GREEN MANURE AND MINERAL FERTILIZATION: RESIDUAL EFFECT ON DRY MATTER PRODUCTION, NUTRIENT EXTRACTION AND NUTRIENTS RECOVERY EFFICIENCY IN TWO SUCCESIVE CROPS †

[ABONO VERDE Y FERTILIZACIÓN MINERAL: EFECTO RESIDUAL EN LA PRODUCCIÓN DE MATERIA SECA, EXTRACCIÓN Y EFICIENCIA DE RECUPERACIÓN DE NUTRIMENTOS EN DOS CULTIVOS SUCESIVOS]

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SUMMARY

Background. Green manures (GM) are an alternative to partially or totally replace mineral fertilizers, in successive crops, but few studies have studied the residual effect (REEF) of GM on the subsequent crops. **Objective.** To determine the REEF of GM (*Mucuna cinerea*, sin. *Stizolobium cinereum*) and mineral fertilization (MF) on dry matter production (DM), nutrient extraction (NEX) and soil solution (SS) characteristics in the *Brachiaria brizantha* (BB). A second objective was to determine the nutrient recovery efficiency (RE) in two successive crops of broccoli and BB. **Methodology.** A single application of GM snd MF was done on broccoli crop, in pots and greenhouse conditions. The treatments were: 1) unfertilized plants (Control). 2) 200% of the recommended dose for broccoli, applied exclusively through MF (MF200). 3) GM at 10 t ha^{-1} (GM10). 4) The same dose of N, P, K, Ca, Mg, and S applied through GM10 was applied through MF (MF=GM10). 5) GM10 + MF to supply the same dose of N, P, K, Ca, Mg, and S supplied with MF200 (GM10+MF). After broccoli harvest, the BB was planted in the same pots, without additional fertilization. **Results.** In BB, greater REEF on DM, NEX (N, P, K, Ca, Mg and S), electrical conductivity and nutrient concentration (N, K, Ca, Mg and S) in SS, was detected with GM10 and GM10+MF. Considering both crops, greater DM and NEX were obtained with MF200 and GM10+MF. Higher RE of P and S was obtained with GM10, while, higher RE of K and Ca was obtained with the MF=GM10. **Implications.** The study shows that the efficiency of nutrients applied through GM at 10 t ha^{-1}, combined with low doses of mineral fertilization, increases in successive crops. **Conclusion.** The REEF on the DM and nutrient extraction of the BB change depending on the fertilizer type and dose used. The REEF on the SS characteristics is only detected in the initial growth phase of the BB. In successive crops, the proportion of DM produced and the amount of nutrients extracted changes depending on the species used. To obtain a similar nutrient recovery between GM and MF is necessary to consider the DM of more of one crop.
Keywords: Broccoli; Fabaceae plants; Fertilization dose; Nitrogen; Soil solution.

RESUMEN

Antecedentes. Los abonos verdes (GM) son una alternativa para reemplazar parcial o totalmente a los fertilizantes minerales en cultivos sucesivos, pero, pocos estudios han estudiado el efecto residual (REEF) del GM en cultivos subsecuentes. **Objetivo.** Determinar el REEF del GM (*Mucuna cinerea*, sin. *Stizolobium cinereum*) y de la fertilización mineral (MF), sobre la producción de materia seca (DM), extracción de nutrientes (NEX) y características de la solución del suelo (SS) en la *Brachiaria brizantha* (BB). Un segundo objetivo fue determinar la eficiencia de recuperación (RE) de nutrientes en los cultivos sucesivos de el brocoli y BB. **Metodología.** Una única aplicación de GM y MF fue realizada al cultivo de brocoli plantado en macetas, en condiciones de invernadero. Los tratamientos fueron: 1) Plantas no fertilizadas (Control). 2) 200% de la dosis recomendada para el broccoli aplicado a través de MF (MF200). 3) GM en dosis de 10 t ha^{-1} (GM10). 4) La misma dosis de N, P, K, Ca, Mg y S aplicado a través de GM10 se aplicó a través de MF (MF=GM10). 5) GM10 + MF para suministrar la misma dosis de N, P, K, Ca, Mg y S que MF200 (GM10+MF). Después de la cosecha del brocoli, la BB fue plantado en las mismas macetas, sin fertilización adicional. **Resultados**. En la BB, mayor REEF en DM, NEX (N, P, K, Ca, Mg y S), conductividad eléctrica y concentración de nutrientes (N, K, Ca, Mg y S) en la SS, se detectó

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con GM10 y GM10+MF. En ambos cultivos, mayor DM y NEX se obtuvo con MF200 y GM10+MF. Mayor RE de P y S se obtuvo con GM10, mientras que, mayor RE de K y Ca se obtuvo con MF=GM10. **Implicaciones.** El studio muestra que la efeciencia de nutrientes aplicados a tráves de 10 t ha^{-1} de GM, combinado con baja dosis de MF, aumenta en cultivos sucesivos. **Conclusión.** El REEF en la producción de DM y NEX de la BB cambia dependiendo del tipo de fertilizante y la dosis utilizada. El REEF en las características de la SS es detectada únicamente en la fase inicial del crecimiento de la BB. En cultivos sucesivos, la proporción de DM producida y cantidad de nutrientes extraídos cambia en función de las especies utilizadas. Para obtener una similar recuperación de nutrientes entre GM y MF es necesario considerar la DM de más de un cultivo.
Palabras claves: Brocoli; Fabaceas; Dosis de fertilización; Nitrógeno; Solución del suelo.

INTRODUCTION

Fertilization is a necessary practice to achieve plant development and the yield desired for crops. In intensive agricultural crop production, mineral fertilizers are the main source of nutrients, although their continued and excessive use has generated negative effects on the environment and the safety of plant foods (Kulkarni and Goswami, 2019). Therefore, other sustainable fertilization alternatives are required to replace or complement to the mineral fertilization (MF). Among these alternatives are different plant species that can be used as green manure (GM). Fabaceae plants stand out as GM, due to their association with nitrogen-fixing bacteria, which allows the fixation of atmospheric nitrogen gas, which is assimilated and used by the plants (Poole *et al*., 2018). When the GM is cut and incorporated into the soil, in addition to nitrogen (N), other nutrients that were obtained from different depths of the soil through its root system are deposited in the surface part of the soil (Valadares *et al*., 2016).

Previous studies have shown that GM applied in doses greater than 5 t ha^{-1} promotes yields and dry matter (DM) production, similar to those obtained with MF (Adekiya *et al*., 2017). When the dose is less than 5 t ha^{-1}, the effect of the GM is less than that of the MF (Bai et al. 2015), and therefore, a better effect is obtained when it is applied in combination with low doses of MF (Xie *et al*., 2016). It is known that GM, in addition to affecting the yield and DM production, also affect the nutritional aspect of the crops, stimulating greater concentration and extraction of nutrients than unfertilized plants and similar to those managed with MF (Dabin *et al*., 2016).

On the other hand, depending on the fertilizer type or fertilization dose used, the growth, yield and nutritional aspect of crops can change (Moreno-Cornejo *et al*., 2017), which generates different efficiency of utilization of the applied nutrients (Diniz *et al*., 2017b). In successive cropping systems, where short-cycle crops are used, the nutrients supplied through fertilization practices are not regularly absorbed by the first crop. Therefore, the nutrients remaining in the soil can be used by other subsequent crops, which is known as the residual effect (REEF). The REEF on other crops depends on the characteristics of the soil, type of fertilizer used, fertilization dose, amount of nutrients that removed by the first crop and the amount of nutrients remaining in the soil (Båth *et al*., 2006; Diniz *et al*., 2017b). Although there are studies where the REEF of fertilization has been observed in subsequent crops, these studies have mainly focused on knowing the effect on the growth and yield of the plant (Diniz *et al*., 2017a), on the nutritional aspect (Båth *et al*., 2006) and the efficiency of nutrient recovery, specifically the N (Vargas *et al*., 2017).

Previous studies have shown that the rates of the nutrient utilization efficiency, is a way to determine the effect of nutrients on plant behavior (Fageria, 2009). One of these indexes is the nutrient recovery efficiency (RE), which indicates the proportion of the applied nutrient that is recovered by the biomass of the crop (Fageria and Baligar, 2005). It is also known that, in successive crop systems, the values of the efficiency indices can change according to the number of crops established in the same production site (Vargas *et al*., 2017). Another way to understand the influence of fertilization on plant development is to know the behavior of nutrients in the soil solution (SS). The SS is very dynamic and quickly reflects the interaction that occurs between the nutrients supplied, soil minerals, and plant requirements (Fernandes *et al*., 2015).

Taking into account the above, an experiment was established to determine the effect of the application of GM and MF, exclusively or in combination, on successive crops. The first crop was broccoli, where the effect of fertilization treatments on plant growth, yield, nutrient extraction (NEX), RE, and chemical characteristics of the SS was demonstrated (Peralta-Antonio et al. 2019). In the present study, it is presented: (1) the REEF of fertilization treatments on the DM and NEX of the second crop (*Brachiaria brizantha* grass). (2) Effect of fertilization treatments on DM, NEX, and RE of N, P, K, Ca, Mg, and S, considering the first and second crops together. (3) The REEF of the treatments on the chemical characteristics of the soil solution in the second crop.

MATERIALS AND METHODS

Green manure

Mucuna cinerea, sin. *Stizolobium cinereum* planted under field conditions at the Universidade Federal de Viçosa (20°45’14” S and 42°52’53” W) and harvested at flowering (15/04/2016) was used as GM. After harvest, the biomass (leaf and stem) were cut into 8 cm segments, a part of the biomass was dried in a forced-air drying oven at 65 °C, and stored in plastic bags until use to avoid the degradation process. The chemical characteristics of the GM were determined from representative samples of leaves (8.13 C/N ratio, 431 g kg^{-1} organic carbon and 53.0, 3.5, 12.0, 16.4, 2.3, 2.3 g kg^{-1} N, P, K, Ca, Mg and S, respectively) and stem (19.9 C/N ratio, 398 g kg^{-1} organic carbon and 20.0, 2.2, 14.0, 7.4, 1.1, 1.0 g kg^{-1} N, P, K, Ca, Mg and S, respectively. Chemical analyses on GM samples were performed according to Tedesco *et al*. (1995).

First crop

The first crop was the Legacy hybrid single-headed broccoli (17/07/2017 - 21/09/2017), was produced in greenhouse conditions, it was planted individually in 20 L plastic pots. The soil used presented the following characteristics: pH 6.2 (in water); 13.1, 130 and 57.4 mg dm^{-3} P (Mehlich 1 extractant), K (Mehlich 1 extractant) and S (monocalcium phosphate extractant); 19.8 mg L^{-1} remaining phosphorus (Premaining after the application of a 60 mg L^{-1} P solution); 2.2, 0.3, 0.0, 1.65, 2.8, 2.8 and 4.5 cmol$_c$ dm^{-3} Ca^{2+} (1 M Kcl extractant), Mg^{2+} (1 M Kcl extractant), Al^{3+} (1 M Kcl extractant) and H+Al (calcium acetate extractant), base sum, effective cation exchange capacity and cation exchange capacity and 63% base saturation.

Treatments and experimental design

The fertilization treatments were based on the N recommendation for the broccoli crop (Fontes 1999). The treatments applied were: 1) unfertilized plants (Control). 2) 200% of the recommended dose for broccoli, applied exclusively through MF (MF200). 3) GM at 10 t ha^{-1} (GM10). 4) The same dose of N, P, K, Ca, Mg, and S applied through GM10 was applied through MF (MF=GM10). 5) GM10 + MF to supply the same dose of N, P, K, Ca, Mg, and S supplied with MF200 (GM10+MF). A dose of 560, 349, 166, 320, 194, and 60 kg ha^{-1} of N, P, K, Ca, Mg, and S were applied with MF200 and GM10+MF. A dose of 365, 28, 130, 119, 17, and 17 kg ha^{-1} of N, P, K, Ca, Mg, and S were applied with GM10 and MF=GM10.

A randomized block design was used. One plant per pot was the experimental unit. Each treatment was replicated four times, giving a total of 20 experimental units.

The recommended dosage of each nutrient was divided between the soil volume of one hectare to 20 cm of depth (2 million of L of soil), multiplied by 20 (volume of soil per pot) to determine the dose per plant for each treatment.
Urea (46% N), Ca $(NO_3)_2$ (14% N, 18% Ca), KCl (58% K_2O), $NH_4H_2PO_4$ (11% N, 60% P_2O_5), dolomite lime (37% CaO, 13% MgO), $MgSO_4$ (9% Mg, 11% S) and MgO (55% Mg) were used to add the macronutrients (N, P, K, Ca, Mg and S) to the treatments. For the MF200 treatment were applied 9.0, 13.4, 3.4, 12.2, 5.4, and 0.8 g pot^{-1} urea, $NH_4H_2PO_4$, KCl, dolomite lime, $MgSO_4$, and MgO, respectively. For the MF=GM10 treatment were applied 4.4, 6.0, 2.6, 1.6, and 6.4 g pot^{-1} urea, $NH_4H_2PO_4$, KCl, $MgSO_4$, and $Ca(NO_3)_2$, respectively. For the GM10+MF treatment were applied 1.4, 12.2, 0.8, 9.4, 4.0, and 1.4 g pot^{-1}, urea, $NH_4H_2PO_4$, KCl, dolomite lime, $MgSO_4$, and MgO, respectively.

All treatments were applied in only dose, one day before transplant. The MF was mixed with the total volume of the soil before filling the pots and the GM biomass was (1:1 leaf/stem ratio) placed as soil cover, according to the corresponding treatment.

Weed control was performed manually whenever necessary. Manual irrigations were carried out according to crop demand. The amount of water used and the application frequency were similar for all pots. In all the pots the drained water was caught and returned.

Residual effect in the second crop

The REEF of the treatments used on broccoli crop was evaluated on *Brachiaria brizantha* grass. After broccoli harvests, seeds of brachiaria grass were sown in the same pots (11/10/2017), without additional fertilization. After seed germination, five brachiaria grass plants were maintained in each pot. Three cuts of brachiara grass biomass were carried out in 44, 72, and 109 days after the sowing date. These correspond to 129, 157, and 191 days after treatment application, respectively.The aerial part of the plant was dried until it reaches a constant weight (in a forced-air drying oven at 65 °C) and weighed at each cut-off date to determine the DM.

Nutrient extraction

The samples of broccoli and brachiaria grass biomass were milled to later determine concentrations of N, P, K, Ca, Mg and S. The chemical analysis was carried out in the laboratory of Plant Mineral Nutrition of the Universidade Federal de Viçosa. N was determined by the Kjeldahl method (Tedesco *et al*., 1995), P by spectrophotometry (wavelength of 725 nm), K in flame photometer, Ca and Mg in atomic absorption spectrophotometer, and S by turbidimetry (Malavolta *et al*., 1997). The extraction of N, P, K, Ca, Mg and S in the total dry matter of the tested plants were obtained by the following equation: Extraction of nutrient (g) = dry matter x (nutrient concentration / 100) (Fageria, 2009). The amount of DM produced and the amount of N, P, K, Ca, Mg, and S extracted by broccoli was added to the amount of DM produced and the amount of N, P, K, Ca, Mg and S extracted by brachiara grass.

Recovery efficiency

The RE of N, P, K, Ca, Mg, and S was determined considering the total extraction of nutrients obtained from the sum of the broccoli and brachiaria grass. Used the following equation: RE (%) = {[(g of extracted nutrient in the fertilized plant - g of extracted nutrient in the non-fertilized plant) / amount of nutrient applied] x 100} (Fageria, 2009).

Soil solution

A SS sampling equipment (with porous terminal capsule) was installed in each pot, in the central part of the pot, and at a depth of 10 cm. The soil located around the sampling equipment was slightly compressed to avoid the formation of air bubbles and interfere with the soil-water sampler system. Weekly SS collections were carried out, initiating seven days after the transplant (DAT) and concluded in the third brachiara grass biomass cut. The plants were irrigated to reach field capacity at each collection date. Six hours after irrigation, a vacuum with pressure of-70 kPa was applied to the sampler equipment. 10 hours after vacuum the SS was collected (with the aid of a syringe and hose) and the samples were stored in a refrigerator until the time of its analysis.

Active acidity, electrical conductivity and macronutrient concentration of soil solution

Values of active acidity (pH) and electrical conductivity (EC) were obtained by a pH and conductivity meter at each sampling date. The chemical analysis was carried out in the laboratory of Plant Mineral Nutrition of the Universidade Federal de Viçosa . Quantification of mineral N (NH_4^+ + NO_3^- + NO_2^-) was realized by the Kjeldahl method (Tedesco *et al*., 1995), using 10 mL of the SS sample. K determination was made by flame photometer, Ca and Mg by atomic absorption spectrophotometer, and S by turbidimetry (Malavolta *et al*., 1997). The volume of 1, 2, 0.5, and 5 mL of SS sample was used for the analysis of K, Ca, Mg, and S, respectively. P determinations were made directly of the SS by spectrophotometry.

Statistical analysis

ANOVA and the Dunnett test (with the statistical software SAS 9.0) were carried out to compare the control treatment with the other fertilization treatments for DM and NEX, and to compare the GM10 treatment with the other fertilization treatments for the variable RE. Comparison of the means test, by contrast, was used to evaluate the fertilization type effect (GM10 vs MF=GM10 and MF200 vs GM10+MF) for the variables DM, NEX and RE. An adjustment was made to the ER data (arcsin transformation) prior to the analysis of variance. In all analyses was considered $p < 0.05$. The arithmetic mean and standard deviation were used to presented values of pH, EC, N, P, K, Ca, Mg, and S of SS and proportion of DM produced and NEX.

RESULTS

Dry matter

GM10 and GM10+MF presented higher DM than the control in the second and third cut dates and the total DM of brachiaria grass (Table 1). Also, the effect of fertilizer type was detected on the brachiaria grass DM, GM10 surpassed MF=GM10 and GM10+MF surpassed MF200 (Table 2). Considering the DM of the two crops (DMBROBRA), higher DMBROBRA was detected with MF200 in comparison with the control (Table 1). The fertilizer type effect was not detected on DMBROBRA (Table 2).

Table 1. Residual effect of fertilization treatments on the dry matter production of brachiaria grass in three cutting dates. Total dry matter produced by broccoli and brachiaria grass (DMBROBRA).

	Brachiaria grass (g)				DMBROBRA (g)
	1st cut	2nd cut	3rd cut	Total	
Control	2.0	5.5	6.2	13.7	150.1
MF200	4.4	6.1	7.6	18.1	237.9*
GM10	3.2	13.2*	16.0*	34.7*	173.2
MF=GM10	2.6	6.2	6.0	14.8	212.3
GM10+MF	6.3*	13.8*	14.7*	34.8*	226.1

Control: unfertilized plants; MF200: mineral fertilizer at 200% of the recommended dose; GM10: 10 t ha^{-1} of the green manure; MF=GM10: mineral fertilizer supplying the same nutrient dose supplied with GM10; GM10+MF: GM10 + mineral fertilizer to supply the same amount of nutrient supplied with MF200. *Different from the control treatment, according to the Dunnett test (p<0.05).

Table 2. Groups of contrasts to compare the effect of the fertilizer type on the dry matter of the brachiaria grass (DMBRA), extraction of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) in the brachiaria grass (NEXBRA), total dry matter obtained from the sum of the broccoli and brachiaria grass (DMBROBRA), the total extraction of nutrients obtained from the sum of the broccoli and brachiaria grass (NEXBROBRA) and recovery efficiency (RE) obtained jointly by broccoli and brachiaria grass.

	GM10 vs MF=MG10		MF200 vs GM10+MF	
	Mean	Difference	Mean	Difference
DMBRA (g)	34.7 – 14.8	19.9 *	18.1 – 34.8	-16.7 *
NEXBRA (g)				
N	0.431 – 0.303	0.128 ns	0.242 – 0.492	-0.249 *
P	0.065 – 0.036	0.029 *	0.062 – 0.097	-0.035 *
K	0.622 – 0.252	0.370 *	0.302 – 0.593	-0.291 *
Ca	0.075 – 0.040	0.036 *	0.050 – 0.093	-0.042 *
Mg	0.045 – 0.023	0.022 *	0.035 – 0.062	-0.027 *
S	0.117 – 0.046	0.071 *	0.056 – 0.093	-0.036 *
DMBROBRA (g)	173.2 – 212.2	-39.0 ns	237.9 – 226.1	11.7 ns
NEXBROBRA (g)				
N	2.131 – 2.423	-0.292 ns	3.051 – 2.491	0.560 *
P	0.297 – 0.270	0.027 ns	0.469 – 0.435	0.034 ns
K	2.837 – 3.124	-0.287 ns	3.026 – 3.328	-0.302 ns
Ca	0.592 – 0.886	-0.294 *	0.926 – 0.762	0.164 ns
Mg	0.136 – 0.136	0.000 ns	0.198 – 0.185	0.013 ns
S	0.940 – 0.776	0.164 *	1.103 – 0.993	0.110 ns
RE (%)				
N			25.5 – 15.5	10.0 ns
P			6.9 – 5.9	0.9 ns
K			46.8 – 65.0	-18.2 ns
Ca			13.0 – 7.9	5.1 ns
Mg			4.8 – 4.1	0.7 ns
S			63.9 – 45.7	18.3 ns

MF200: mineral fertilizer at 200% of the recommended dose for broccoli; GM10: 10 t ha^{-1} of the green manure; MF=GM10: mineral fertilizer supplying the same nutrient dose supplied with GM10; GM10+MF: GM10 + mineral fertilizer to supply the same amount of nutrient supplied with MF200. ns: not significant according to the F test ($P < 0.05$); *Different by F test ($P < 0.05$).

Nutrient extraction

In brachiaria grass, higher extraction of N, P, K, Ca, Mg, and S were obtained with GM10 and GM10+MF compared to control. Higher extraction of P and Mg were obtained with MF200 compared with the control (Table 3). Regarding the effect of the fertilizer type, GM10 had a higher extraction of P, K, Ca, Mg, and S than MF=GM10, also, GM10+MF had a higher extraction of N, P, K, Ca, Mg and S than MF200 (Table 2).

Considering the two crops, in comparison to the control, MF200 extracted more N, P, Ca, Mg and S, GM10 extracted more P, Mg and S, MF=GM10 extracted more N, K, Ca and Mg, and GM10+MF extracted more N, P, K, Ca, Mg and S than the control (Table 3). The effect of the fertilizer type was detected, where, GM10 extracted less and more Ca and S than MF=GM10, respectively. On the other hand, MF200 extracted more N than GM10+MF (Table 2).

Recovery efficiency

The RE of N was not affected. Less RE of P and Mg were obtained with MF200 and MF=GM10 compared to GM10. Greater RE of K and Ca was obtained with MF=GM10 compared to GM10. Less RE of S was obtained with MF200, MF=GM10, and GM10+MF in comparison with GM10 (Table 3). The effect of the fertilizer type was not detected (Table 2).

Table 3. Extraction and recovery efficiency of the nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) obtained in three cutting dates of brachiaria grass jointly to the extracted on the broccoli crop.

	N	P	K	Ca	Mg	S
			Brachiaria grass (g)[1]			
Control	0.194	0.029	0.222	0.038	0.021	0.047
MF200	0.242	0.062*	0.302	0.050	0.034*	0.056
GM10	0.430*	0.065*	0.622*	0.075*	0.044*	0.116*
MF=GM10	0.302	0.036	0.251	0.039	0.022	0.045
GM10+MF	0.491*	0.097*	0.592*	0.092*	0.061*	0.092*
			Brachiaria grass + broccoli (g)[1]			
Control	1.622	0.225	2.248	0.509	0.105	0.719
MF200	3.051*	0.468*	3.026	0.925*	0.198*	1.103*
GM10	2.131	0.296*	2.837	0.591	0.136*	0.939*
MF=GM10	2.423*	0.269	3.124*	0.886*	0.135*	0.775
GM10+MF	2.490*	0.434*	3.327*	0.761*	0.185*	0.993*
			Recovery efficience broccoli + Brachiaria grass (%)[2]			
MF200	26	7*	47	13	5*	64*
GM10	14	25	38	7	18	130
MF=GM10	22	15	89*	38*	21	45*
GM10+MF	16	6*	65	8	4*	46*

The treatments were applied in the first crop (broccoli). Control: unfertilized plants; MF200: mineral fertilizer at 200% of the recommended dose; GM10: 10 t ha^{-1} of the green manure velvet bean; MF=GM10: mineral fertilizer supplying the same nutrient dose supplied with GM10; GM10+MF: GM10 + mineral fertilizer to supply the same amount of nutrient supplied with MF200.[1]Different from the control treatment, according to the Dunnett test ($p<0.05$). [2]Different from the GM10 treatment, according to the Dunnett test ($p<0.05$).

Active acidity, electrical conductivity and macronutrient concentration of soil solution

Changes in pH were observed during the growth cycle of the brachiaria grass. Differences in pH between treatments occurred mainly between 107 and 121 days after application of the treatments (DAAT), with MF200 presenting the lowest pH values (Figure 1 a).

The highest EC values in all treatments were before 135 DAAT (before the first brachiaria grass cut). Differences between treatments occurred between 114 and 135 DAAT, where GM10 showed higher EC values than MF200 (Figure 1 b).

Similar behavior over time was observed in the concentration of N, K, Ca and Mg, where the highest values of these nutrients were observed before 149 DAAT, in this period there were also differences between treatments, with GM10 and GM10+MF presenting the highest values (Figure 1 c, d, and Figure 2 a, b).

Different behavior presented the S concentration, the lowest value in all treatments was observed at 107 DAAT, after that date the concentration increased in all treatments. Differences between treatments were observed specifically at the 93, 177, and 191 DAAT, at the three dates, MF=GM10 had lower values than GM10+MF (Figure 2 c).

DISCUSSION

Dry matter

The REEF of the treatments applied to the broccoli was confirmed and favored to the GM, alone or combined with MF, since GM10 exceeded MF=GM10 and, GM10+MF exceeded MF200. Lower REEF with MF is attributed to lower soil nutrient reserves in response to the higher amount of nutrient removed in the first crop (Peralta-Antonio *et al.*, 2019), since the proportion of N, K, Ca and Mg absorbed by the first crop compared to that initially applied was 58%, 221%, 64%, and 65% with MF=GM10, values that were higher at 47%, 171%, 42% and 53% of GM10. A similar situation occurred at the highest dose of fertilization, where, the proportion of N, P, Ca, Mg, and S absorbed by the first crop concerning that initially applied with MF200 were 50%, 12%, 28%, 8%, and 1750%, values higher at 36%, 10%, 21%, 6% and 1500%, obtained with GM10+MF, respectively. According to the DMBROBRA, the results suggest that, in short-cycle crops, although, GM10 did not affect the dry matter in the first crop (Peralta-Antonio et al. 2019), in the second crop its effect was superior to

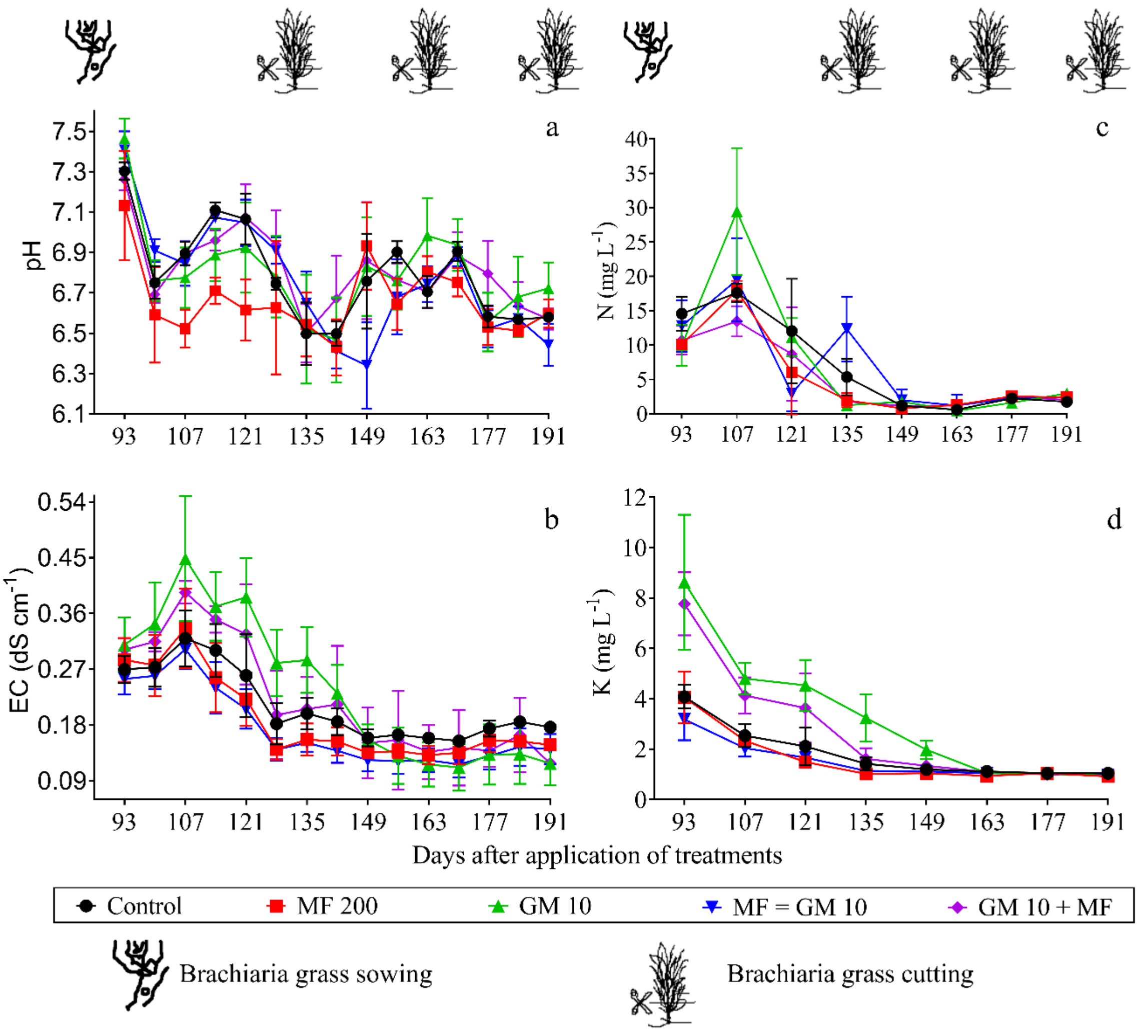

Figure 1. Residual effect of fertilization treatments on the active acidity (pH) (a), electrical conductivity (EC) (b), concentrations of nitrogen (N) (c), and potassium (K) (d) of the soil solution during the growth of the brachiaria grass. The treatments were applied in the first crop (broccoli). Control: unfertilized plants; MF200: mineral fertilizer at 200% of the recommended dose; GM10: 10 t ha^{-1} of the green manure; MF=GM10: mineral fertilizer supplying the same nutrient dose supplied with GM10; GM10+MF: GM10 + mineral fertilizer to supply the same amount of nutrient supplied with MF200. Vertical bars represent the standard deviation of the means (n = 4).

superior to mineral fertilization applied alone (MF200 or MF=GM5) and similar to combined fertilization (GM10+MF). On the other hand, the MF only affected the DMBROBRA when applied at 200% of the recommended dose, therefore, to obtain DM production in successive crops the best option is to mix low dose GM with low dose MF, as has been reported in other studies (Perin *et al*., 2004; Diniz *et al*., 2017b).

Nutrient extraction

The REEF of GM on the nutrient extraction in brachiaria grass changed depending on the dose and fertilizer type used. In both cases, GM was favored, since GM10 and GM10+MF extracted greater N, P, K, Ca, Mg, and S than the control and mineral fertilization (MF=GM10 and MF200). Considering that the extraction of nutrients is related to the amount of dry matter produced (Peralta-Antonio *et al*., 2019), the greater nutrient extraction in plants that received GM is then attributed to the greater amount of biomass produced. Of the total N, P, K, Ca, Mg, and S extracted in the two crops, in the first crop more than 80% was extracted when MF was used and more than 67% with GM. This situation is similar to that observed in the successive crops of broccoli single head and zucchini, where increasing doses of *Crotalaria juncea* (3, 6 and 9 t ha^{-1}) together with 12 t ha^{-1} of compost stimulated greater nitrogen recovery in the first crop (Vargas *et al*., 2017). It was also shown that GM requires more time to achieve the effect of MF on nutrient extraction, since the lower extraction of N, K and Mg obtained with GM compared to MF detected in the first crop (Peralta-Antonio *et al*., 2019), disappeared when considering the total extracrion of nutrients in both crops.

Recovery efficiency

Both the dose of fertilization and the type of fertilizer used can influence the recovery of nutrients

(Conversa *et al*., 2019). The results indicate that, with successive crops in medium-fertility soil level, such as that used in this research (Alvarez *et al*., 1999), the mineral fertilization (MF=GM10) only promotes greater RE of K and Ca compared to GM10. It also indicates that GM10 was more efficient in recovering P, Mg, and S compared to high dose fertilization, regardless of how the nutrient was supplied (FM200 or GM10+MF). Although taking into account the yield of the first crop, DM production, and NEX, the best option was the combined fertilization, as it managed to stimulate higher yield in broccoli crop (Peralta-Antonio *et al*., 2019) and higher DM production and NEX in the second crop.

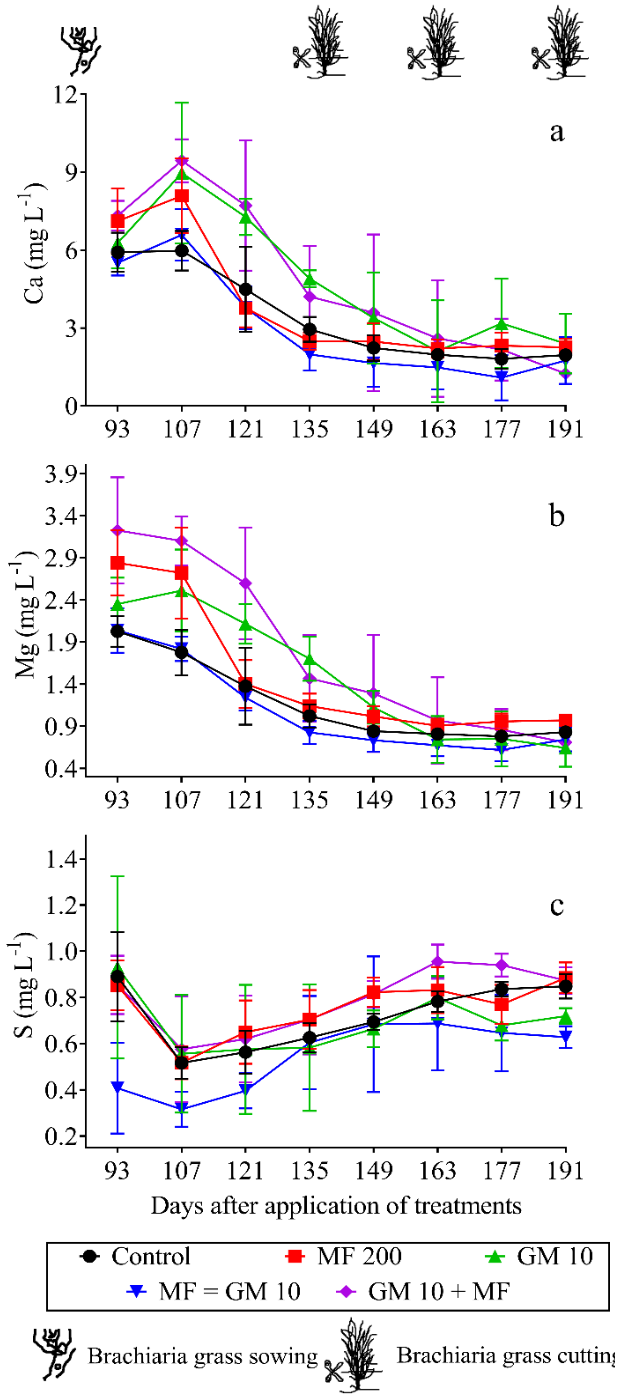

Figure 2. Residual effect of fertilization treatments on the concentrations of calcium (Ca) (a), magnesium (Mg) (b), and sulfur (S) (c) of the soil solution during the growth of the brachiaria grass. The treatments were applied in the first crop (broccoli). Control: unfertilized plants; MF200: mineral fertilizer at 200% of the recommended dose; GM10: 10 t ha^{-1} of the green manure; MF=GM10: mineral fertilizer supplying the same nutrient dose supplied with GM10; GM10+MF: GM10 + mineral fertilizer to supply the same amount of nutrient supplied with MF200. Vertical bars represent the standard deviation of the means (n = 4).

Active acidity, electrical conductivity, and macronutrient concentration of soil solution

The pH of the soil may change depending on the dose and fertilizer type used, soil characteristics and established crop (Veçozzi *et al*., 2018). In this study, the effect of the fertilizer type was only confirmed during 20 days, specifically, before the first cut of the brachiaria grass. In this period, lower pH with MF200 is attributed to the remaining amounts of urea, because it can promote the nitrification process, releasing H^+ to the soil solution. A different situation occurred with GM, which kept the pH similar to non-fertilized soils, possibly due to the capacity of organic matter to delay soil acidification (Shi *et al*., 2019). On the other hand, the pH of the soil changed due to the nutrient demand of the brachiaria grass, lower pH values were observed in the initial stage of plant growth, specifically after sowing and after brachiaria cuts. Lower pH in this stage was similar to that observed in the first crop (broccoli), where a negative correlation was observed between pH with plant growth and ion concentration in the soil solution (Peralta-Antonio *et al*., 2019). The previous indicates that, regardless of the plant species, the pH behavior of the soil solution continues to depend on the development of the plant, and the soil characteristic.

High EC values in all treatments, in the first three weeks after sowing or after brachiaria cutting, are attributed to the low rate of ion absorption due to the low nutrient demand in this growth phase (Garay *et al*., 2017). Higher EC values with GM10 compared to mineral fertilization (MF200 and FM=GM10) even before the first cut of the brachiaria grass, are attributed to the higher nutrient reserve maintained in the biomass of the GM. Of the total GM biomass initially applied, 52% was mineralized in the first crop (Peralta-Antonio *et al*., 2019) and the remaining 48% continued its decomposition process from the time of sowing of brachiaria grass, resulting in ion release in the soil solution.

The results suggest that the concentration of nutrients in the soil solution was influenced by the crop nutrient requirements, fertilizer type and fertilization dose used, similar to the reported by Miranda *et al*. (2006). The behavior of N, K, Ca, and Mg in the soil solution indicates that these nutrients presented the same nutrient absorption behavior in brachiaria grass, from germination to the last cut date. The behavior of S concentration was different from the other nutrients, since its value was constant or increased during the growth of brachiaria grass, indicating that it was less required by the crop. The effect of the fertilizer type and fertilization dose was detected by the difference in the concentration of N, K, Ca, Mg, and S between GM10 and mineral fertilization (MF=GM10 and MF200). The nutrients supplied with MF were quickly absorbed by the brachiaria grass because they were applied in inorganic form, whereas with GM, the nutrients were in the organic form and before being released into the soil solution need to go through the mineralization process (Lorensini *et al*., 2014). Thus, the lower concentration of nutrients with MF is attributed to the possible lower nutrient reserve in the soil, since in the first crop more nutrients were extracted in plants that received MF. Taking into account that the mineralization speed in GM occurs faster in the most labile fraction of the plant (Diniz *et al*., 2014), it is deduced that in the broccoli the leaf of the GM was the main source of nutrients for the plants, while in the brachiaria grass the main source of nutrients was the stem.

CONCLUSIONS

The residual effect on dry matter and nutrient extraction of brachiaria grass change depending on the fertilizer type and dose used. The residual effect of the fertilization treatments on the soil solution characteristics is only detected in the initial growth phase of brachiaria grass. In successive crops, the proportion of dry matter produced and the amount of nutrients extracted changes depending on the species used. To obtain similar nutrient recovery between green manure and mineral fertilization is necessary to consider the dry matter of more of one crop.

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