. phaseoloides* presented the highest nutrient concentrations. *C. mucunoides* mass decomposed rapidly and influenced the chemical properties of the soil, with a greater role of soil microorganisms in the biochemical process of decomposition of the organic residues. The best-developed and highest yielding plants with the highest leaf nutrient content were obtained for *C. adamantium* grown with the green manures *C. mucunoides* and *S. macrocephala*. The results showed that *C. adamantium* responded positively to the use of the green manure *C. mucunoides* with increased leaf production. This agroecological cultivation contributes for the preservation of *C. adamantium* and the appropriate use of the natural resources of the Cerrado.

Index terms: Guavira; *Stylosanthes macrocephala*; *Pueraria phaseoloides*; *Calopogonium mucunoides*; *Cajanus cajan*.

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Crescimento de *Campomanesia adamantium* (Cambess.) O. Berg. nativa do Cerrado brasileiro, com adubação verde em sistema agroecológico, contribui para a preservação da espécie

Resumo - A *Campomanesia adamantium* (guavira) é nativa do Cerrado brasileiro e utilizada como alimentícia e medicinal. A planta tem sido explorada indiscriminadamente em seu habitat, o que, somado às queimadas e ao desmatamento, levam-na ao risco de extinção. Para a preservação da espécie, é essencial seu manejo *in situ* e *ex situ*, e as práticas agroecológicas associadas à adubação verde são um sistema recomendado. Neste estudo, investigamos o desenvolvimento da *C. adamantium* cultivada com os adubos verdes *Stylosanthes macrocephala*, *Pueraria phaseoloides*, *Calopogonium mucunoides* e *Cajanus cajan*, além dos atributos químicos e microbiológicos do solo. Os adubos verdes tiveram maiores produções de massas frescas e secas no segundo corte, e as plantas de *C. mucunoides*, *S. macrocephala* e *P. phaseoloides* tiveram maiores concentrações de nutrientes. A massa do *C. mucunoides* teve rápida decomposição e influenciou os atributos químicos do solo, havendo maior atuação dos microrganismos do solo no processo bioquímico de decomposição dos resíduos orgânicos. As plantas de *C. adamantium* mais bem desenvolvidas e com maiores produções e teores de nutrientes nas folhas foram cultivadas com os adubos verdes *C. mucunoides* e *S. macrocephala*. Os resultados mostraram que as plantas de *C. adamantium* respondem positivamente ao uso do adubo verde *C. mucunoides*, com aumento da produção de folhas. Este cultivo agroecológico contribui para a preservação de *C. adamantium* e para o uso adequado dos recursos naturais do Cerrado.

Termos para indexação: Guavira; *Stylosanthes macrocephala*; *Pueraria phaseoloides*; *Calopogonium mucunoides*; *Cajanus cajan*.

Introduction

The risk of extinction of plants native to Brazilian biomes results mainly from the expanse of agriculture, the introduction and dispersion of exotic species, deforestation, burning, and the degradation of natural resources (FERREIRA et al., 2018). One example is the commercial exploitation of leaves and fruits of *Campomanesia adamantium* (Cambess.) O. Berg. (Myrtaceae), a native shrub of the Brazilian Cerrado, for food and medicinal uses, making the species susceptible to extinction (LORENZI, 2006; FERNANDES et al., 2016). Studies on the domestication of native species of economic potential are fundamental for the preservation of the Cerrado. The biome is the second largest Brazilian vegetation

unit and has the greatest abundance of plant species compared with the world's savannas (LEÃO-ARAÚJO et al., 2019).

Several studies attest to *C. adamantium* leaves having therapeutic actions including antimicrobial (ALVES et al., 2019), antibacterial (OLIVEIRA et al., 2016), anti-inflammatory and anti-diarrheal (MARTELLO et al., 2016), anti-flu (LESCANO et al., 2016), and being protective against cardiometabolic diseases (ALVES et al., 2019). The species has attracted the attention of the pharmaceutical industry because of the antioxidant substances, i.e., essential oils that can be extracted from leaves and other parts of the plant (FERREIRA et al., 2018). Ripe fruits are sweet and traded mainly as fresh produce, as well as preserves

and ice cream (AJALLA et al., 2014; FERNANDES et al., 2016; ARAÚJO et al., 2019). Due to its high appreciation, *C. adamantium* became a symbol of the state of Mato Grosso do Sul - Law 5.082 / 2017 - being included in all tourism publications of the State.

The preservation of *C. adamantium* requires adequate *in situ* management and *ex situ* cultivation (MIRANDA et al., 2016) by means of domestication of the species in agroecological farming system with the minimum use of chemical inputs, avoiding environmental degradation and contributing to the preservation and proper use of the Cerrado's natural resources (AJALLA et al., 2014; EMER et al., 2020). Furthermore, growing *C. adamantium* in an agroecological system can reduce the contamination of plant material used as a medicinal source by chemical residues and synthetic fertilizers.

The main challenges of agroecological cultivation for the growth of native species are soil fertilization and soil structuring, control of spontaneous species, low content of organic matter, and the long-term response in growth and physiology of native species of the Cerrado (GONDIM et al., 2020). A strategy used by family farmers to overcome these variables in agroecological systems is the use of plants as green manure to maximize the use of space, land cover, moisture retention, and nutrient cycling (CALHEIROS et al., 2013; MAZZETTO et al., 2016; SOLATI et al., 2017; ARAÚJO et al., 2017; CHEN et al., 2019). These conditions improve the environment within a relatively short time (CLERMONT-DAUPHIN et al., 2016; ARAÚJO et al., 2017).

The cultivation of *C. adamantium* with green manures is still poorly studied. Gondim et al. (2020) evaluated in an 11-month cultivation

cycle the intercropping and the spacing of *C. adamantium* with legumes. The authors found that the wider spacing (2.00 m x 1.20 m) and intercropping with the mixture of *Crotalaria breviflora* and *Cajanus cajan*, plants of *C. adamantium* were taller, with larger canopy area and larger biomass.

To meet the demands of studies and due to the nutritional and medicinal potential of *C. adamantium*, the objective of this work was to evaluate growth, physiological parameters, and the leaf production of the plants of *C. adamantium* cultivated with green manures and the influence on the chemical and microbiological properties of the soil.

Material and Methods

The experiment was carried out in the field, in the Medicinal Plants Botanical Garden - MPBG (22°11'44"S and 54°56'08"W, 430 m altitude), of the Federal University of Grande Dourados (UFGD), Dourados, Mato Grosso do Sul (MS), Brazil. The experiment was conducted according to the principles of organic farming for medicinal plants. This area has been managed in the organic system for over 20 years. The soil is classified as a dystrophic Red Latosol, originally under Cerrado vegetation (SANTOS et al., 2013) with the chemical attributes (in the layer 0 - 0.10 m) before the sowing of the green manures as follows: K = 3.54; Al⁺³ = 27; Ca = 17.04; Mg = 9.46; H+Al = 104.65; BS = 34.43, and CEC = 139.08 as mmol_c dm⁻³ and V (%) = 25, pH (H₂O) = 4.11, P (mg dm⁻³) = 8,02, and OM = 18,12 g dm⁻³. The climate is classified as humid mesothermal, with hot rainy summers and dry winters (Cwa) (FIETZ et al., 2017). The temperature and rainfall that occurred during the development of the experiment are shown in Figure 1.

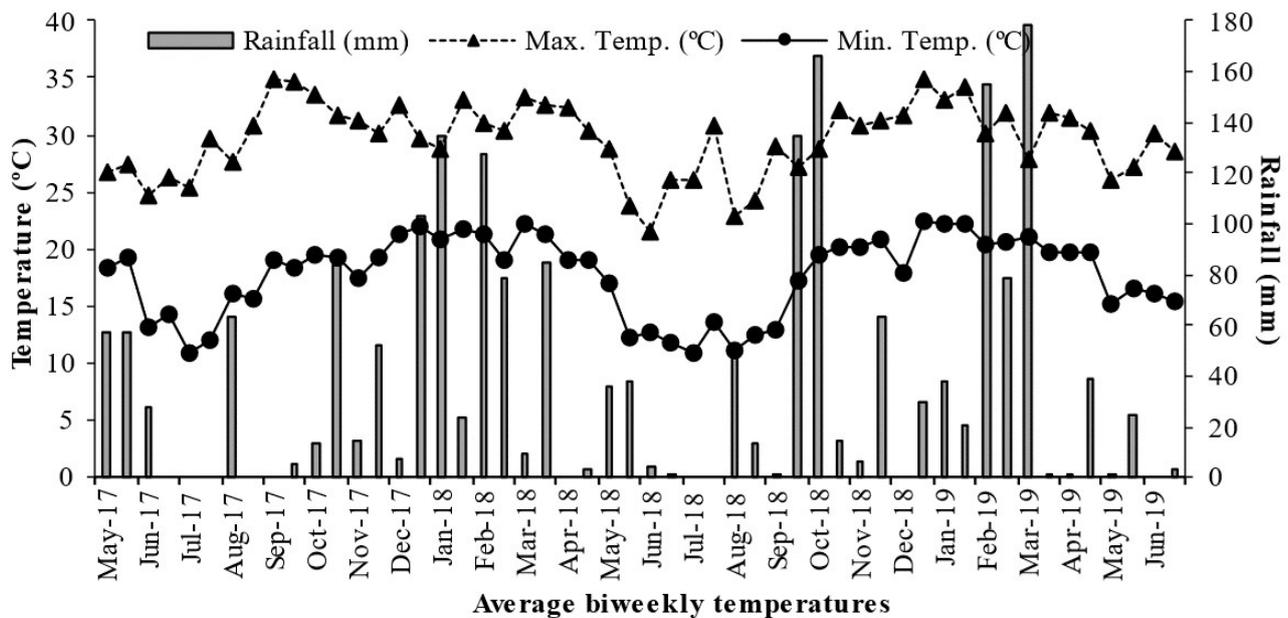


Figure 1. Maximum and minimum temperatures and rainfall, biweekly averages, in part of the cultivation cycle of *C. adamantium* (Source: clima.cpao.embrapa.br).

The treatments of the study included covering the soil with three species of perennial green manures: *Stylosanthes macrocephala* M.B.Ferreira & Sousa Costa (stylo), *Pueraria phaseoloides* (Roxb.) Benth. (tropical kudzu), and *Calopogonium mucunoides* Desv. (calopogonium); a semi-perennial species *Cajanus cajan* (L.) Huth (pigeon pea). In addition, two controls were formed: one covered with spontaneous vegetation and the other with bare soil (weeding). The six treatments were arranged in a randomized block design with four replications. The plots were 3.60 m wide and 2.0 m long.

The cultural practices included sprinkler irrigation, in the afternoon, to keep the soil with $\pm 70\%$ of the field capacity. The spontaneous plants between the green manures were hand pulled, when necessary, except for the area maintained with spontaneous vegetation. To keep the bare soil, spontaneous plants were removed with a hoe, when they were about 10 cm in height.

Seeds of the cover crops were hand sown direct into the plots in eight 0.40-m-apart rows;

0.2 m on the sides and 2 cm depth. Thinning was carried out 15 days after seedling emergence. Stands were established with 30 plants m^{-2} on average of *C. mucunoides*, *P. phaseoloides*, and *C. cajan* (AMABILE et al., 2000) and 25 plants m^{-2} of *S. macrocephala* (TEODORO et al., 2011). Green manures were cut twice, the first cut at 180 days after sowing (DAS), when the plants showed approximately 70% flowering and 90% soil cover and the second cut (regrowth) at 360 DAS. To evaluate fresh and dry mass, 1-square-meter metal quadrat was randomly tossed in each plot and within the quadrat, the enclosed bunch of the green manure and spontaneous vegetation were cut at about 15cm stalk height and total fresh weight was measured.

The fresh vegetable material was weighed, and then two samples of the plant material from each plot were separated: one sample of 400 g to evaluate the decomposition rate and one sample of 200 g to determine the dry mass, the unused material was returned to the experimental area. For dry mass determination, the material was packed in a paper

bag and placed in a forced air circulation oven at 60 ± 5 °C, to constant mass. The dry samples were ground in a Willey mill, homogenized, and the nutrient contents determined (MALAVOLTA et al., 1997). The plants remaining in the field were cut at about 15 cm above ground using a brush cutter and all the material was left on the soil.

The decomposition rate of each green manure was measured by the mass loss of the fresh samples placed in four litterbags [4-mm nylon mesh decomposition bags of 0.05 m² (0.20 x 0.25 m)]. Each litterbag contained 100 g of sample and was placed on the ground of each plot in the field, after the cuts. The loss rates of the dry mass and nutrient were assessed by weighing and analyzing the material in the litterbag randomly removed from the plots at 30, 60, 90, and 120 days after the start of the evaluation, following each cut (ESPINDOLA et al., 2006). The unused material was removed from the litterbags, dried in forced air circulation oven at 60 ± 5 °C to constant mass (to measure dry mass), ground in a Willey mill, homogenized, and nutrient contents were determined according to Malavolta et al. (1997).

The decomposition rate was quantified using the equation adapted from Wiegert and Evans (1964), with the exponential model:

$$k = \ln(X / X_0) e^{-kt}$$

Where:

k = mass of material remaining on the soil surface (g kg⁻¹);

t - time in days (days⁻¹);

x = mass of material remaining on the soil surface after 120 days (g kg⁻¹);

x₀ = mass of dry material placed in the bags at time zero (t = 0) (g kg⁻¹);

kt = mass of dry material remaining after t days (g kg⁻¹).

The half-life time $t_{(1/2)}$ of the remaining mass and the macronutrient content, i.e., the time required for decomposing half the mass and nutrients, was calculated according to Wiegert and Evans (1964): $t_{(1/2)} = \ln(2)/k$.

Specimens of the spontaneous vegetation species present in the area were collected 180 days after sowing (DAS) of the green manures, the taxonomic identification was carried out using herbarium records, consultations with specialists, and classified according to the Angiosperm Phylogeny Group (CHASE et al., 2016). The scientific names of the species and classification were confirmed by consulting the database of the World Flora Online (<http://www.worldfloraonline.org/>) and classified according to their photosynthetic pathway. The vouchers were incorporated into the Herbarium of the Federal University of Grande Dourados (DDMS) (Table 1).

The chemical attributes of the soil were evaluated 60 days after each cut of the green manures. Samples were collected in the 0.00-0.10 m-deep layer, using a Dutch auger, at six random points in each plot, homogenized, and chemically analyzed (MALAVOLTA et al., 1997).

The analysis of soil microbial biomass carbon (SMBC) was according the fumigation-extraction method by Vance et al. (1987). Organic carbon was determined by the Yeomans and Bremner (1988) method, and the basal respiration (C-CO₂) by the respirometric method (CO₂ evolution). The microbial quotient ($qMIC$) was calculated by the formula (SMBC/Corg) x 100 and the metabolic (qCO_2) was calculated by the ratio basal respiration by microbial carbon ($\mu CO_2/\mu g SMBC h^{-1}$).

Table 1. Botanical family, scientific and Brazilian common names; photosynthetic pathway (PP), and voucher of spontaneous species deposited at the DDMS Herbarium, UFGD.

Family	Scientific name	Brazilian common name	PP	Voucher
Apiaceae	<i>Cyclosporum leptophyllum</i> (Pers.) Sprague ex Britton & P. Wilson	Aipo bravo	C4	6428
Asteraceae	<i>Gnaphalium purpureum</i> P. Dusen	Macela fina	C3	6288
Asteraceae	<i>Ambrosia artemisiifolia</i> L.	Cravo-da-roça	C3	6430
Asteraceae	<i>Acanthospermum hispidum</i> DC.	Carrapicho-de- carneiro	C3	6432
Asteraceae	<i>Emilia fosbergii</i> Nicolson	Falsa serralha	C3	6433
Asteraceae	<i>Conyza bonariensis</i> L. Cronquist	Buva	C3	2259
Commelinaceae	<i>Commelina erecta</i> L.	Trapoeaba	C4	5751
Cyperaceae	<i>Cyperus rotundus</i> L.	Tiririca	C4	6289
Cyperaceae	<i>Hypolytrum pulchrum</i> (Rudge) H. Pfeiff.	Navalha-de-macaco	C3	6426
Cyperaceae	<i>Cyperus</i> sp.	Junquilha	C3	6429
Phyllanthaceae	<i>Phyllanthus tenellus</i> Roxb.	Quebra-pedra	C3	6284
Lamiaceae	<i>Leucas martinicensis</i> (Jacq.) R.Br.	Falsa menta	C4	6287
Malvaceae	<i>Sida rhombifolia</i> L.	Guanxuma	C3	6286
Plantaginaceae	<i>Plantago major</i> L.	Tansagem	C3	5644
Poaceae	<i>Digitaria insularis</i> (L.) Fedde	Capim amargoso	C4	6343
Poaceae	<i>Digitaria sanguinalis</i> (L.) Scop.	Capim colchão	C4	6339
Poaceae	<i>Eleusine indica</i> (L.) Gaertn.	Pé-de-galinha	C4	6340
Poaceae	<i>Paspalum pumilum</i> Nees	Grama baixa	C4	6338
Poaceae	<i>Paspalum virgatum</i> L.	Capim navalha	C4	6342
Poaceae	<i>Urochloa humidicola</i> (Rendle) Morrone & Zuloaga	Brachiarão	C4	6341
Poaceae	<i>Urochloa plantaginea</i> (Link) R.D. Webster	Capim marmelada	C4	6336
Portulacaceae	<i>Portulaca oleracea</i> L.	Beldroega	C3	6332
Rubiaceae	<i>Richardia brasiliensis</i> Gomes	Poaia branca	C4	5037
Solanaceae	<i>Cestrum axillare</i> Vell.	Dama-danoite	C3	6290
Verbenaceae	<i>Stachytarpheta cayennensis</i> (Rich.) Vahl	Gervão	C3	6335

The analysis of the humic fractions of the soil organic matter (SOM) was based on the Kononova-Belchikova method (KONONOVA, 1982). The carbon stock was calculated using the equation described by Elbert and Bettany (1995):

$$\text{STOC} = (\text{OC} \times \text{SBD} \times t) / 10.$$

Where:

STOC = stock of organic C at a given depth (Mg ha^{-1})

OC = total organic C content at the depth sampled (g kg^{-1})

SBD = soil density in the depth (g cm^{-3})

t = thickness of the layer considered (cm)

The seedlings were produced by indirect sowing into 128-cell polystyrene trays, filled with commercial substrate Tropstrato[®]. Seeds were removed from ripe fruits randomly harvested from a natural population of a native area of Cerrado in Santa Madalena farm (22°08'23.24"S and 55°08'16.84"W; 487 m altitude) in Dourados, Mato Grosso do Sul, Brazil. The access to plant material in this study, the authors followed all Brazilian legal frameworks and can be accessed in the National System of Genetic Resource Management and Associated Traditional Knowledge (SISGEN nº A9CDAAE). A voucher specimen

was deposited in the Herbarium of the Federal University of Grande Dourados (DDMS), with number 4653. Two-year old seedlings with 37 cm average height were transplanted 15 days after the first cut and 195 DAS of green manures. Three rows of plants 1.20 m apart with 0.50 m between plants (VIEIRA et al., 2011) and plots 2.0 m long and 3.6 m wide were used for the planting.

During the cultivation cycle of guavira, starting at 30 days after transplanting (DAT), with intervals of 30 days, and going up to 630 DAT, height and stem diameter of the main stem were measured at ground level in all trees of the plot. Gas exchange and the chlorophyll index of the plants were measured from 90 DAT, every 90 days, up to 630 DAT, between 08:00 a.m. and 11:00 a.m. Four plants were evaluated per plot, using a physiologically mature non-shaded leaf from each plant.

CO₂ assimilation rate – photosynthesis (*A*), stomatal conductance (*gs*), intercellular CO₂ concentration (*C_i*), transpiration (*E*), water-use efficiency (*WUE*), instant carboxylation efficiency (*CE_i*), and intrinsic water-use efficiency (*WUE_i*) were analyzed with an Infra Red Gas Analyzer - IRGA (LCIPro - SD ADC BioScientific Ltda), 300 mL min⁻¹ air flow and light source of 995 μmol m⁻² s⁻¹. The chlorophyll index was calculated using a Falker portable SPAD chlorophyll meter.

The stems and branches of the plants were counted and two *C. adamantium* plants were collected per plot by cutting close to the ground, at 690 DAT. Leaves and stems were separated and weighed to obtain fresh mass. Leaf area was measured using the LI-COR 3100 C area meter, placed in a forced air circulation oven at 60 ± 5 °C to constant mass. Leaf nutrient contents were determined (MALAVOLTA et al., 1997).

Means of fresh and dry mass, dry mass-to-fresh mass percentages, accumulated macronutrient levels in green manures, and chemical and microbiological attributes of the soil were subjected to analysis of variance as split plots (green manure in the plot and cuts in the subplot) and if significant by the F test, means were compared by the Tukey's test, all up to 5% probability.

The means of the remaining dry mass, nutrient contents released by the remaining mass of green manures, decomposition constant, half-life, and surface temperature of the soil were subjected to analysis of variance as split-split-plots (green manures in the plot, evaluation time in the subplot, and cuts in the sub-subplot) and if significant by the F test, they were analyzed by regression analysis, all up to 5% probability.

Means of the physiological parameters, plant height, and stem diameter of *C. adamantium* were subjected to analysis of variance as subdivided plots (green manures in the main plot and evaluation times in the subplot) and if significant by the F test, they were analyzed by regression analysis or compared using the Tukey's test, all up to 5% probability.

The means of branch number, fresh and dry mass of leaves and stems, leaf area and macro and micronutrient contents of the leaves of *C. adamantium* were subjected to analysis of variance and if significant by the F test, they were compared by the Tukey's test, all up to 5% probability.

Principal component analysis was used to understand the associations between the plants used as green manure and the plants of *C. adamantium*. The selection of number

of main components was based on the analysis of the quality of approximation to the correlation matrix, showing only the components associated with eigenvalues greater than 1 (SNEATH; SOKAL, 1973).

Results

The spontaneous vegetation produced significantly more fresh mass at the first cut than *S. macrocephala* and *C. cajan*. The largest production of fresh and dry masses was recorded for the cover crops *C. mucunoides* and *P. phaseoloides* and the spontaneous vegetation, all at the second cut. In general, the largest production of fresh and dry

masses of green manures occurred at the second cut, after regrowth, when they were already growing with the guavira plants. The lowest fresh and dry masses were recorded for *S. macrocephala* and *C. cajan*, in both cuts (Table 2).

The shoot nutrient contents in the green manures *C. mucunoides*, *S. macrocephala*, *P. phaseoloides*, and *C. cajan* showed higher levels of nitrogen at the first cut, while *P. phaseoloides* had higher levels at the second cut. The highest phosphorus content was found in *C. mucunoides*, in both cuts. *C. cajan* had lower phosphorus and potassium at the first cut (Table 3).

Table 2. Fresh and dry mass of green manures at two cuts.

Green manure	Fresh mass		Dry mass	
	1 st Cut	2 nd Cut	1 st Cut	2 nd Cut
	Mg ha ⁻¹			
<i>S. macrocephala</i>	8.57 bB	13.73 bA	1.85 aB	3.32 bA
<i>C. mucunoides</i>	11.31 abB	27.28 aA	2.28 aB	6.27 aA
<i>P. phaseoloides</i>	10.90 abB	22.81 aA	1.97 aB	4.94 abA
<i>C. cajan</i>	5.46 bB	12.52 bA	1.85 aB	3.83 bA
Spont. vegetation	16.01 aB	22.56 aA	3.58 aB	6.30 aA
C. V. (%)	20.40	20.40	23.02	23.02

*Means followed by the same small letter in the columns and capital letters in the rows between the cut times are not significantly different by the Tukey's test ($p < 0.05$) and F ($p < 0.05$). C.V. - coefficient of variation.

Table 3. Macronutrient content of green manures at two cuts.

Green manure	Nitrogen		Phosphorus		Potassium	
	1 st Cut	2 nd Cut	1 st Cut	2 nd Cut	1 st Cut	2 nd Cut
	g kg ⁻¹					
<i>S. macrocephala</i>	23.80 aB	24.76 bcA	3.27 abA	3.79 abA	2.03 aA	1.28 aA
<i>C. mucunoides</i>	24.50 aB	26.25 abA	4.03 aA	4.37 aA	2.14 aA	1.40 aA
<i>P. phaseoloides</i>	22.75 aB	30.10 aA	3.13 abA	3.96 abA	2.14 aA	1.49 aA
<i>C. cajan</i>	21.52 aB	26.95 abA	1.78 cB	3.33 bA	1.37 bA	1.15 aA
Spont. vegetation	16.80 bB	21.26 cA	2.76 bA	3.23 bA	2.40 aA	1.11 aA
C. V. (%)	7.34	10.61	12.58	13.66	15.94	13.53

*Means followed by the same small letter in the columns in each cut and capital letters between the cuts are not significantly different by the Tukey's test ($p < 0.05$) and F test ($p < 0.05$). C.V. - coefficient of variation.

The green manures showed different decomposition dynamics according to the plant species and followed exponential behavior with rapid decomposition up to 30 days, irrespective of the cuts (Figure 2). *C. mucunoides* showed a fast decomposition in less time, while *C. cajan* showed a slow decomposition, remaining on the soil surface for a longer time, irrespective of the cuts (Figures 2A and 2B).

The release of nitrogen from the decomposing mass was faster at the first cut for *C. mucunoides* and *P. phaseoloides* (Figure 3A) and at the second cut for *C. mucunoides* (Figure 3B). The spontaneous vegetation released the least nitrogen, irrespective of the cuts (Figures 3A and 3B). The mass of *C. mucunoides* rapidly released phosphorus at the second cut (Figure 3D). Potassium release was rapid, irrespective of the factors in study (Figures 3E and 3F).

The chemical attributes of the soil evaluated after the green manure cuts varied in nutrient availability, among them, *S. macrocephala*, *P. phaseoloides*, and *C. mucunoides* contributed the most to increase soil fertility,

mainly after the second cut, and decrease aluminum content. However, soil fertility was lower under the weeding treatment. The chemical attributes of the soil did not vary between cutting times (Table 4).

The potassium content was higher in the soil after the first cut of *P. phaseoloides* and after the second cut of *C. cajan* and spontaneous vegetation (Table 4). Cultivation of *C. mucunoides* and *S. macrocephala* resulted in a higher content of calcium and magnesium, irrespective of the cuts. The highest content of organic matter was recorded in the soil cultivated with *C. mucunoides*, *S. macrocephala*, and *P. phaseoloides* after both two cuts.

Phosphorus was higher in the soil cultivated with *C. mucunoides* and *P. phaseoloides* after the second cut. After the second cut of *S. macrocephala*, there was a variation in the levels of calcium (24.40 mmolc dm⁻³), phosphorus (8.93 mg dm⁻³), and organic matter (24.11 g dm⁻³) in relation to the initial analysis. After the second cut of *C. mucunoides*, there was an increase of 75.37% in the content of organic matter in relation to the initial analysis (Table 4).

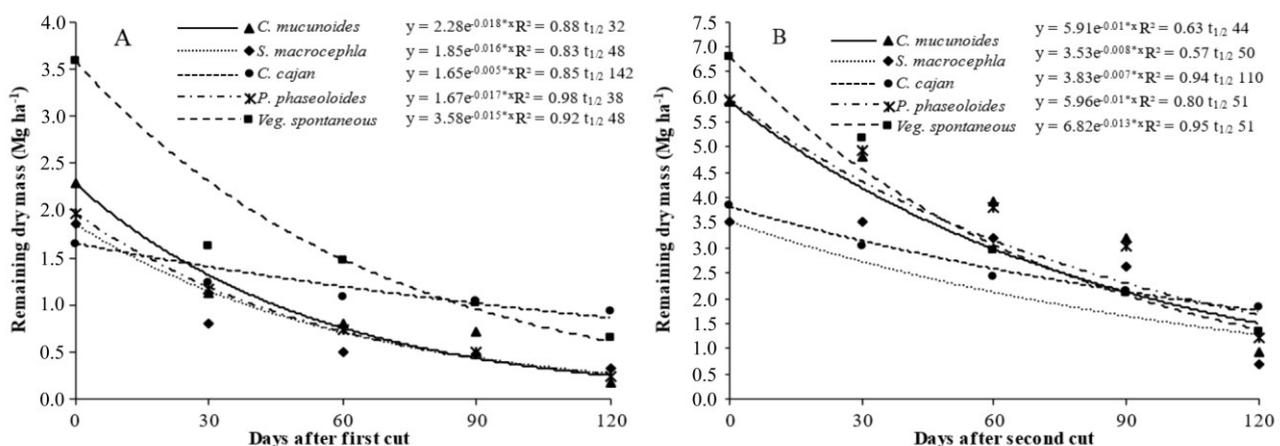
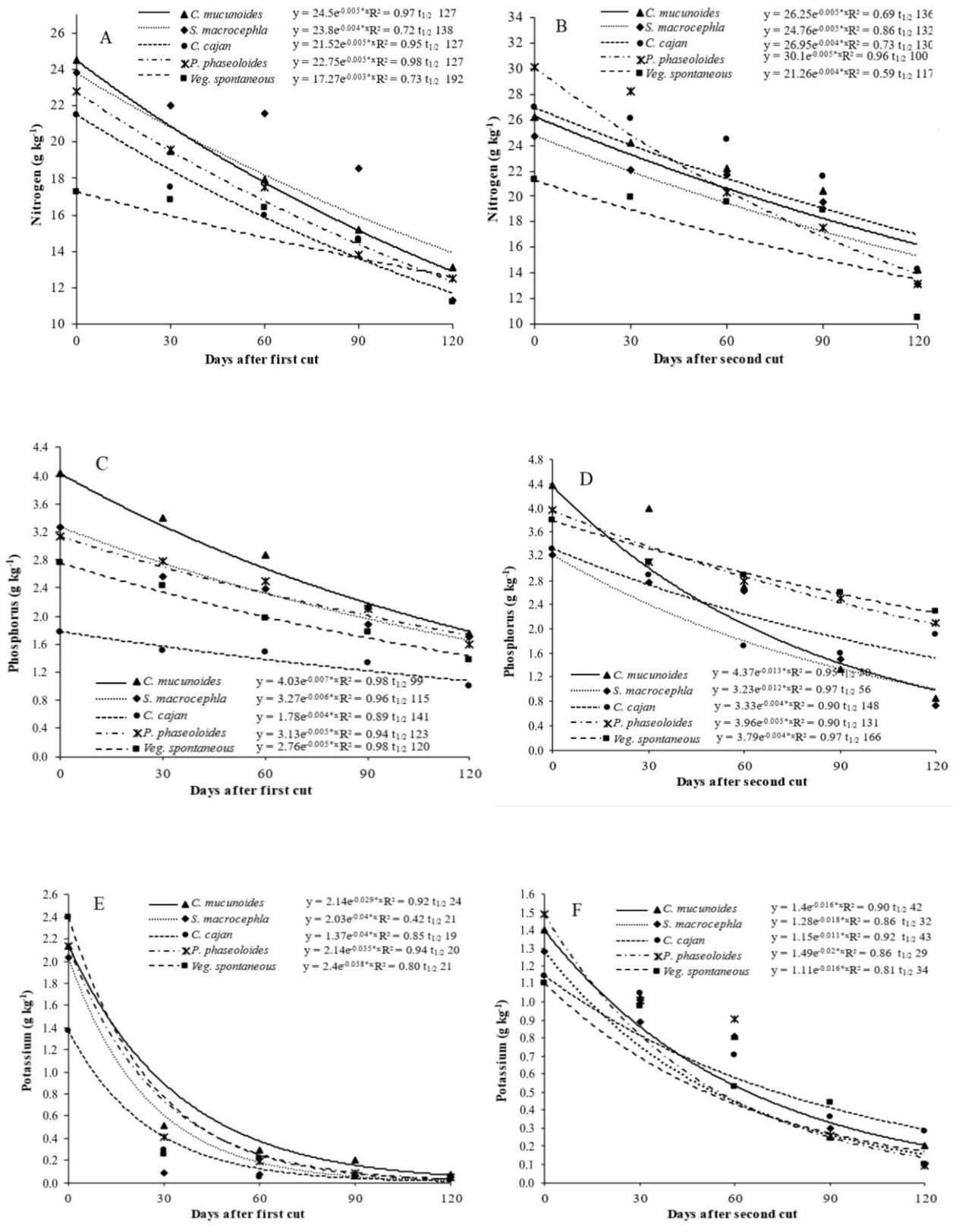


Figure 2. Remaining dry mass of green manures as a function of time, at the first (A) and second (B) cuts.



Figures. 3. Release of nitrogen (A and B), phosphorus (C and D), and potassium (E and F) from the remaining biomass of green manures as a function of time at first and second cuts.

Table 4. Chemical attributes of the soil after the first and second cuts of green manures

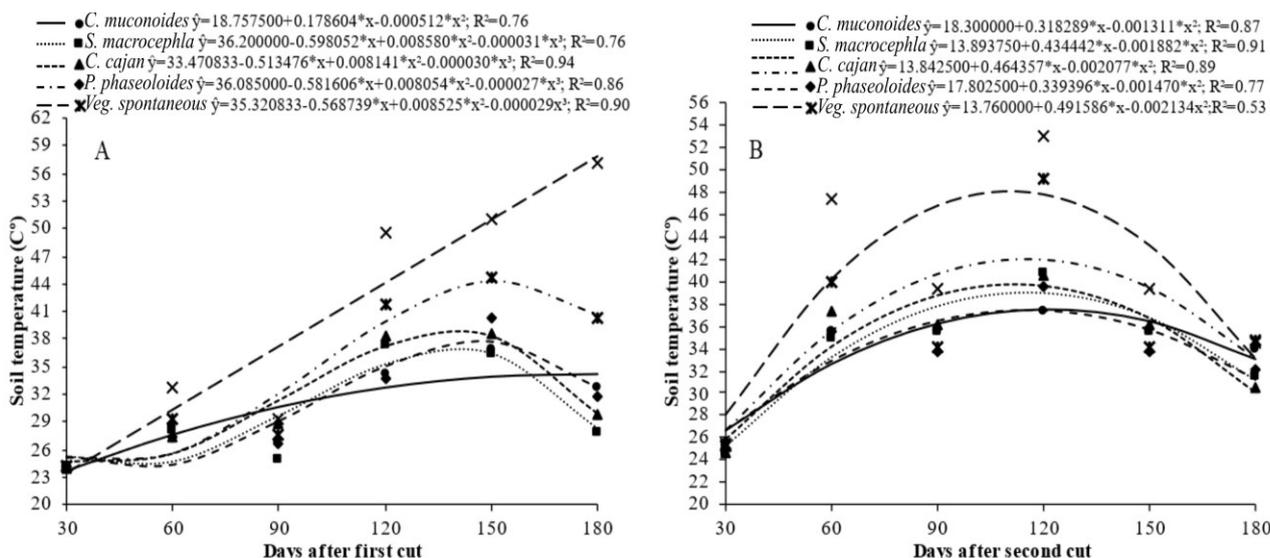
Attribute	First cut						C.V. (%)
	<i>S. macrocephala</i>	<i>C. mucunoides</i>	<i>P. phaseoloides</i>	<i>C. cajan</i>	Spontaneous vegetation	Bare soil	
P ¹	6.33 a	5.39 a	6.58 a	5.58 a	5.77 a	5.40 a	12.89
K ²	3.10 ab	2.80 b	4.50 a	3.60 ab	3.30 ab	2.20 b	20.01
Ca ²	17.80 ab	18.80 a	14.80 abc	8.00 c	13.90 abc	17.0 bc	24.71
Mg ²	12.20 a	12.50 a	9.00 ab	5.30 b	8.30 ab	6.10 b	24.25
Cu ²	12.32 a	12.64 a	11.23 a	12.03 a	11.72 a	11.40 a	6.17
Mn ²	69.62 a	67.95 a	59.31 a	51.23 a	61.52 a	57.08 a	22.35
Fe ²	95.86 a	118.28 a	105.83 a	107.31 a	102.10 a	102.47 a	10.89
Zn ²	1.42 a	1.25 a	1.18 a	1.05 a	1.36 a	1.14 a	20.45
OM ³	17.63 a	18.04 a	18.52 a	16.20 ab	16.38 ab	11.38 b	15.41
pH (H ₂ O)	4.07 a	4.05 a	3.97 a	3.94 a	4.00 a	4.00 a	3.50
Al ²	21.30 b	23.10 b	32.40 a	30.90 a	30.03 a	33.50 a	8.29
H+Al ²	123.30 a	143.70 a	144.50 a	149.70 a	141.20 a	137.60 a	19.09
SB ²	26.90 ab	28.60 ab	25.90 ab	21.80 b	35.80 a	17.50 b	22.10
CEC ²	147.50 a	161.70 a	168.30 a	171.60 a	177.20 a	156.10 a	18.31
V%	16.42 ab	18.03 ab	14.29 ab	13.01 ab	20.00 a	11.80 b	22.64

Attribute	Secondcut						C.V. (%)
	<i>S. macrocephala</i>	<i>C. mucunoides</i>	<i>P. phaseoloides</i>	<i>C. cajan</i>	Spontaneous vegetation	Bare soil	
P ¹	7.67 ab	8.93 a	9.24 a	7.67 ab	6.92 bc	5.25 c	19.89
K ²	2.90 ab	2.60 ab	2.20 ab	3.60 a	3.50 a	1.30 b	26.32
Ca ²	24.40 a	18.30 ab	17.80 ab	12.10 b	16.80 ab	10.00 b	24.88
Mg ²	8.30 a	7.50 a	6.40 ab	4.20 ab	6.20 ab	1.10 b	41.18
Cu ¹	14.00 a	12.84 a	12.65 a	12.54 a	13.37 a	13.52 a	6.61
Mn ¹	63.00 a	51.46 a	43.83 a	44.24 a	50.65 a	46.36 a	22.81
Fe ¹	114.13 a	94.68 a	94.28 a	113.13 a	110.99 a	103.59 a	12.89
Zn ¹	1.26 a	1.38 a	1.19 a	1.03 a	1.05 a	1.12 a	22.90
OM ³	24.11 a	23.72 a	24.04 a	22.22 ab	22.14 ab	18.66 b	8.40
pH (H ₂ O)	4.08 a	4.04 a	3.99 a	3.93 a	4.08 a	4.11 a	2.48
Al ²	15.90 b	17.40 b	21.00 ab	21.90 ab	20.10 b	28.70 a	17.06
H+Al ²	118.10 a	125.10 a	125.70 a	126.30 a	123.00 a	114.70 a	17.94
SB ²	33.90 a	28.40 ab	27.20 ab	19.90 bc	26.50 ab	13.00 c	21.44
CEC ²	148.60 a	153.60 a	150.30 a	146.30 a	149.60 a	115.30 a	12.03
V%	24.71 a	18.75 ab	18.94 ab	13.66 b	17.95 ab	12.17 b	25.54

1) mg dm⁻³ 2) mmolc dm⁻³ 3) g dm⁻³ ** Means followed by the same small letters on the row are not significantly different by the Tukey's test (p < 0.05). Phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), manganese (Mn), iron (Fe), zinc (Zn), organic matter (OM), hydrogen potential (pH), aluminum (Al), potential acidity (H + Al), sum of bases (SB), cation exchange capacity (CEC) and bases per saturation (V%). C.V. - coefficient of variation.

The microbial biomass of the soil was in equilibrium and in early stages of development due to the low biomass production and nutrient release after the first cut of green manures (Figure 4). However, at the second cut, an influence on the levels of calcium, phosphorus and organic matter was detected, which contributed to the perfor-

mance of microorganisms in the biochemical process of decomposition of organic residue. Basal respiration (C-CO₂) of microorganisms was 24.10% more efficient at the second cut of *C. mucunoides* than of the spontaneous vegetation (Table 5). The microbiological attributes of the soil showed no variation with the cut times.



Figures 4. Temperature of the soil cultivated with green manures, over time, after the first cut (A) and the second cut (B).

Table 5. Microbial biomass carbon (MBC), basal respiration (C-CO₂), metabolic quotient (*q*CO₂), and microbial quotient (*q*MIC) of the soil under green manures and bare soil after the first and second cuts.

Green manure	MBC (µg C g ⁻¹)		C-CO ₂ (µg g ⁻¹ day ⁻¹ of C-CO ₂)		<i>q</i> CO ₂ (µg CO ₂ /µg Cmic h ⁻¹)		<i>q</i> MIC (%)	
	1st Cut	2nd Cut	1st Cut	2nd Cut	1st Cut	2nd Cut	1st Cut	2nd Cut
<i>C. mucunoides</i>	210.92 a	454.74 a	51.89 a	12.78 a	118.54 a	12.44 ab	2.04 a	3.37 a
<i>S. macrocephala</i>	259.86 a	548.85 a	53.27 a	8.99 ab	88.76 a	7.00 ab	2.61 a	3.93 a
<i>P. phaseoloides</i>	181.36 a	429.30 a	51.38 a	7.12 ab	137.87 a	8.22 ab	1.71 a	3.13 a
<i>C. cajan</i>	256.12 a	503.03 a	42.58 a	7.50 ab	85.94 a	6.10 ab	2.68 a	3.90 a
Spont. vegetation	225.80 a	465.56 a	48.44 a	11.97 a	95.28 a	11.54 ab	2.45 a	3.64 a
Bare soil	126.96 a	432.47 a	42.42 a	3.08 b	183.17 a	13.40 ab	1.88 a	3.09 a
C.V. (%)	41.49	21.98	19.22	33.58	59.90	54.28	40.79	23.30

* Means followed by the same small letters in the column are not significantly different by Tukey's test (p < 0.05). C.V. - coefficient of variation.

The soil temperature was highest in the bare soil, regardless of the time of cuts of green manure (Figure 4), with an average increase of 10 °C at 120 DAT in relation to the soil with *C. mucunoides* after the second cut (Figure 4B). The second cut of green manure contributed to the reduction of soil temperature after 120 days (Figure 4B).

The physiological parameters of *C. adamantium* were influenced separately by the green manures and evaluation times. The photosynthetic rate (*A*) and stomatal conductance

(*g_s*) were higher in *C. adamantium* grown with green manures than with spontaneous vegetation and bare soil. The intercellular CO₂ concentration (*C_i*) was not influenced by the growing conditions and the highest transpiration rate (*E*) was recorded for *C. cajan*. The water-use efficiency (*WUE*), and the intrinsic water-use efficiency (*iWUE*) were higher with *S. macrocephala* than bare soil. The instant carboxylation efficiency (*CE_i*) was higher in *C. adamantium* grown with *C. mucunoides* and *C. cajan* (Table 6).

Table 6. Physiological parameters of *C. adamantium* grown with green manure and in bare soil.

Parameter	A ($\mu\text{mol CO}_2$ $\text{m}^{-2} \text{s}^{-1}$)	Gs ($\text{mmol H}_2\text{O}$ $\text{m}^{-2} \text{s}^{-1}$)	C _i ($\mu\text{mol CO}_2$ $\text{m}^{-2} \text{s}^{-1}$)	E ($\text{mmol H}_2\text{O}$ $\text{m}^{-2} \text{s}^{-1}$)	WUE ($\mu\text{mol CO}_2$ $\text{mmol}^{-1} \text{H}_2\text{O}$ $\text{m}^{-2} \text{s}^{-1}$)	CE _i ($\mu\text{mol CO}_2$ $\text{m}^{-2} \text{s}^{-1}$)	iWUE ($\mu\text{mol CO}_2$ $\text{mmol}^{-1} \text{H}_2\text{O}$ $\text{m}^{-2} \text{s}^{-1}$)
<i>S. macrocephala</i>	11.22 a	0.20 a	274.41 a	3.92 bc	3.33 a	0.040 ab	70.19 a
<i>C. mucunoides</i>	11.39 a	0.21 a	272.92 a	4.53 ab	2.63 ab	0.043 a	60.27 ab
<i>P. phaseoloides</i>	10.06 a	0.20 a	284.31 a	4.69 ab	2.66 ab	0.037 ab	60.24 ab
<i>C. cajan</i>	11.22 a	0.20 a	268.40 a	5.26 a	2.74 ab	0.046 a	61.61 ab
Spont. vegetation	7.22 b	0.16 b	277.41 a	3.29 c	2.50 ab	0.028 bc	61.17 ab
Bare soil	6.40 b	0.14 b	290.62 a	3.96 bc	1.83 b	0.024 c	54.34 b
C. V. (%)	26.61	22.17	12.97	25.88	41.57	38.69	28.34

*Means followed by same letters in the columns are not significantly different by the Tukey's test ($p < 0.05$). Intercellular CO_2 concentration (C_i), transpiration rate (E), stomatal conductance (g_s), photosynthetic rate (A), instant carboxylation efficiency (CE_i), water-use efficiency (WUE), and intrinsic water-use efficiency ($iWUE$). Data as function of times were grouped. C.V. - coefficient of variation.

The maximum photosynthetic rate (A) ($15.13 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was recorded at 483 DAT ($\hat{y} = 5.798810 + 0.019322 * x + 0.000020 * x^2$; $R^2 = 0.73$) and minimum intercellular CO_2 concentration (C_i) ($144.54 \mu\text{mol CO}_2 \text{ mol}^{-1}$) at 392 DAT ($\hat{y} = 364.189524 - 0.560596 * x + 0.000714 * x^2$; $R^2 = 0.68$). The minimum transpiration rate (E) of *C. adamantium* ($5.79 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) was recorded at 82 DAT ($\hat{y} = 4.519762 + 0.015520 * x - 0.000093 * x^2 + 0.0000001 * x^3$; $R^2 = 0.74$) and the minimum stomatal conductance (G_s) ($0.22 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) at 55 DAT ($\hat{y} = 0.1993452 + 0.000607 * x - 0.000004 * x^2 + 0.000001 * x^3$; $R^2 = 0.64$). The maximum instant carboxylation efficiency (CE_i) ($0.05 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was determined at 450 DAT ($\hat{y} = 0.009881 + 0.000155 * x - 0.000001 * x^2$; $R^2 = 0.74$). *C. adamantium* had the minimum chlorophyll index a (13.25) at 430 DAT ($\hat{y} = 37.306548 - 0.055957 * x + 0.000065 * x^2$; $R^2 = 0.61$) and minimum total chlorophyll index (19.25) at 418 DAT ($\hat{y} = 49.360714 - 0.071985 * x + 0.000086 * x^2$; $R^2 = 0.68$).

The leaves of *C. adamantium* grown with *C. mucunoides* had a higher nitrogen content than the bare soil. The highest phosphorus content was found in plants grown with *S.*

macrocephala, *C. cajan*, and spontaneous vegetation. The cultivation of green manures provided a higher potassium content in *C. adamantium* than in bare soil (Table 7).

Times of evaluation and green manures influenced stem height and stem diameter of *C. adamantium*. The growth was linear, ascending to average height of 86 cm at 630 DAT ($\hat{y} = 31.874405 + 0.078194 * x$; $R^2 = 0.92$), following the model of apical dominance, which is a characteristic of this native species. The plants reached an average stem diameter of 17.58 mm at 630 DAT ($\hat{y} = 2.500595 + 0.019136 * x$; $R^2 = 0.88$).

Height and diameter of *C. adamantium* stems were greater when cultivated with *C. mucunoides* and *S. macrocephala*. In this study, branching of *C. adamantium* was greater when cultivated with *P. phaseoloides* than with spontaneous vegetation (Table 8).

Plants of *C. adamantium* had higher fresh and dry leaf masses when grown with *C. mucunoides* than with spontaneous vegetation. The highest fresh and dry mass of stem were determined in plants cultivated with *S. macrocephala*, differing only from spontaneous vegetation and bare soil. Leaf area of *C.*

adamantium was larger in cultivation with *C. mucunoides* than with spontaneous vegetation and bare soil. The lowest fresh and dry

masses of leaves and stems were determined in plants cultivated with spontaneous vegetation (Table 9).

Table 7. Macro and micronutrient contents in *C. adamantium* leaves.

Green manure	N	P	K	Ca	Mg	Cu	Mn	Fe	Zn
	g kg ⁻¹					mg kg ⁻¹			
<i>S. macrocephala</i>	18.55 ab	1.40 a	0.61 a	4.76 a	2.85 a	9.77 a	173.20 a	217.96 a	18.02 a
<i>C. mucunoides</i>	19.60 a	1.22 ab	0.78 a	5.12 a	2.80 a	10.97 a	238.21 a	158.11 a	22.28 a
<i>P. phaseoloides</i>	18.90 ab	1.27 ab	0.73 a	4.44 a	3.00 a	9.50 a	460.64 a	147.03 a	20.07 a
<i>C. cajan</i>	17.15 ab	1.42 a	0.66 a	4.37 a	2.55 a	11.34 a	445.28 a	144.14 a	21.62 a
Spont.vegetation	17.15 ab	1.42 a	0.73 a	3.86 a	2.51 a	11.70 a	223.64 a	160.15 a	19.83 a
Bare soil	11.90 b	0.89 b	0.36 b	3.77 a	2.39 a	14.65 a	312.86 a	179.80 a	14.74 a
C. V. (%)	18.41	13.02	18.90	21.36	24.11	44.08	45.94	34.38	23.31

*Means followed by same letters in the columns are not significantly different by the Tukey's test ($p < 0.05$). C.V. - coefficient of variation.

Table 8. Height, stem diameter, and number of branches of *C. adamantium* plants as a function of green manure species.

Green manure	Height (cm)	Stem diameter (mm)	Number of branches
<i>S. macrocephala</i>	67.14 a	10.47 a	8.16 ab
<i>C. mucunoides</i>	60.59 b	10.33 a	8.30 ab
<i>P. phaseoloides</i>	62.72 b	9.38 ab	9.72 a
<i>C. cajan</i>	56.77 c	8.63 bc	8.93 ab
Spont. vegetation	47.34 e	6.92 d	5.39 b
Bare soil	51.48 d	7.14 cd	8.86 ab
C. V. (%)	14.01	40.30	20.27

*Means followed by same letters in the columns are not significantly different by the Tukey's test ($p < 0.05$). C.V. - coefficient of variation.

Table 9. Leaf fresh mass (LFM), leaf dry mass (LDM), stem fresh mass (SFM), stem dry mass (SDM), and leaf area (LA) of *C. adamantium* plants grown in succession to green manure and bare soil.

Green manure	LFM	LDM	SFM	SDM	LA
	g plant ⁻¹				cm ² plant ⁻¹
<i>S. macrocephala</i>	360.51 ab	150.28 ab	273.26 a	146.41 a	11312.45 ab
<i>C. mucunoides</i>	373.36 a	158.28 a	243.70 ab	124.77 ab	12055.11 a
<i>P. phaseoloides</i>	273.15 ab	123.92 ab	188.41 abc	98.44 abc	9849.67 abc
<i>C. cajan</i>	180.15 ab	78.75 ab	130.70 abc	68.89 bc	6294.75 abc
Spont. vegetation	103.93 b	42.83 b	72.52 c	37.54 c	3837.49 c
Bare soil	150.81 ab	72.65 ab	104.66 bc	55.68 bc	4554.57 bc
C. V. (%)	38.29	37.25	33.26	29.74	38.89

*Means followed by same small letters in the columns are not significantly different by the Tukey's test ($p < 0.05$). C.V. - coefficient of variation.

The principal component analysis (PCA) examined the characteristics studied of the green manure effect on the soil and *C. adamantium* plants. Most of the variation in green manure data was explained in 63.62% at the first principal component (PC1) and

22.62% at the second component (PC2), totaling 86.24% of the total data variability. In PC1, the characteristics with highest factor loadings in descending order were N green manures, P green manures, OM, Al, *Gs*, *A*, *WUE*, Diameter, SFM, and SDM, while in PC2,

the characteristics in descending order were potassium in soil (K Soil), *C. adamantium* branches, phosphorus in *C. adamantium* (PG),

dry mass of green manures at second cut, C-CO₂, LFM, qMIC, height, LDM, SB, and leaf area of the *C. adamantium* plants (Figure 5).

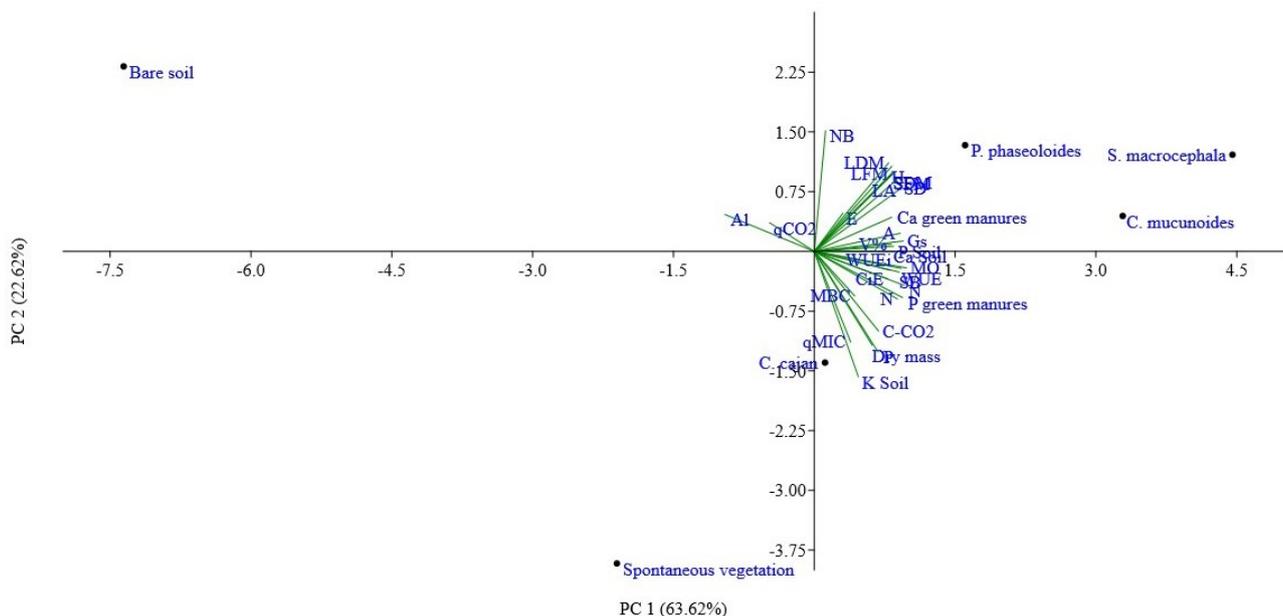


Figure 5. Principal components (PC) of variables related to the influence of green manures on chemical attributes of soil, nutrients accumulated in plant, and physiological parameters of *C. adamantium*.

2nd DM = dry mass of green manures at second cut; N = concentration of nitrogen and phosphorus in green manure mass; OM = organic matter, K soil = potassium content in soil; SB = sum of bases; Al = aluminum; qCO₂ = metabolic quotient; qMIC = soil microbial quotient; Gs = stomatal conductance; A = photosynthetic rate; Height, Diameter, and Branches = *C. adamantium* plants; WUE = instantaneous water use efficiency; LFM and LDM = fresh and dry mass of *C. adamantium* leaves; SFM and SDM = fresh and dry mass of *C. adamantium* stems; LA = leaf area of *C. adamantium*; N *C. adamantium* and P *C. adamantium* = concentration of nitrogen and phosphorus in *C. adamantium* leaves.

The characteristics evaluated under the effect of green manures were separated into four response groups: Group 1 comprised *C. mucunoides*, *S. macrocephala*, and *P. phaseoloides* and is explained by P green manures, Gs, A, WUE, Diameter, SFM, SDM, Branches, P *C. adamantium*, 2nd DM, LFM, Height, LDM, LA; Group 2 included *C. cajan* and is explained by the variables N *C. adamantium*, OM, K soil, C-CO₂, qMIC, and SB; Group 3 consisted of the bare soil and is explained by Al; and Group 4 consisted of the spontaneous vegetation, without characteristics (Fig. 5).

Discussion

The well-developed roots of green manures (LIMA FILHO et al., 2014), increase in organic

matter, and increase in soil cover (FERNANDES, 2006; CALHEIROS et al., 2013) contributed to the higher productivity at the second cut (Table 2). Furthermore, the vigorous, fast growing cover crops *C. mucunoides* and *P. phaseoloides* (ARAÚJO et al., 2017) were adapted to the high temperatures and soil characteristics of the Cerrado. These results show green manures as an alternative for soil covering in early stages of agroecological systems, providing favorable environmental conditions for guavira plants.

The production of spontaneous vegetation fresh mass at the first cut was in consequence of the fallow period that favored the germination of plants in the seed bank and occupied the entire net plot. was owing to

the fallow area that favored the development of the seed bank and occupying the entire useful area.

The nitrogen content of green manures is related to the N_2 biological fixation capacity of the *Rhizobium* native strains (CAVALCANTE et al., 2012; CLERMONT-DAUPHIN et al., 2016) in the roots of Fabaceae. Furthermore, it has a direct relationship with dry mass production (Table 2); thus, *P. phaseoloides* provided $30.10 \text{ } 148.69 \text{ g kg}^{-1} \text{ N}$ at the second cut (Table 3). The *C. mucunoides* showed potential for phosphorus cycling by exploration of the soil (LIMA FILHO et al., 2014). The production of morphological components of green manures at the second cut indicates a greater use of potassium (Table 3).

C. mucunoides and *P. phaseoloides* released higher nitrogen (Figures 3A and 3B) during the decomposition of plant material, indicating that mineralization was greater than immobilization (SOLATI et al., 2017). The rapid release of phosphorus by the biomass of *C. mucunoides* (Figures. 3C and 3D) occurred because of the use of soluble compounds by soil microorganisms (GUIMARÃES et al., 2017). Potassium is not associated with any structural component of plant tissue and has high mobility in the plant (TAIZ et al., 2017), which is why it was quickly released from the biomass of green fertilizers (Figures 3E and 3F) and made it available in the soil. At the second cut, green manures increased soil cover, moisture retention, and organic matter content, which favored soil microbial activity accelerating the mineralization of biomass nutrients (SOLATI et al., 2017).

The low K values both in green manures and in *C. adamantium* plants had low values compared to other species can be explained by the fact that the cutting of the leaves of

the green manures was carried out in full bloom, that is, considering that the K presents high mobility in the plant, this was directed to the draining organs in formation. In addition, we emphasize that *C. adamantium*, being a native species, with no record of ex situ cultivation, may be a species that presents good efficiency in the use of the nutrient under these cultivation conditions.

Adsorption of calcium and magnesium by soil colloids and decomposition of organic matter (ZHAO et al., 2015; ZHONG et al., 2018; CHEN et al., 2019) may be due to the improved activity of microorganisms and protozoa in the solo (RAIJ, 2017; SILVA et al., 2017). The levels of nutrients in the soil (Table 4) are within the range suitable for the development of Cerrado plants such as: P ($1.4 - 1.9 \text{ g kg}^{-1}$), K ($13 - 20 \text{ g kg}^{-1}$), Ca ($7 - 15 \text{ g kg}^{-1}$), Mg ($2.4 - 4.0 \text{ g kg}^{-1}$), Cu ($10 - 40 \text{ mg dm}^{-3}$), Mn ($40 - 250 \text{ mg dm}^{-3}$), and Zn ($25 - 35 \text{ mg dm}^{-3}$) (SOUSA; LOBATO, 2002).

The increased plant material from the green manures on the soil surface was used as a source of energy and nutrients, providing favorable conditions for microorganisms (GUIMARÃES et al., 2017). The carbon of the microbial biomass was 46.38% more efficient after the second cut of *C. mucunoides* than after the first cut (Table 5), indicating that the nutrients were temporarily immobilized, resulting in less losses in the soil-plant system (MAZZETTO et al., 2016). After removal of the spontaneous vegetation, the soil showed microbial activity close to values of the soils covered with green manures. This result is due to the minimal disturbance of the surface layer of the soil exposed during the clearing of vegetation, which may have stimulated the activity of soil microorganisms (ARAÚJO et al., 2019).

The efficiency of microbial basal respiration (C-CO₂) with *C. mucunoides* (Table 5) indicates greater biological activity and organic matter content, consequently, the rapid release of nutrients (MAZZETTO et al., 2016; GUIMARÃES et al., 2017). These results demonstrate that green manures influence soil quality, even in the short term (ARAÚJO et al., 2019). The $qMIC$ after the second cut (Table 5) indicates the good quality of the organic matter, ranging from 1 to 4% (JAKELAITIS et al., 2008). The bare soil remained without anthropic activity, which may have favored immobilization in soil organic matter (MAZZETTO et al., 2016; GUIMARÃES et al., 2017).

The second cut of green manures provided greater soil covering, which hindered solar radiation from directly reaching the soil and kept an average temperature of 33.81 °C (Figure 4B). This condition is favorable to the microbial efficiency (Table 5), and thereby there was a greater speed of decomposition and release of nutrients (Figure 3) (LIMA FILHO et al., 2014). However, the bare soil had high temperatures (Figure 4), and probably the amount of global energy available in the atmosphere was transferred to the deep layers (LIMA FILHO et al., 2014) and impaired the soil microbial quality. In general, green manures contributed to soil cover and nutrient supply, which are important findings for the development of agroecological farming systems, with low chemical inputs.

Green manures were grown before and during the growth cycle of *C. adamantium*, which was the main crop in this study. Then, it was verified that green manures influenced *C. adamantium* physiological parameters, with increase in transpiration rate (E) when

grown with *C. cajan* (Table 6). This fact favored CO₂ entry, maximizing the photosynthetic rate and photoassimilate production (TAIZ et al., 2017). The nutrients released by the decomposition of *S. macrocephala* biomass (Figure 3) may have contributed to the transport and absorption of ions and to the speed of enzymatic reactions in the Krebs cycle, resulting in higher water-use efficiency (WUE) and the intrinsic water-use efficiency ($iWUE$) of *C. adamantium* (Table 6).

The higher instant carboxylation efficiency (CE) of *C. adamantium* (Table 6) results from the elevated CO₂ concentration that causes increased photosynthetic rates. Besides, *C. adamantium* had the ability to regulate gas exchange by decreasing stomatal conductance and transpiration more than CO₂ assimilation, which conserves water for each molecule of CO₂ assimilated (TAIZ et al., 2017). Furthermore, the production of metabolic energy and synthesis of nucleic acids increase (TAIZ et al., 2017), therefore, the growth of *C. adamantium* shoots favored leaf exposure to solar radiation and production of photoassimilates (LEÃO-ARAÚJO et al., 2019).

C. adamantium plants had the ability to continue photosynthetic metabolism even at reduced transpiration rate (E), stomatal conductance (G_s), and chlorophyll indices. They also had high carboxylation capacity even at low CO₂ availability because of the high instant carboxylation efficiency (CE), high photosynthetic rates (A), and low internal CO₂ concentration (C_i). The reduction in chlorophyll indices at 430 DAT may be related to post-transplant stress in the field, although it has not impaired photosynthetic rates (TAIZ et al., 2017), which shows the

adaptive capacity of the plants enabling resilience to the growing environment. Furthermore, *C. adamantium* is a deciduous plant native to the Cerrado that shed leaves during dry season (FERNANDES et al., 2016), between June and July (Figure 1).

The nitrogen levels found in *C. adamantium* are close to those reported by Vieira et al. (2011). These authors compared chemical fertilizers and green manures as nitrogen source and concluded that green manures are a sustainable alternative that can replace chemical fertilization. *C. adamantium* efficiently used the phosphorus present in low concentrations in the soil solution (Table 4) because of the adsorption characteristics to the iron and aluminum oxides in the clay fraction (RAIJ, 2017). Moreover, potassium contributed to cellular osmotic regulation, activation of enzymes in the photosynthesis, and respiration of plants (TAIZ et al., 2017).

C. adamantium plants growth was greater when cultivated with *S. macrocephala* and *C. mucunoides* (Table 8), because of the adaptation of these green manures to Cerrado soils with rapid nutrient release (CALHEIROS et al., 2013; BISI et al., 2019). Then, *S. macrocephala* plants become an alternative for improving soil structure and decompression, owing to the vigorous growth and deep root system, up to 1.5 m depth (LIMA FILHO et al., 2014). In turn, they are more efficient as green manure, as they bring to the surface layers of the soil some nutrients that would be lost by leaching and make them available to *C. adamantium* plants (ARAÚJO et al., 2017).

The biomass of *S. macrocephala* and *C. mucunoides* (Table 2), with nitrogen accumulation, served as a source of energy and nu-

trients for soil microorganisms (Table 5). Consequently, the decomposition of plant material was accelerated (Figure 2), improving the chemical attributes of the soil (Table 4), resulting in greater production of metabolic energy and increased synthesis of nucleic acids, which contributed to shoot growth of *C. adamantium*. Gondim et al. (2020) found that fast-growing Fabaceae such as *Crotalaria breviflora* and *C. cajan* contributed to the mass gain of *C. adamantium*.

The largest fresh and dry masses of leaves and leaf area of *C. adamantium*, part with potential for medicinal use, grown with *C. mucunoides* (Table 9), may result from a greater absorption of nutrients such as phosphorus and potassium accumulated and released into the soil by the green manure (Table 3). The production of fresh and dry masses of stems of *C. adamantium* plants grown with *S. macrocephala* (Table 9) was similar to the tallest stems with the largest diameters (Table 8). This result is owing to the accumulation of mass in the leaves that transport metabolic energy to the stem (TAIZ et al., 2017). In turn, the potential of green manures for maintaining soil cover with moisture retention and soil temperature reduction (Figure 4B) (SILVA et al., 2017; BISI et al., 2019) shows the beneficial effect of *C. mucunoides* and *S. macrocephala* over time for the production of *C. adamantium* in the agroecological system.

The response groups in the principal component analysis including the characteristics of the green manures *C. mucunoides*, *S. macrocephala* and *P. phaseoloides* (Figure 5) resulted from the greater mass production that influenced the chemical and microbiological

attributes of the soil (BISI et al., 2019). Nutrients such as nitrogen, phosphorus, and potassium became available to plants of *C. adamantium* and, consequently, there was a greater growth and leaf yield (EMER et al., 2020). However, *C. adamantium* plants had growth reduced with spontaneous vegetation and bare soil due to the competition between the plants and the low organic matter content (SÁ et al., 2017), limiting nutrient availability to plants.

Conclusions

The green manures had higher production of fresh and dry masses at the second cut and *C. mucunoides*, *S. macrocephala*, and *P. phaseoloides* presented the highest concentrations of nutrients. The *C. mucunoides* mass decomposed rapidly and influenced the chemical attributes of the soil, with a greater role of soil microorganisms in the bi-

ochemical process of decomposition of organic residues.

Plants of *C. adamantium* showed better photosynthetic responses, higher concentrations of leaf nutrients and growth when cultivated with *C. mucunoides* and *S. macrocephala*. Furthermore, they responded positively to the green manure *C. mucunoides* by increasing leaf production, which resulted in a greater production of the plant that is used as medicinal material.

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