Physical Properties of Soil and Glyphosate Residue as a Function of Cassava Weed Management by Cover Crops in the Amazon Ecosystem

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Abstract. Cassava production in Amazonas state deserves to be highlighted due to its great historical, social, and economic importance. Weed competition severely constrains cassava production in Amazonas. The use of cover crops is safe and very efficient at eliminating weeds while keeping the soil covered. The objective of this study was to evaluate physical properties of soil and glyphosate residues in storage roots as a function of the weed management in cassava. The experiment was carried out in a randomized complete block design with five treatments and five repetitions. The treatments were biological control with two species of cover plants (*Brachiaria ruziziensis* and *Mucuna pruriens*), chemical control, mechanical control, and treatment with no weed control. The cover crops characteristics evaluated were dry weight, the percentage of cover, and rate of decomposition of plant residues. In the soil, the bulk density and total porosity were determined. The contamination of the storage roots was evaluated based on the analysis of glyphosate residue. *Brachiaria ruziziensis* presented more dry weight and higher percentage of cover compared with *M. pruriens*, and both cover crops showed very similar decomposition rates. The physical properties of soil were unaffected by any treatment evaluated. There was no detection of glyphosate and its metabolite, aminomethylphosphonic acid (AMPA), in any treatment evaluated. Chemical control with glyphosate is not able to contaminate cassava storage roots.

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The conventional agricultural model, based on the intensive use of natural resources, combined with the use of pesticides, allowed to increase the production and productivity of crops, reducing prices, and guaranteeing access to food, but on the other hand, the indiscriminate use can promote the degradation of natural and human resources, contributing to food insecurity. Therefore, it is important to carry out studies that promote sustainable alternatives to the use of pesticides in agricultural systems. Cassava (*Manihot esculenta*) is a native plant to Amazon region, which has the highest genetic variability, and belongs to the

Euphorbiaceae family and the genus *Manihot* (Rocha et al., 2020a). Due to the ease adaptation and the lower technological requirements for its production, it is a species that plays an important role against the hunger, especially in developing and underdeveloped countries. In Brazil, cassava has a great historical and socio-economic importance due most of the producers are formed by small farmers and intended for subsistence and animal consumption. As the weeds promote intense competitive pressure on cassava crops, the producers are required to carry out weeding periodically in his crops, being the main problem faced by Amazonian cassava producers. Furthermore, the scarcity of labor in rural areas and the climatic conditions of high temperature, high relative humidity, and intense solar radiation, becomes the weeding an inefficient and prejudicial practice to the farmer's quality of life. Regarding to chemical control, the main difficulties are related to the lack of knowledge on the safe use of herbicides, related to the use of registered products and the appropriate application. Thus, the use of cover crops is an alternative for suppressing weeds, reducing the demand for herbicides and the risks of food contamination (Ghahremani et al., 2021; Mennan et al., 2020; Proctor, 2021). Moreover, cover crops as legumes and grasses can improve the physical properties of soil in different forms and several studies have shown these benefits (Demir and Işık, 2020; Rós and Hirata, 2019; Soares et al., 2021). Although cassava is known worldwide for its rusticity and low nutritional requirements, physical properties of soil, such as bulk density and porosity, can be crucial for this crop, since these properties are directly related with the resistance of penetration and expansion of roots in the soil. Thus, knowing cover crops species capable of improve physical properties of soil is of great interest to cassava farmers in Amazonas. The objective of this research was to analyze the effects of cover crops and chemical control on physical properties of soil and glyphosate residues in cassava storage roots, to incorporate good agricultural practices in the cassava production system, promoting sustainability, and food security.

Material and Methods

Site description and treatments application. The trials were conducted at the Experimental Farm of the Federal University of Amazonas (lat. 02°37'17" 1" and 02°39'41" 4" S; long. 60°03'29" 1" and 60°07'57" 5" W), Amazonas state, Brazil, in two crop seasons (2017–18 and 2018–19). The climate is humid tropical, corresponding to the Am type in the Köppen classification, with relative humidity of air around 75% and 86% and annual rainfall from 2000 to 2600 mm (Dubreuil et al., 2018; Vieira and D'avila Junior, 2020). The area was prepared with a light harrowing and received fertilization based on the recommendation for cassava crop in the region (Dias et al., 2004). The cultivar used was Manteiga, a sweet cassava that presents a cycle of 12 months, an average

yield of 15 t·ha⁻¹ and hydrocyanic acid content below 50 mg·kg⁻¹ (Oliveira e Barreto, 2020). Stem cuttings 10 to 15 cm longer, with 3 to 6 growth buds, were planted in furrows ≈10 cm deep, arranged horizontally, and covered with soil. The spacing of 1.0 m between lines and plants was adopted, totaling 10,000 plants per hectare. Each plot consisted of five planting lines, with six plants per line. The useful area of the plot comprised the three central lines, totaling 12 useful plants for evaluation. The experiment was established in a randomized complete block design with five treatments and five repetitions. The treatments were biological control with two species of cover crops (*Brachiaria ruziziensis* and *Mucuna pruriens*), chemical control with herbicide, mechanical control with hoe, and treatment with no weed control. These controls started 3 months after the planting of cassava and were carried out every 2 months after the first control.

Cover crops. The cover crops were planted 3 months after cassava planting to avoid competition with cassava plants since several studies have related the slow initial growth that cassava has (Pinheiro et al., 2021; Silva et al., 2020; Soares et al., 2019). The planting densities were 9 kg·ha⁻¹ for *B. ruziziensis* and 80 kg·ha⁻¹ for *M. pruriens*. The *B. ruziziensis* was planted in furrows between the cassava plants lines, whereas the legume, *M. pruriens*, was planted in pits 40 cm apart. Cover crops were sown at a distance of 30 cm from cassava.

To quantify the cover crops' dry weight, a random collection was carried out 90 d after the cover crops planting. For this purpose, samplers with an area of 0.12 m² were randomly placed twice at each plot to collect the cover crops biomasses samples. The material collected was dried in an air-circulating oven at 65 °C, until the constant weight was obtained, then the data were transformed in t·ha⁻¹.

The percentage of soil cover was performed at 30, 60, and 90 d after the cover crops planting adapting the linear transection method described by Lafen et al. (1981), calculating the percentage of the points, in the line, with cover crops, weeds, or uncovered soil, resulting in a 2 × 3 factorial scheme with two cover crops and three observation times, respectively.

The cover crops were mowed after 3 months of planting and before its flowering, according to the greatest nutritional input in these plants and to avoid the possibility of infestation.

To measure the cover crops residue decomposition, cover crops biomasses were packed in little nylon bags, with a mesh of 2 mm and dimensions of 0.04 m². The little bags were randomly distributed in each plot, totaling five little bags per plot, being collected one at a time at intervals 30, 60, 90, 120, and 150 d after the mowing of the cover crops. Each bag contained 20 g of plant material of each species, resulting in a 2 × 5 factorial scheme with two cover crops and five collection times, respectively.

Weed control. For chemical control, the postemergence application of glyphosate (480 g ae/ha) was carried out using an automated backpack sprayer, pump pressure of 40 to 60 lb/inch, nozzle 80.04, and the dose of 3.5 L of commercial product per ha. For mechanical control, weeding was carried out using a hoe.

Physical properties of soil. For the soil analysis, two undisturbed soil samples were collected in each plot, at depths of 0–10 cm, at the beginning and at the end of the experiment, to assess the physical attributes of the soil. The bulk density (*Ds*) was evaluated by the volumetric ring method (Blake and Hartge, 1986), the particle density or real (*Dp*) by the volumetric balloon method and the total porosity (VTP) by calculating VTP = 100 × (*Dp* – *Ds*)/*Dp* (EMBRAPA, 1997).

Glyphosate residues. For the analysis of glyphosate and its metabolite AMPA residues, samples of cassava roots, in the median portion, were collected from each useful plant in each plot and stored at a temperature of –20 °C. The analysis was carried out by the Massachusetts Pesticide Analysis Laboratory, through its Standard Operating Procedures (SOP) for glyphosate, which consists of using the derivatizing agent 9-fluorenylmethyl chloroformate (FMOC-Cl) and liquid chromatography coupled to triple quadrupole mass spectrometer (LC/TQD).

Statistical analysis. Using the Sisvar software (Version 5.6, Build 90), all data were subjected to normality test, analysis of variance, and *F* test (5% probability). The percentage of coverage and the decomposition rate of the cover crops were submitted to the *t* test and regression analysis, respectively. To analyze the dry weight of the two species of cover crops and the treatment with no weed control, as well as to analyze the influence of the five treatments on the physical properties of the soil, the mean values of the plots were submitted to the Scott–Knott test (Ferreira, 2011).

Results

Cover crops dry weight. There was a difference between the cover crops. *M. pruriens* showed less dry weight in comparison with *B. ruziziensis* and the treatment with no weed control, which were statistically equal (Table 1).

Percentage of cover. In 2017–18, the factors species of cover crops and time of analysis (months) were significant. The interaction between species of cover crops and time of

analysis was significant for percentage of coverage, uncovered soil, but not significant for percentage of weeds. From the second month on, there was a difference between the cover species, in which *B. ruziziensis* showed highest percentage of cover, in comparison with *M. pruriens*, to cover most of the plot and leave a smaller percentage of soil uncovered. In the 2018–19, the interaction of factors was significant for all parameters studied, with *B. ruziziensis* showing greater percentage of cover compared with *M. pruriens* since the first month, leaving a lower percentage of soil without cover in the second and third months (Table 2).

Cover crops residue decomposition. Both *B. ruziziensis* and *M. pruriens* showed very similar decomposition rates. In general, before the first collection, carried out at 30 d after mowing, the residues had already reached half-life (*t*^{1/2}), that is, more than 50% of its biomass was lost (Fig. 1).

Physical properties of soil. None of the treatment influence the physical properties of soil in terms of bulk density and total porosity (Table 3).

Analysis of glyphosate residues. There was no detection of glyphosate and its metabolite, AMPA, in any treatment evaluated (Table 4).

Discussion

Cover crops dry weight. The Amazon region is characterized by high temperatures and rainfall, which are factors favorable to the rapid growth of weeds. The weeds in Amazonas quickly cover the soil, being an obstacle in agricultural production. In this sense, it is important to highlight the main species of weed identified in the experimental area of this research (Table 5), such as *Mimosa pudica*, *Stachytarpheta cayennensis*, and *Axonopus affinis*, known for their high biomass production and aggressiveness. For the dry weight, the treatment with no weed control was statistically equal to the treatment with *B. ruziziensis*, which suggests the competitive advantage of this cover species with weeds (Table 1). These results are similar to those obtained by Gama et al. (2020), carried out in Amazonas, in which species of the genus *Brachiaria* had the highest production of dry weight in relation to the other cover crops studied (5.95 t·ha⁻¹ and 6.35 t·ha⁻¹, respectively). For *M. pruriens*, it was observed the lower dry weight production, around 3.50 t·ha⁻¹ in the average of the 2 years. However, it can be considered satisfactory, according to the high aggressiveness of this species, it is possible that the production of dry weight above 4 t·ha⁻¹ represents significant reductions in terms of production when the species is intercropped with other crops of agricultural interest. As reported by Bressanin et al., (2016), *M. pruriens* showed accumulation of dry weight throughout all the period of evaluation and reduced sugar cane productivity by up to 50%. The dry weight production of cover crops must be carefully analyzed, because when the

Table 1. Cover crops dry weight (t·ha⁻¹) in cassava crop system under different managements in 2017–18 and 2018–19.

Treatments	Dry wt (t·ha ⁻¹)	
	2017–18	2018–19
<i>B. ruziziensis</i>	6.00 a	4.78 a
<i>M. pruriens</i>	3.71 b	3.30 b
No weed control	6.88 a	4.96 a

Means followed by the same letters, per column, do not differ at 5% probability by the Scott–Knott test.

Table 2. Coverage (C), weeds (W), and uncovered soil (U) in the first, second, and third months after cover crops planting, *B. ruziziensis* and *M. pruriens*, in cassava cropping system in 2017–18 and 2018–19.

Cover Crops	C (%)			W (%)			U (%)		
	First mo.	Second mo.	Third mo.	First mo.	Second mo.	Third mo.	First mo.	Second mo.	Third mo.
2017–18									
<i>B. ruziziensis</i>	56.25 Ba	81.25 Aa	78.75 Aa	23.75 Aa	13.75 Aa	18.75 Ab	20.00 Aa	5.00 Bb	2.50 Bb
<i>M. pruriens</i>	53.75 Aa	60.00 Ab	53.75 Ab	26.25 Aa	18.75 Ba	30.00 Aa	17.50 Aa	21.25 Aa	16.25 Aa
2018–19									
<i>B. ruziziensis</i>	50.00 Ba	73.75 Aa	76.25 Aa	28.75 Aa	14.50 Ba	18.75 Ba	21.25 Aa	12.00 Bb	5.00 Bb
<i>M. pruriens</i>	40.00 Bb	41.25 Bb	62.50 Ab	31.25 Aa	22.50 Aa	23.75 Aa	28.75 Aa	36.25 Aa	13.75 Ba

Means followed by the same lowercase letters do not differ in relation to the type of cover (*B. ruziziensis* and *M. pruriens*) and means followed by the same uppercase letters do not differ in relation to the time (first, second, and third months) for the parameters of percentage of cover crops, invasive plants, and soil.

objective is only to use it as green manure or mulch, the greater production of dry weight does not represent risks and is always desired; however, in intercropped systems, the high production of dry weight can result in increased competitive pressure between the cover crops and the main crop.

Percentage of cover. *B. ruziziensis* showed a higher percentage of cover, leaving the soil less uncovered in comparison with *M. pruriens* (Table 2). These results are similar with those obtained by Gama et al. (2020), when studying cover plants in guarana crop system observed that at 90 d after planting, *B. ruziziensis* covered 100% of the soil, showing its high capacity development, lower values of coverage for *M. deeringiana* were also observed, around 70%. In general, *B. ruziziensis* promoted coverage greater than 75% in both crop seasons analyzed, which can contribute to the conservation of water in the soil, in addition to reducing erosion processes and the consequent loss of nutrients in the soil. It is noteworthy that the prostrate growth habit of *M. pruriens* favors the rapid expansion of its leaves and branches close to the soil when used for mulching or green manure. Cantanhede et al. (2018), studying *M. pruriens* as green manure, observed that the species showed a percentage of coverage of $\approx 90\%$ at 90 d after seedling emergence. However, when this species is intercropped with other crops of agricultural interest, it tends to present itself as an aggressive plant, climbing on the surrounding plants, investing in vertical growth and, consequently, leaving more space in the soil when compared with other species with low growth habit. From the above, *B. ruziziensis* showed a higher percentage of cover with less space with the soil uncovered.

Cover crops residue decomposition. *B. ruziziensis* and *M. pruriens* reached $t^{1/2}$ between 30 and 38 d after cutting, in both crop seasons (Fig. 1). Although similar, *M. pruriens* showed slightly faster decomposition than *B. ruziziensis*. In general, legumes tend to show faster decomposition due to the lower C/N ratio, when compared with grasses, which due to the higher C/N ratio tend to remain in the environment for longer. As the cultivar Manteiga has a cycle of 12 months and the cover cut occurred when cassava was ≈ 6 months old, the rapid nutrient cycling is highly desirable. According to

Thomas and Asakawa (1993), the shorter time required for the residue to be decomposed implies less time for the residue to remain and, therefore, releases nutrients more quickly. Although the rapid decomposition of cover crops residues may favor the emergence of weeds, after the sixth month of cultivation, it is expected that the effects of weed interference on cassava until harvest are not significant, most because the canopy closure puts seeds and emerging weeds in the shade avoiding their growth, being after the critical period of weed interference in cassava found in the literature (Soares et al., 2019). The decomposition rate of the cover species presents great discrepancies in the literature, according to Silva Filho et al. (2018), these differences are mainly related to the dry weight production capacity, the chemical compositions of the cover species, the greater contact of the straw with the soil, and the combination of high temperature and rainfall. It is noteworthy that the amount of material used in sampling also appears to be an important factor in $t^{1/2}$ determination. Silva Filho et al. (2018), using samples with 40 g of crop residue, found $t^{1/2}$ of 12 d for brachiaria; Gama et al. (2020), using samples with 100 g of residue, obtained a $t^{1/2}$ of 85 d for *B. ruziziensis* and 114 d for mucuna; and Ramos et al. (2018), studying decomposition of different types of legumes, with samples of 200 g of crop residue, obtained a $t^{1/2}$ of 105 d for mucuna. In relation to the effects of rapid decomposition, *B. ruziziensis*, as a perennial species, showed the ability to regrow after cutting, whereas *M. pruriens*, being an annual species, after cutting was completely replaced by weeds. Under the conditions of the present work, the coverings showed satisfactory decomposition rates, but this process needs to be better understood.

Physical properties of soil. Regarding the physical properties of soil, there was no difference between treatments for density and total porosity (Table 3). Studies about the effects of cover crops on the physical attributes of the soil such as bulk density and total porosity are incipient, and most of these studies suggest that the physical attributes of the soil are not influenced by cover crops in the first years of use. De Carvalho et al. (2020), when studying mucuna and brachiaria as cover plants, concluded that the root system of cover plants, evaluated 90 d after planting,

are not able to change the physical properties of soil. Similarly, Rocha et al. (2020b), when studying the influence of cover crops on the physical attributes of a soil grown with black pepper, observed that there was no significant difference for bulk density, total porosity, and the other variables studied. Especially for cassava, changes on the physical property of soil may be more interesting than eventual changes in the chemical properties, since cassava has low nutritional requirements and considering that physical properties such as bulk density and porosity are directly related to the capacity of penetration and expansion of the roots, and the level of water content in the soil, which are crucial for the development of cassava storage roots (Thomas et al., 2020). Changes in the physical properties of soil involve complex biotic and abiotic factors not yet fully understood and, in general, demand long periods of time, thus, the use of cover plants to improve these properties may demand several years. However, it is possible to find studies in which cover species were responsible for the decrease of soil density and for the increase of total porosity, which suggests that the influence of cover plants on the physical attributes of the soil must be analyzed specifically, in regarding the characteristics of each soil type and cover crops. Rós and Hirata (2019) observed that the incorporation of *Crotalaria ochroleuca* and the weed community reduced the bulk density and increased the total porosity of the soil in relation to *C. ochroleuca* mowed and weeded control. Therefore, for the conditions of the present experiment, the cover crops were not able to promote significant changes in the physical properties of soil evaluated. Longer term studies are needed to determine cover crops effects on soil physical properties such as density and porosity.

Analysis of glyphosate residues. Glyphosate and its metabolite, AMPA, were not found in any treatment, even with five applications per year. It is important to highlight that the plants with developed rhizomes such as cassava tend to have a lower translocation of glyphosate, it occurs because the starch accumulation hinders translocation and accumulation of herbicides at its site of action (Machado et al., 2008). Bhattacharjee and Dikshit (2017), when studying the glyphosate residues in mango orchard soil and their subsequent absorption by the fruit, concluded

Fig. 1. Decomposition rate of cover crops residues in cassava crop system under different managements in (A) 2017–18 and (B) 2018–19.

that even with the application of twice the recommended dose, the glyphosate residues were below the limit detectable in ripe fruits, due to the glyphosate being strongly adsorbed by the soil, being practically immobile, where it is quickly degraded. Blackshaw and Harker

(2016), when studying glyphosate and AMPA residues in the soil, concluded that even with the application of high doses of glyphosates for several years, the possibility of contamination or injury to wheat, pea, and canola plants soil is low. Zoller et al. (2018)

Table 3. Density and total porosity of the soil in cassava crop system under different managements in 2017–18 and 2018–19, at layer 0.00–0.10 m.

Treatments	Density (g/cm ³)	Total porosity (%)
2017–18		
Mechanical control	1.71 ^{ns}	31.43 ^{ns}
No weed control	1.69 ^{ns}	35.67 ^{ns}
<i>B. ruziziensis</i>	1.70 ^{ns}	29.06 ^{ns}
<i>M. pruriens</i>	1.68 ^{ns}	27.97 ^{ns}
Chemical control	1.73 ^{ns}	28.93 ^{ns}
2018–19		
Mechanical control	1.77 ^{ns}	30.01 ^{ns}
No weed control	1.74 ^{ns}	34.07 ^{ns}
<i>B. ruziziensis</i>	1.68 ^{ns}	31.25 ^{ns}
<i>M. pruriens</i>	1.70 ^{ns}	29.75 ^{ns}
Chemical control	1.75 ^{ns}	30.55 ^{ns}

^{ns}Treatments did not differ at 5% probability by the *F* test.

analyzing the presence of glyphosate and AMPA in a total of 243 samples of different fruits, did not find samples with residues above the maximum allowed limit, even in samples where high levels of residues were expected. In a different way, Wood (2019), when studying residues of glyphosate and AMPA in the aerial part, roots and fruits of certain plant species, found residues of glyphosate in the aerial part, roots and fruits, even 1 year after application, being the highest levels found in the roots of perennial herbaceous plants. In this sense, Qiao et al. (2020), studying the behavior and influence of glyphosate in a peach orchard, found AMPA residues in peach leaves and fruits, even with the application of the herbicide only in the soil. The same authors evaluated that the speed of degradation of glyphosate is influenced by the type of soil, being enhanced mainly by pH and humidity. Thus, the behavior and risks of contamination by glyphosate residues can vary widely depending on factors such as climate, precipitation, soil characteristics, doses, and frequency of application, among others, requiring specific assessments for each type of management. The results found in this study suggest that glyphosate is not able to contaminate cassava roots by contact via soil, probably due to its strong adsorption and consequent low mobility, and that there is also no translocation or accumulation of glyphosate and AMPA in the cassava roots to above the detectable limits, being safe the consumption of the roots. As the objective of this work was to evaluate the presence of glyphosate and AMPA residues in cassava storage roots, the effects of this herbicide were not evaluated in relation to the biological

Table 4. Cassava root analysis for glyphosate and its metabolite, aminomethylphosphonic acid (AMPA).

Treatments	Glyphosate	AMPA
No weed control	ND ^z	ND
Chemical control	ND	ND

^zND: not detected. The detection limit was 10 µg/kg (ppb) of cassava for glyphosate, and 20 µg/kg (ppb) of cassava for AMPA.

Table 5. Weeds identified in in cassava crop system under different managements in 2017–18 and 2018–19.

Family	Species		
	Scientific name	Code	Common name
Amaranthaceae	<i>Alternanthera tenella</i>	ALRTE	Apaga-fogo
Commelinaceae	<i>Commelina erecta</i>	COMER	Trapoeiraba
Cyperaceae	<i>Cyperus rotundus</i>	CYPRO	Tiririca
	<i>Cyperus diffusus</i>	CYPDF	Capim-agreste
Euphorbiaceae	<i>Croton glandulosus</i>	CVNGL	Gervão-branco
Fabaceae	<i>Mimosa pudica</i>	MIMPU	Mimosa
	<i>Pueraria phaseoloides</i>	PUEPH	Puerária
Poaceae	<i>Axonopus affinis</i>	AXOAF	Grama-tapete
	<i>Eleusine indica</i>	ELEIN	Capim-pé-de-galinha
	<i>Homolepis aturensis</i>	HOPAT	Capim-arroz
	<i>Paspalum multicaule</i>	PASMA	Capim-taripucu
	<i>Paspalum virgatum</i>	PASVI	Capim-navalha
Rubiaceae	<i>Spermacoce verticillata</i>	BOIVE	Vassourinha-de-botão
Solanaceae	<i>Solanum stramonifolium</i>	SOLST	Jurubeba
Verbenaceae	<i>Lantana camara</i>	LANCA	Chumbinho
	<i>Stachytarpheta cayennensis</i>	STCDI	Gervão

properties of soil or on aspects of the environment in general, not being possible, only with based on the data obtained in this research, recommend its use for cassava culture.

We recommend *B. ruziziensis* as a cover plant in cassava production systems in the Amazon, due to the advantage of being perennial, without the need for replanting during the crop cycle, high biomass production with slow degradation, in addition to its decumbent growth, prostrating itself to the soil with good coverage rate. The physical properties of the soil were unaffected by the management of weeds in the two harvests. We suggest future work to determine whether these control methods will have an effect on maintaining the physical quality of the soil in the long term, considering the edaphoclimatic conditions of the Amazon, knowing that the process of soil transformation can take time. The absence of contamination of the cassava roots by glyphosate and its metabolite, AMPA, contributes to food security and suggests that damage to food and, consequently, to the health of the farmer and consumer, is more likely by use undue in terms of dose and time of application. However, future studies in the medium and/or long term can determine whether there is a cumulative effect. We emphasize that the use of *B. ruziziensis* as a cover crop can be a potential alternative control for sustainable agroecological management in the Amazon; however, further studies are needed to verify the impacts on cassava yield.

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