Bovine manure and rock powder and their influences on the chemical characteristics of a Latossolo soil type (yellow oxisols) under butter kale (*Brassica oleracea* L. var. acephala) cultivation

Estiércol bovino y polvo de roca y sus influencias sobre las características químicas de un tipo de suelo Latossolo (oxisoles amarillos) bajo cultivo de col rizada de mantequilla (*Brassica oleracea* L. var. acephala)



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Butter kale experiment view.

Photo: M.B. Pereira

# **ABSTRACT**

Chemical soil quality is one of the factors more quickly affected by anthropogenic degradation processes and is one of the more important components for the development of agriculture. Thus, this study aimed to evaluate the effects of different doses of cattle manure and rock powder on the chemical characteristics of soil cultivated with butter kale. The treatments were arranged in five randomized blocks in a  $4\times4$  factorial for the different doses of bovine manure (60, 120, 180 and 240 g/plant) combined with doses of rock powder (6, 12, 18 and 24 g/plant). Each block was composed of three plots, 18 m long and 1 m wide. The bed was composed of six portions, and each experimental plot consisted of 14 plants spaced at  $0.40\times0.40$  m. At the end of the experiment, the following were analyzed: pH, organic matter, P, K, Na, Mg, exchangeable acidity, cation exchange capacity (CEC), sum of base and base saturation. The doses of bovine manure and MB-4 provided an increase in pH, organic matter, concentration of phosphorus, potassium, sodium, calcium, magnesium,



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exchangeable acidity, cation exchange capacity, sum of the base and saturation of the soil base. The doses of 240 g of cattle manure and 24 g of rock dust generated an increase in the chemical properties of the soil.



Additional key words: biofertilizers; organic amendments; organomineral fertilizers; nutrient availability; soil suitability.

#### RESUMEN

La calidad química del suelo es uno de los factores más rápidamente afectados por los procesos de degradación antropogénica, y este es uno de los componentes más importantes para el desarrollo de la agricultura. Por lo tanto, este trabajo tuvo como objetivo evaluar los efectos de las diferentes dosis de estiércol de ganado y polvo de roca en las características químicas del suelo en cultivo de col rizada de mantequilla. Los tratamientos se organizaron en cinco bloques aleatorizados en un factorial de 4×4 en referencia a las diferentes dosis de estiércol bovino (60, 120, 180 y 240 g/planta) combinado con dosis de polvo de roca (6, 12, 18 y 24 g/planta). Cada bloque estuvo constituido por tres parcelas de 18 m de largo y 1 m de ancho, el lecho estuvo compuesto por seis porciones y cada parcela experimental constaba de 14 plantas espaciadas en 0,40×0,40 m. Al final del experimento se analizaron: pH, materia orgánica, P, K, Na, Mg, acidez intercambiable, capacidad de intercambio catiónico (CIC), suma de base y saturación por base. Las dosis de estiércol bovino y MB-4 proporcionaron un aumento en el pH, materia orgánica, concentración de fósforo, potasio, sodio, calcio, magnesio, acidez intercambiable, capacidad de intercambio catiónico, suma de la base y saturación de la base del suelo. Las dosis de 240 g de estiércol de ganado y 24 g de polvo de roca generaron un aumento en las propiedades químicas del suelo.

Palabras clave adicionales: biofertizantes; enmiendas orgánicas; organomineral fertilizers; disponibilidad de nutrientes; aptitud del suelo.

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### INTRODUCTION

Growing vegetables is done mostly conventionally. However, alternative systems such as an ecological basis using organic fertilizers such as manure are gaining prominence, especially because of both the quality of food and the environment concern (Vergel et al., 2015). This concern also extends to the forms of cultivation, driving global demand for techniques and technologies that replace the use of agricultural-industrial inputs with high energy, economic and social costs with low-cost organic inputs. The use of organic fertilizers contributes to environmental sustainability and increases in agricultural production (Dick and Gregorich, 2004; Conway and Barbier, 2013; Souza and Resende, 2014; Li et al., 2017).

Trying to find solutions to minimize impacts on agricultural production systems which uses techniques of conventional production generating significant losses of arable land, in recent years empirical efforts

of practices were carried out in ecological family production systems (Gliessman, 2000; Meirelles, 2004). The use of materials from natural sources in agricultural fertilizer has been highlighted as a way to meet and balance nutritional, chemical and biological soil needs.

The growth of organic production and ecological bases worldwide is a response to the demand from society for a sustainable environment and production practices that generate safer and healthier products for fairer social relations and trade (Lima-Filho and Quevedo-Silva, 2012; Albuquerque Júnior *et al.*, 2013; Braga Júnior *et al.*, 2014).

Organic fertilizers are an alternative that is easy to acquire and low cost, with several benefits, such as increased water holding capacity and infiltration, increased pH and ability to exchange cations (CEC),

providing nutrients (Clemente et al., 2012; Silva et al., 2013; Dutra et al., 2015) that provide higher productivity of various vegetables, including brassica (Candian et al., 2015).

Among the materials commonly used in the composition of natural fertilizers, manure and rock powders have been widely used. Manure mainly stands out as a nitrogen source, and rock powder is an excellent alternative of calcium, magnesium, potassium and phosphorus, as well as all essential plant micronutrients (Clemente et al., 2012; Silva et al., 2013; Dutra et al., 2015; Nascimento et al., 2015, 2017).

Manure is the preferred source for supplying organic matter to soil cultivated with vegetables. Organic carbon is a power source for the microbial mass in soil; it improves soil physical, chemical and biological properties and is an important option to maintain sustainable agricultural practices (Ceretta *et al.*, 2010; Silva *et al.*, 2010; Yang *et al.*, 2016).

Rock powder is a good fertilization alternative because, depending on availability, it has a low market value. It is the material that remains after breaking rocks, mainly in quarries and mining. Usually, it contains nutrients in varied amounts and quality, depending on the source of the material or rock mother.

The material can be used as a nutritional supplement, releasing nutrients over years, and, in some cases, releasing nutrients quickly (Ribeiro *et al.*, 2010).

Therefore, the aim of this study was to evaluate the effect of different doses of manure and rock powder on the chemical characteristics of soil cultivated with butter kale

## **MATERIALS AND METHODS**

This experiment was conducted on Campus III of the experimental area of the Federal University of Paraíba, in the municipality of Bananeiras-PB (CCHSA/UFPB), located at 6°45′4″ S and 35°38′0″ W.

According to the Koppen classification, climate is considered Type As - Tropical rainy, dry summer, irregular annual rainfall distribution (1,174.7 mm), with a maximum annual temperature of 27°C and minimum of 18.8°C, and annual average of 22°C. The meteorological data for the experimental period are shown in Tab. 1.

The treatments were arranged in five randomized blocks in a  $4\times4$  factorial for the doses of bovine manure (BM) (60, 120, 180 and 240 g/plant) combined

Table 1. Representation of relative humidity, soil temperature, rainfall and average air temperature in the period from October 2014 to February 2015. Data from the weather station, EFSA, PB (2015).

Relative humidity (%)					
	October	November	December	January	February
	87.64	84.39	84.36	82.32	82.12
Soil temperature (°C)					
Layer	October	November	December	January	February
10 cm	22.79	23.67	23.58	24.06	23.12
20 cm	22.94	24	23.62	24.17	25.14
40 cm	22.96	23.78	23.68	23.78	24.65
Rainfall (mm)					
	October	November	December	January	February
	70.9	30.3	27.3	34.9	35.1
Average air temperature (°C)					
	October	November	December	January	February
Maximum	22.31	23.01	23.4	23.87	22.32
Average	21.52	22.46	22.58	22.95	21.56
Minimum	20.81	21.7	22.01	22.31	20.85

with powder rock doses (6, 12, 18 and 24 g/plant). The doses were selected based on the organic fertilization recommendation for leaf cabbage crops (Trani et al., 2015). Each block was composed of three plots, 18 m long and 1 m wide. The bed was composed of six portions, and each experimental plot consisted of 14 plants spaced at  $0.40 \times 0.40$  m, for a total of 62,500 plants/ha, 85 portions.

The soil of the prevailing experimental area corresponded to a yellow Oxisol with a medium texture, soft curly relief, deep profile, good drainage, moderate moisture retention capacity and Sandy Clay Loam textual class.

Before conducting the experiment, 25 soil simple samples were collected in the experimental area (0-20 cm). The simple samples were homogenized in containers forming five composite samples specimens; afterwards, the chemical and physical characterizations were made using the methodology suggested by Embrapa (2017). The samples had the following characteristics: sand  $(g kg^{-1}) = 740$ ; silte  $(g kg^{-1}) =$ 120; clay (g kg<sup>-1</sup>) = 140; clay dispersed in water (g  $kg^{-1}$ ) = 78; degree of flocculation (%) = 30; dispersion index (%) = 56; soil density (kg dm<sup>-3</sup>) = 1.61; particle density (kg dm<sup>-3</sup>) = 2.60; total porosity (m<sup>3</sup> m<sup>-3</sup>) = 0.49; macroporosity ( $m^3 m^{-3}$ ) = 0.049; microporosity  $(m^3 m^{-3}) = 0.442$ ; field humidity capacity to 0.01 to 1.5 MPa ( $m^3 m^{-3}$ ) = 0.209; wilting point tensions to  $0.01 \text{ to } 1.5 \text{ MPa } (\text{m}^3 \text{ m}^{-3}) = 0.134$ ; available water (g  $kg^{-1}$ ) = 0.074; pH in H<sub>2</sub>O (1.0:2.5) = 6.50; P (mg dm<sup>-1</sup>  $^{3}$ ) = 69.5; K<sup>+</sup> (cmol<sub>c</sub> dm<sup>-3</sup>) = 0.20; Ca<sup>2+</sup> (cmol<sub>c</sub> dm<sup>-3</sup>) = 3.50; Mg<sup>2+</sup> (cmol<sub>c</sub> dm<sup>-3</sup>) = 2.20; Na<sup>+</sup> (cmol<sub>c</sub> dm<sup>-3</sup>) = 0.20;  $H^+ + Al^{3+}$  (cmol<sub>c</sub> dm<sup>-3</sup>) = 1.50;  $Al^{3+}$  (cmol<sub>c</sub>  $dm^{-3}$ ) = 0.00; bases sum (cmol<sub>c</sub> dm<sup>-3</sup>) = 6.1; cation exchange capacity in pH7 (cmol<sub>c</sub> dm<sup>-3</sup>) = 7.6; effective acidity (aluminum saturation) (%) = 0.00; V (%) = 80.26 and organic matter (g dm<sup>-3</sup>) 24.96.

Butter kale seedlings of the *Brassica oleracea* L. var. acephala were produced in the substrate composed of vegetable soil (60%), sand (30%) and bovine manure (10%) and planted in disposable cups. During the production of seedlings, a simple biofertilizer foliar application was done (Silva *et al.*, 2012) to supply nutrients.

Conventional soil tillage preparation was used with plowing, harrowing and a subsequent lifting of the beds. Holes were made at 0.20 m in the transplanting seedling stage when the fertilizer was also added using bovine manure and rock powder (MB-4®) (Mineração Barreto SA - Arapiraca, Alagoas-Brasil) (Mibasa,

2007) according to the proposed treatments. The bovine manure was analyzed and had the following characteristics: pH in  $H_2O$  (1.0:2.5) = 8.25; P (mg dm<sup>-3</sup>) = 5.06; K<sup>+</sup> (cmol<sub>c</sub> dm<sup>-3</sup>) = 0.0018; Ca<sup>2+</sup> (cmol<sub>c</sub> dm<sup>-3</sup>) = 4.00; Mg<sup>2+</sup> (cmol<sub>c</sub> dm<sup>-3</sup>) = 3.90; Na<sup>+</sup> (cmol<sub>c</sub> dm<sup>-3</sup>) = 1.08; H<sup>+</sup> + Al<sup>3+</sup> (cmol<sub>c</sub> dm<sup>-3</sup>) = 0.495; Al<sup>3+</sup> (cmol<sub>c</sub> dm<sup>-3</sup>) = 0.00; Ca<sup>2+</sup> + Mg<sup>2+</sup> = 7.90; electrical conductivity (dS m<sup>-1</sup>) = 5.48; cation exchange capacity (cmol<sub>c</sub> dm<sup>-3</sup>) = 9.47; base saturation (%) = 5.10; base sum = 9.69; and organic matter (g kg<sup>-1</sup>) = 100.82.

The rock powder was analyzed and had the following characteristics: pH = 6.80; P (mg dm<sup>-3</sup>) = 42.82; K<sup>+</sup> (cmol<sub>c</sub> dm<sup>-3</sup>) = 0.12; Ca<sup>2+</sup> (cmol<sub>c</sub> dm<sup>-3</sup>) = 5.65; Mg<sup>2+</sup> (cmol<sub>c</sub> dm<sup>-3</sup>) = 1.95; Na<sup>+</sup> (cmol<sub>c</sub> dm<sup>-3</sup>) = 0.30; H<sup>+</sup> + Al<sup>3+</sup> (cmol<sub>c</sub> dm<sup>-3</sup>) = 1.70; Al<sup>3+</sup> (cmol<sub>c</sub> dm<sup>-3</sup>) = 0.00; Ca<sup>2+</sup> + Mg<sup>2+</sup> = 7.60; cation exchange capacity (cmol<sub>c</sub> dm<sup>-3</sup>) = 9.72; base saturation (%) = 97.77; base sum = 74.56 and organic matter (g kg<sup>-1</sup>) = 43.85. The rock powder came from Neoproterosóica Brasiliane s.I - Granitoids (Santo *et al.*, 2002), composed of 55% sand, 43% silt and 2% clay.

After transplantation and fertilization, the beds were covered with straw, *Brachiaria decumbens*, using a 2 cm thick layer in order to enhance the development of biological activities, control spontaneous plants, and maintain soil moisture.

Irrigation was done with a drip system according to the water needs of the butter kale culture, adopting shifts of 9 h irrigation in the initial phase when the crop was established, increasing up to 1 d in the final stage, corresponding to the last 20 d of the crop cycle. Self-cleaning drippers with self-compensating emitters with a flow of  $6.0 \text{ L} \text{ h}^{-1}$  were used, 0.40 m spaced between themselves and distributed along the lines. The methodology used for the irrigation calculation (Cleves *et al.*, 2016) was based on Etc = Kc x ETo (crop evapotranspiration = crop coefficient × evapotranspiration of reference (mm d<sup>-1</sup>).

At the end of the experiment, 60 d after transplantation, the following were measured: pH, organic matter (OM), phosphorus content (P), potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), acid exchangeable (H and Al), cation exchange capacity (CEC), base sum (BS) and base saturation (V). For the soil fertility analysis, soil samples were collected in the 0 to 20 cm layer. The analyses were performed at the Soil Laboratory of the Federal University of Paraiba, Campus III, Bananeiras (CP), with the method suggested by Embrapa (2017).

The data were analyzed with analysis of variance and regression using Sisvar, and the means were compared with the Tukey test, up to 5% probability. The choice of the regression model was based on the significance of the regression coefficients using the t-test at 5% probability, the coefficient of determination ( $R^2 = SQReg / SQtrat$ ) and a biological behavior study.

### **RESULTS AND DISCUSSION**

It was observed that the pH, organic matter, phosphorus content, potassium, sodium, calcium, magnesium, exchangeable acidity, CEC, amount of base and base saturation presented a significant interaction between the doses of bovine manure (BM) and doses of rock powder (MB-4). The manure doses significantly influenced the variables, except pH and magnesium, and the rock powder doses affected the pH, organic matter, potassium, sodium and sum of base.

There was an increase in pH (Fig. 1A) in the treatments with BM and MB-4 interactions. The pH of the soil treated with the doses of 120 g of BM + 18 g of MB-4 had a higher value (7.07), and the minimum value (6.71) was observed with BM 120 g + 6 g MB-4, meaning the pH increased as the MB-4 amount increased. Comparing the pH values before the experiment, which was 6.50, and after the experiment, the values increased with the fertilization used, but it was observed that the rising pH not harmed the development of butter kale.

The higher pH values were probably due to the buffering of bicarbonates and elemental cations introduced by the bovine manure and rock powder, such as Ca<sup>2+</sup> and Mg<sup>2+</sup> (Mkhabela *et al.*, 2005). Yang *et al.* (2016), when working with watermelon, observed a higher pH in plants fertilized with 80 t ha<sup>-1</sup> of bovine manure. Resende *et al.* (2006) also observed an increase in the pH of clayey soil that received doses of alkaline ultramafic rock powder.

For the phosphorus content, the highest value (169.94 mg dm $^{-3}$ ) was found in the soil treated with 60 g of BM + 6 g of MB-4, corresponding to the lower doses, while the smaller levels were found with the dose 120 + 18 g MB-4, presenting a decrease of 77.35 mg dm $^{-3}$  from the higher values found with fertilization (Fig. 1B).

It was also found that the phosphorus content had significant differences between the treatments, with higher levels observed in the soil at the end of the experiment (Tab. 1), when the phosphorus presented 69.5 mg dm<sup>3</sup>. The increase in phosphorus in the soil may have occurred because of the increase in organic matter, as OM is an important controller of phosphorus synthesis in the soil and the availability of this element for plants depends on its fixation (Harrison, 1987; Andrade *et al.*, 2003; Dao, 2004).

The interaction of BM and MB- 4 doses provided a quadratic effect on the potassium content (Fig. 1C), sodium (Fig. 1D) and calcium (Fig. 1E). For these elements, it was observed that the maximum values in the soil was obtained with the 120 g BM dose, leading to a dose reduction from 180 to 240 BM.

The potassium content reached maximum values in the treatment with BM 120 g + 18 g of MB-4, 0.409 mg dm<sup>-3</sup> (Fig. 1C), twice that was measured at the beginning of the experiment (0.20 mg dm<sup>-3</sup>). Sodium presented the highest level in the treatment with BM 120 g + 6 g of MB-4, obtaining 0.333 cmol<sub>c</sub> dm<sup>-3</sup> (Fig. 1D). Thus, both the potassium and sodium values obtained with the soil analysis were higher after the completion of the experiment.

The lower sodium levels were observed at doses of BM 240 g and 6 g of MB-4 (Fig. 1D), indicating that doses higher than 4-MB can cause soil salinization, making it unsuitable for cultivation.

For the calcium content, it was found that the doses of 240 g of BM + 12 g of MB-4 provided an increase of 0.40 cmol<sub>c</sub> dm<sup>-3</sup> (Fig. 1E) when compared with the results of the soil analysis performed at the beginning of the experiment. After the experiment, the soil calcium content was  $3.8 \text{ cmol}_c \text{ dm}^{-3}$ .

The magnesium content in the soil increased as the BM doses increased, with maximum values of 3.96 cmol<sub>c</sub> dm<sup>-3</sup> in the soil fertilized with 240 g of BM and 12 g of MB-4, leading to a reduction of 1.03 cmol<sub>c</sub> dm<sup>-3</sup> as a result of the application of maximum MB-4 doses. After the completion of the experiment, an increase of 1.76 cmol<sub>c</sub> dm<sup>-3</sup> of magnesium was observed in the soil, as compared to that observed before the experiment, with 2.20 cmol<sub>c</sub> dm<sup>-3</sup> (Fig. 1F).

The increases verified in the soil for the potassium, phosphorus, sodium, calcium and magnesium can be associated with the joint application of bovine manure and MB-4 since the application of bovine manure and rock powder can increase the availability of nutrients in soil because of the greater solubility of rock powder and mineralization of bovine manure

(Camargo et al., 2012). As seen in the present study, an increase in nutrient contents in soil has been observed in different species grown under conditions of fertilization with bovine manure and/or rock dust, such as *Raphanus sativus* L. (Tito et al., 2019), *Annona muricata* L. (Malta et al., 2019), *Phaselous vulgaris* L. (Gotz et al., 2016); and *Helianthus annuus* L., Sol Noturno variety (Andrade et al., 2015).

The bovine manure and rock powder had a broadspectrum effect by acting on the chemical, physical and biological mechanisms of the soil. Because bovine manure is a source of organic matter that, when added to soil in proper amounts, promotes mineralization, that is, it is easily released to the soil solution, absorbed by the plants or leachate (Clemente *et al.*, 2012; Silva *et al.*, 2013).

The interaction between the doses of BM and MB-4 provided an increasing effect of exchangeable soil acidity on all analyzed treatments (Fig. 2A). However, almost all observed values were lower than the

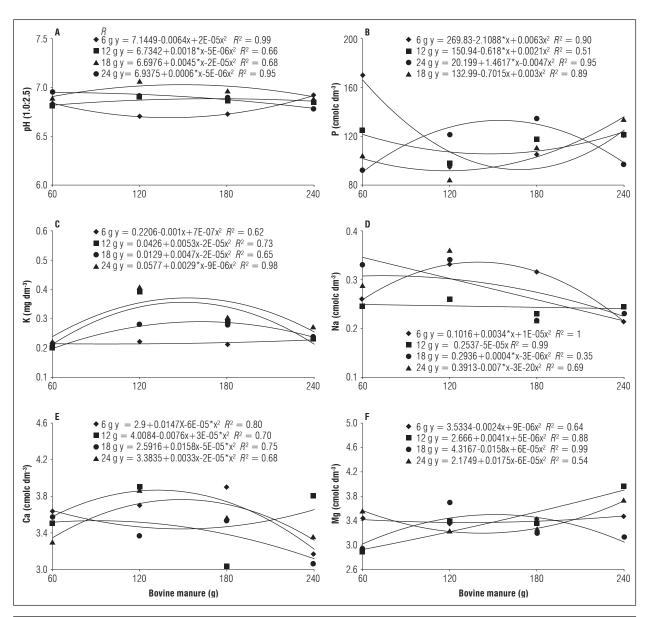


Figure 1. Effect of bovine manure doses and rock powder on the pH (A), phosphorus content (B), potassium (C), sodium (D), calcium (E) and magnesium (F) in yellow Oxisol under butter kale cultivation.

values in the soil analysis before the experiment, in Tab. 1, except for treatments 240 g of BM + 12 g MB-4, 240 g of BM + 18 g MB-4 and 240 g of BM + 24 g of MB-4, which presented slightly higher values for the initial results, respectively, 1.54 and 1.59 cmol<sub>c</sub> dm<sup>-3</sup>.

The effects of organic compounds on the exchangeable soil acidity can be associated with the complexation of Al by organic matter, which promotes the removal of that element from the soil solution and the formation of the dissolved complex by the dissolved organic carbon. The replacement of Al<sup>3+</sup> with Ca<sup>2+</sup> in the cationic complex immobilizes Al<sup>3+</sup> with organic ligands, and BM reduced toxic aluminum from the ground (Hargrove and Thomas, 1981; Cancès *et al.*, 2003; Zambrosi *et al.*, 2007). Thus, during the decomposition of organic matter, aluminum can be complexed in the soil solution with fulvic acids (Anghinoni and Salet, 2000) or organic acids with a low molecular weight, such as citric, oxalic and tartaric acids.

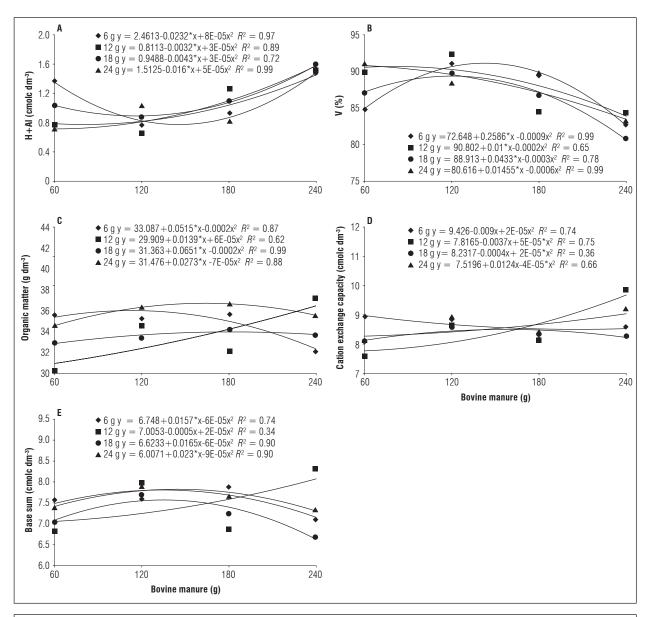


Figure 2. Effect of bovine manure and rock powder on exchangeable acidity (A), base saturation (B), organic matter (C), cation exchange capacity (D) and sum bases (E) in yellow Oxisol under butter kale cultivation.

However, the increase of the exchangeable acidity as a result of the increase of bovine manure can also be associated with the increase of the MB-4 doses or with material mineralization processes or through removing a greater amount of nutrients by cultivated plants (Hanisch *et al.*, 2013; Ramos *et al.*, 2014).

For the base saturation, it was found that the interaction between the doses of BM and MB-4 gave the highest interaction values, with up to 92% doses of 120 g BM + 12 g of MB-4, with a subsequent reduction of the doses of 240 to 180 g BM (Fig. 2B). This result shows excellent soil fertility indices, especially for the contents of the Ca, Mg, K and Na in the soil. May *et al.* (2007) emphasized that the values of the base saturation, which are for the butter kale between 70 and 80%, are relevant for the productivity of this culture.

The organic matter content (Fig. 2C), cation exchange capacity (Fig. 2D) and values of the base sum (Fig. 2E) presented superior results than those observed in the initial soil characterization. And doses of 240 g BM + 12 g MB-4 increased more than those values.

Increases in organic matter levels, cation exchange capacity and base sum values as a function of fertilization with BM and or MB-4 were also observed by Barcellos *et al.* (2015) when analyzing a typical dystrophic latosol Bruno, Silva *et al.* (2018) and evaluating a culture of *Brachiaria brizantha* cv. Marandú, Batista *et al.* (2017) in a red-yellow latosol under soybean and sorghum cultivation, and by Alovisi *et al.* (2017) when analyzing the availability of nutrients in soil during three incubation periods with rock dust.

The BS, CEC and V parameters were calculated with a base on Ca, Mg, K, and  $H^+ + Al^3$ . Therefore, as in the treatments with the greatest presence of bovine manure (120 to 240 g), there was greater availability of Ca, Mg, K and a reduction of  $H^+ + Al^3$ . The BS, CEC and V values had significant changes.

The observed results are similar to those obtained by Silva *et al.* (2012), where the same, testing fertilization with 6 types of rocks in a red-yellow oxisol observed that the pH values increased with the rock powder. For Ca, Mg, P and K, according to these authors, there was also an increased release of these elements, which was related to the mineralogical composition of the studied rocks, where the rock powders reacted when in contact with the soil and thus were released. They also observed that the

analyzed rock powders provided an increase in the CEC and V values of the soil.

Ribeiro *et al.* (2010), in a greenhouse, applied different doses of phlogopite powder, ultramafic alcaline and pyroclastic gap in a sample yellow Oxisol dystrophic and found that the rock powders released higher amounts of K and provided P. These rock powders promoted further correction in the soil acidity and increased the base saturation as a result of a possible release of bases contained in the minerals of these rocks.

### CONCLUSION

The doses of bovine manure and MB-4 provided an increase in pH, organic matter, concentration of phosphorus, potassium, sodium, calcium, magnesium, exchangeable acidity, cation exchange capacity, sum of the base and saturation of the soil base.

The doses of 240 g of cattle manure and 24 g of rock dust generated an increase in the chemical properties of the soil.

**Conflict of interests:** The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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