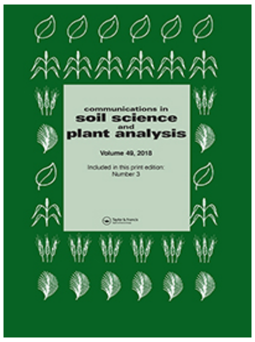

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The Impact of Organic Biofertilizer Application in Dairy Cattle Manure on the Chemical Properties of the Soil and the Growth and Nutritional Status of Urochroa Grass

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ABSTRACT

The management of nitrogen (N) fertilization in pasture has great importance for yield and maintenance of Brazilian livestock. The objective of this study was to evaluate soil chemical attributes, shoot dry weight yield (SDWY) and roots dry weight yield (RDWY), nutritional status and nutritive value of Mulato II *Urochloa* grass that received organic biofertilizer rates as N source. The treatments were arranged in completely randomized blocks, were fertilized with six organic biofertilizer rates (0, 25, 50, 100, 200, and 400 m^3 ha^{-1}) and five replicates during four harvest cycles. There was a quadratic response in the accumulated SDWY for up to a rate of 400 m^3 ha^{-1} and RDWY with 330 m^3 ha^{-1} of organic biofertilizer. The organic biofertilizer influenced the soil chemical attributes and foliar nutrient concentration. However, only the crude protein content presented a quadratic response, and neutral detergent fiber and acid detergent fiber levels were not affected by the influence of organic biofertilizer applied to the soil.

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KEYWORDS
Cattle manure; crude protein; nutritional status; roots yield; shoot dry weigh yield

Introduction

The ruminant's production in pasture is undoubtedly one of the most efficient forms of animal feed. However, maintaining the compatibility of forage productivity with soil and climatic conditions in order to keep the system sustainable over time is one of the major problems in livestock farming, because of degradation of these ecosystems. In this sense, the search for a solution of the problem involves not only the identification of suitable forage materials for different conditions, but also the optimal levels of fertilization that guarantee perennial yield.

Due to the high nitrogen (N) requirement for crops and the current need for this nutrient to be used organically, alternative sources of N, that is, organic biofertilizer, such as cattle manure, for pasture fertilization are being used and studied to reduce environmental pollution, as well as to reduce production costs, building on sustainable management. Furthermore, the resolution CONAMA (1997) requires that the nutrients in all animal manures are taken into account in farm nutrient management plans.

The Mulato II *Urochloa* grass (commercialized in Brazil as Convert HD 364 since 2009) was developed from three generations of hybridization between ruzigrass (clone 44–6) and signalgrass (*U. decumbens* (Stapf) R. D. Webster (syn. *U. decumbens* (Stapf) R. D. Webster) (cultivar Basilisk)), where the first generation was exposed to open pollination from lines of *U. brizantha*, including Marandu palisadegrass (Argel et al. 2007).

The Mulato II *Urochroa* grass is characterized by a number of leaves ranging from nine to ten per tiller with a high tillering potential, which contributes to good soil cover (Argel et al. 2007). This grass

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has a high potential for forage production, depending mainly on the soil characteristics in which it will be planted. The use or lack of fertilization, whether with nitrogen or other sources, will also influence the productivity of the grass. It is a grass that easily adapts to tropical and subtropical conditions and tolerates soils with drainage deficiencies, if the flooding is not permanent, but requires medium to high fertility soils with good drainage (Argel et al. 2007). The introduction of new grass cultivars or hybrids on the market generates expectations for cattle ranchers as new options for establishment and maintenance of pastures (Bonfim Silva et al. 2014; Moreira et al. 2010).

On the other hand, the maintenance and productivity of forage plants can be maximized by increasing the use of fertilizers. In this sense, fertilization, especially with nitrogen, is fundamental for the increase of biomass production. The increase in the N content in the soil through fertilization is one way to increase productivity in pastures, especially when the forage responds to N application (Martuscello et al. 2005). However, the N efficient use by the plant depends on several factors, among them, source, form and application time, N rates and fractionation, soil and climatic conditions, the potential response of the plant, and the presence and animal stocking dose, among others.

Bovine biofertilizer from anaerobic biodigestors contains N in greater quantities. Soares Filho et al. (2015) applied bovine biofertilizer to Tierra Verde bermudagrass (*Cynodon dactylon* (L.) Pers.) and Lemes et al. (2013) to alfalfa (*Medicago sativa*), and the authors observed that it positively influenced the soil and leaf chemical contents and the nutritional quality of alfalfa (Lemes et al. 2016). In Brazil, there is a considerable part of cattle production conducted in intensive rearing systems, resulting in a large volume of liquid slurry, with high fertilizer potential that, when properly stored and correctly used, can supply nutrients to the plants and improve soil chemical conditions.

Because grassland species can respond differently to fertilizer application, it was hypothesized that the response of grassland species to animal manure would also vary. This was tested by assessing the responses of *Urochroa* grass to different doses of dairy cattle biofertilizer. The objective of this work was to evaluate soil chemical attributes, dry mass production of shoot and roots, macro and micronutrient contents in the plant and the chemical composition of Mulato II *Urochloa* grass submitted to doses of organic biofertilizer as the N source.

Materials and methods

Growth conditions, biofertilizer availability and experimental design

The experiment was conducted during the spring and summer in a greenhouse conditions (average temperature of 26.4°C and photoperiod of 14/10 h, day/night) in plastic pots with 5 liters of capacity, cultivating the forage Mulato II *Urochloa* grass, at São Paulo State University (UNESP-FMVA) in Araçatuba County, São Paulo State, Brazil (21°8′ LS, 50°25′ LW, 415 m a.s.l.).

The organic biofertilizer used in the experiment comes from manure and cow urine from dairy cows after being fermented anaerobically in a bio-digester. The effluent presented the following chemical attributes: organic matter (OM): 32.90 *Urochloa* grass total N 0.97 g L^{-1}, phosphorus (P) in P_2O_5: 1.27 g L^{-1}, potassium (K) in K_2O: 0.68 g L^{-1}, sulfur (S): 0.09 g L^{-1}, calcium (Ca): 1.27 g L^{-1}, magnesium (Mg): 0.35 g L^{-1}, copper (Cu): 1325 g L^{-1}, manganese (Mn): 1215 g L^{-1}, zinc (Zn): 1663 g L^{-1}, iron (Fe): 8382 g L^{-1}, density: 1010 g mL^{-1}, and pH 7.5.

An Ultisol (Embrapa 2013), collected in the 0–20 cm depth were used, with the following chemical attributes: P (resin) = 3 mg dm^{-3}; OM = 17 g dm^{-3}; pH in $CaCl_2$ = 4.6; K = 1.6 $mmol_c$ dm^{-3}; Ca = 13 $mmol_c$ dm^{-3}; Mg = 6 $mmol_c$ dm^{-3}; H + Al = 34 $mmol_c$ dm^{-3}; base sum (SB) = 20.6 $mmol_c$ dm^{-3}; cation exchange capacity (CEC) = 54.6 $mmol_c$ dm^{-3} and base saturation (V): 37.7%. The need for liming was determined by the base saturation method to reach a value of 60% (Quaggio, Raij, and Malavolta 1985). The soil of each pot was incubated with calcium carbonate ($CaCO_3$): magnesium carbonate ($MgCO_3$), using a 3:1 ratio, for 30 days before planting, receiving constant irrigation during this period, to maintain at 80% of field capacity.

After the incubation period, the soil was dried again for seven days and placed in plastic trays and the following nutrients were added: monocalcium phosphate [$Ca(H_2PO_4)_2$], 200 mg dm^{-3} of P; potassium sulfate (K_2SO_4), 150 mg dm^{-3} of K and 61.53 mg dm^{-3} S; boric acid (H_3BO_3), 0.5 mg dm^{-3} boron (B); copper sulfate ($CuSO_4$) 1.0 mg dm^{-3} copper (Cu); molybdic acid (H_2MoO_4), 0.1 mg dm^{-3} molybdenum (Mo); manganese sulfate ($MnSO_4$), 3 mg dm^{-3} manganese (Mn), and zinc sulfate ($ZnSO_4$), 2.0 mg dm^{-3} of zinc (Zn). The organic biofertilizer and the other nutrients were applied with a graduated pipette, applied one fifth at sowing, at intervals of seven days in the first cycle. In the other cycles were five applications, with the first one after the harvest and the others at intervals of 5 days. After four days, the sowing of Mulato II *Urochloa* grass was carried out. The experimental design was a completely randomized with five replicates, arranged in subdivided plots, with repeated measures in time (four growth cycles). In the main plots were different organic biofertilizer rates (0, 25, 50, 100, 200, and 400 m^3 ha^{-1}).

Plant harvest and measurements of productive and nutritional parameters

Two weeks after the emergence of Mulato II *Urochloa* grass, thinning was performed to keep five uniform plants per pot. Deionized water for irrigation was used. The grass was evaluated when it reached an average height of 35 cm, and the harvest was done to 15 cm of the surface of the ground. Four growth cycles with a 6-week interval were evaluated. After each harvest, the material was identified, weighed and oven dried at ±60°C until it reached constant mass, and subsequently weighed on a precision balance to quantify SDWY. After drying, the samples were ground in a Wiley type mill the bromatological analyses were carried out, and the concentrations of the foliar nutrients (N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn) were determined according to Malavolta, Vitti, and Oliveira (1997).

The roots were collected at the end of the experiment and washed in running water until all existing soil was removed, using 2-mm mesh sieves. To determine the root dry mass the samples were properly identified bagged, and the material was then dried as described above. After they were dried in forced ventilation at ±60°C until reaching a constant mass, they were weighed on a precision balance to quantify the RDWY. All plant material collected during the harvests was dried in a forced-ventilation oven at 60°C until it reached a constant mass, and subsequently weighed on a precision balance to quantify the aboveground and RDWY. After weighing, each component of the plant was ground separately in a Wiley-type mill to determine concentrations in plant tissues.

The CP (crude protein) concentration was determined according to AOAC (1990) by multiplying the N concentration (%) by 6.25. The fractions of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the method of Campos, Nussio, and Nussio (2004). After four growth cycles, soil samples were collected at the end of the experiment to perform a chemical analysis according to Van Raij et al. (2001).

Statistical analysis

The statistical analysis was carried out with Assistat version 7.7 beta statistical software. An analysis of variance (ANOVA) was performed. When F-test revealed significant differences between biofertilizer rates, linear and quadratic regressions were carried out. The linear and quadratic regressions used to estimate each data set were chosen by taking into consideration the criteria of the higher significance level of the entire mathematical model ($Pr \geq F$), the significance level of the regressions coefficients and the higher value of the coefficient of determination (R^2) of the regression.

Results

Shoot dry weight and roots dry weight yield

Higher organic biofertilizer rates affected the SDWY of Mulato II *Urochroa* grass, changing the SDWY and RDWY in forage of harvests in both growth periods (Figure 1) and the SDWY

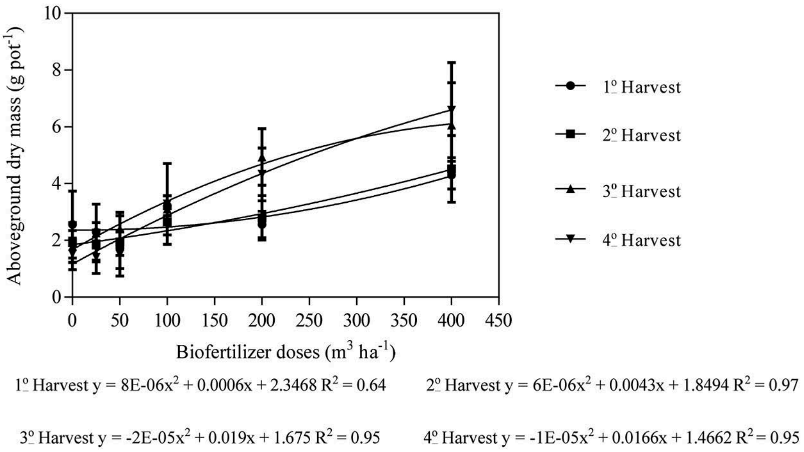

Figure 1. Development of the interaction for medium shoot dry weight yield reduction, as a function of harvests and organic biofertilizer rates in Mulato II *Urochroa* grass. The vertical bars associated with symbols represent the mean standard error (n = 5). All equation models and their coefficients are significant at 1% level.

accumulation (Figure 2a). The highest numbers were obtained with a biofertilizer supply of 400 m^3 ha^{-1}, and at the highest level, the third and fourth harvests were, on average, 55% higher, compared to the two initial harvests (Figure 1). During the initial growth (first and second harvests), plants showed quadratic increases in the SDWY response of lower magnitude up to 200 m^3 ha^{-1}, but showed quadratic increases with a biofertilizer supply of 400 m^3 ha^{-1}.

The SDWY as a function of the four harvests of Mulato II *Urochloa* grass was significant in the ANOVA for rates, harvests, and interaction of rates and harvests. The biofertilizer rate positively and quadratically affected the accumulated SDWY, when evaluated by harvests and RDWY (Figures 1 and 2). The response in SDWY was cumulative and higher for the third and fourth growth harvests, with the first two harvests having quadratic responses of smaller magnitude. The quadratic behavior of organic biofertilizer dose on forage SDWY is an indication that the plants responded positively to the application after the harvest and the interval between harvests was enough for the plants to reach their maximum accumulated yield (Figure 2a).

In first two harvests, the applied biofertilizer rates were insufficient to increase the SDWY. However, because the organic biofertilizer was applied again after each forage harvest, nutrient accumulation occurred in the pots, which resulted in a higher N input for the plants to express a greater response potential during the experimental period (Figure 1). The third and fourth harvests

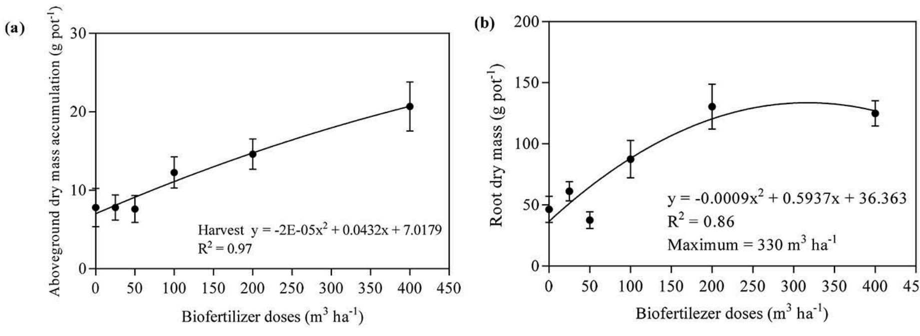

Figure 2. Accumulation aboveground growth DM (a) and roots (b) with biofertilizer rates in Mulato II *Urochroa* grass. The vertical bars associated with symbols represent the mean standard error (n = 5). All equation models and their coefficients are significant at 1% level.

showed that the SDWY of Mulato II *Urochroa* grass would support an even greater response if higher biofertilizer rates were applied up to 830 m^3 ha^{-1} (value estimated by the regression equation in the fourth harvest). The accumulated SDWY presented significant result from the ANOVA for the organic biofertilizer rates (Figure 2a). The biofertilizer rates positively affected the accumulated total dry weight yield, with a quadratic effect up to 400 m^3 ha^{-1}.

The supply of organic biofertilizer resulted in significant changes in the root growth performance (Figure 2b). For the RDWY after the harvest at the end of the experiment, there was a significant effect on the biofertilizer rates (Figure 2b). From the ANOVA, the quadratic effect of the N fertilization on RDWY was verified, and the maximum yield was obtained with 330 m^3 ha^{-1} (Figure 2b). RDWY and total dry weight yield (RDWY+SDWY) were positively correlated with a shoot/root dry weight of 0.78, a shoot/total dry weight of 0.83 and a root/total dry weight of 0.99.

Soil chemical properties

As for the soil chemical attributes at the end of experiment, a significant difference was observed with the application of increasing organic biofertilizer rates for the variables OM, pH, K^+, Ca^{2+}, Mg^{2+}, SB, $S\text{-}SO_4^{2-}$, CEC, and V. The P content in the soil did not present a significant difference with organic biofertilizer application. The P content was very low, varied from 5.4- to 6.6 g dm^{-3}. The highest OM content was found in the treatment that did not receive the fertilizer and in the initial rates of 25 and 50 m^3 ha^{-1} (Figure 3a). Each pot that received the increasing biofertilizer rates had a small reduction in these contents. The soil pH varied from 5.8 to 6.3 and had a quadratic effect with little variation with the biofertilizer application with a tendency to increase with higher applications (Figure 3b).

The K^+ content presented a quadratic response with organic biofertilizer application up to 400 m^3 ha^{-1}, and the contents varied from 2.3- to 6.3 $mmol_c$ dm^{-3}, presenting a value considered high in the soil (3.1–6.0 $mmol_c$ dm^{-3}) (Figure 3c). The Ca^{2+} content had a gradual but steady increase with the highest biofertilizer rates, ranging from 19.2- to 31.4 $mmol_c$ dm^{-3} presenting a value also considered high (above 7 $mmol_c$ dm^{-3}) (Figure 3d). The Mg^{2+} content presented the same quadratic behavior with a positive response tendency at the higher rates and varied from 13.0- to 18.6 $mmol_c$ dm^{-3}, presenting very high values (above 8.0 $mmol_c$ dm^{-3}). The increase in the $S\text{-}SO_4^{2-}$ content occurred in a quadratic way in the soil until 400 m^3 ha^{-1}, which was an alternative to increase the nutrient concentration in the soil (Figure 3e).

The CEC and base saturation (V) showed the same quadratic behavior with a positive response trend, with V values varying from average (up to 70) to high (71–90) in the range of 63.2% to 75.6% (Figure 3f and 3g, respectively). For soil micronutrients, only available boron (B) and iron (Fe) were significantlyinfluenced. The available B content presented a linear response in relation to the control treatment (Figure 3h). The available Fe content in the soil presented with little variation and a negative linear tendency with the applied rates, varying from 23.8- to 31.2 mg dm^{-3}. Available copper (Cu) presented values that varied from 0.68- to 0.84 mg dm^{-3}, available zinc (Zn) ranged from 0.46- to 0.56 mg dm^{-3}, and available manganese contents ranged from 2.20- to 5.64 mg dm^{-3}.

Nutrients concentrations in leaves

The organic biofertilizer application resulted in significant variation in the mineral content of Mulato II *Urochroa* grass. There was a significant increase in the N, P, K, Fe, and Zn concentration in the forage and a significant decrease in leaf Ca, S and B concentration. The leaf N concentration corresponded in a quadratic form up to (maximum point) the rate of 148 m^3 $ha^{-1}$and ranged from 7.5- to 8.8 g kg^{-1} (Figure 4a). The P concentration responded positively and quadratically (Figure 4b). In the evaluation of the four harvests (growth cycles), there was an increase in leaf P concentration after organic biofertilizer application.

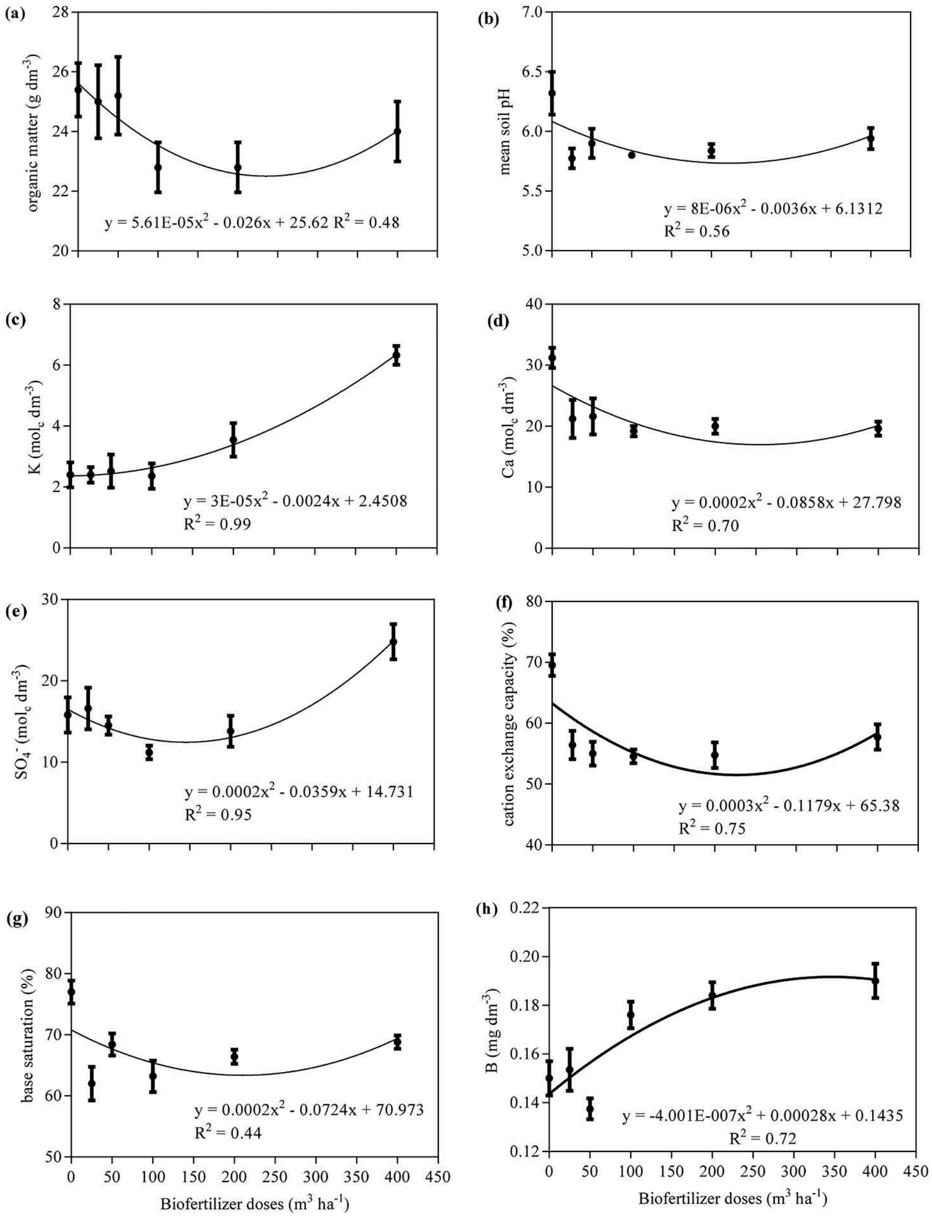

Figure 3. Chemical properties in the soil as a function of biofertilizer rates in Mulato II *Urochroa* grass. The vertical bars associated with symbols represent the mean standard error (n = 5). All equation models and their coefficients are significant at 1% level.

The K concentration increased with the organic biofertilizer application, until the dose of 323 g kg^{-1} was achieved and was compared to that of the control treatment (Figure 4c). The concentration ranged from 15.0- to 24.0 g kg^{-1}, and the nutrient range of 12.0- to 30.0 g $kg^{-1}$was considered adequate. The Ca concentration had a linear decreasing effect and ranged from 2.9- to 4.0 g kg^{-1}, which was considered adequate (3.0- to 6.0 g kg^{-1}) (Figure 4d). The Mg concentration was not significant but was considered in the ideal range (1.5- to 4.0 g kg^{-1}), ranging from 3.1- to 3.5 g kg^{-1}.

Sulfur concentration decreased linearly with the increasing of biofertilizer rates, due to the effect of dilution on the dry weight yield (SDW). Values ranged from 1.1- to 3.4 g $kg^{-1}$and these results are within the optimum range for Palisadegrass, which is 0.8- to 2.5 g kg^{-1} (Figure 4e). The foliar B

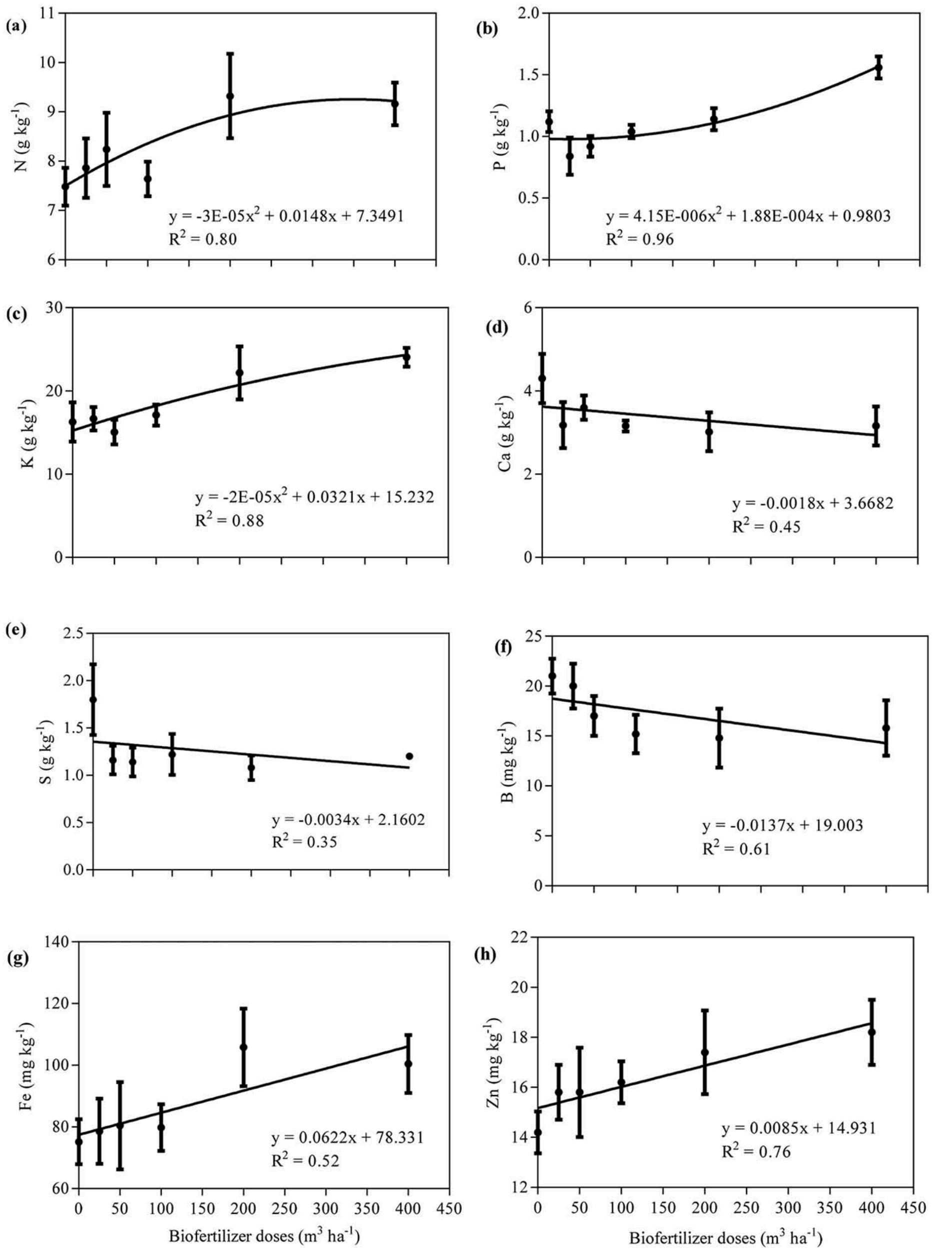

Figure 4. Macro contents and foliar micronutrients according to the biofertilizer rates in Mulato II *Urochroa* grass. The vertical bars associated with symbols represent the mean standard error (n = 5). All equation models and their coefficients are significant at 1% level.

concentration decreased linearly and varied from 14.8- to 21.0 mg kg^{-1} and this concentration was within the appropriate range (10.0- to 25.0 mg kg^{-1}) (Figure 4f). Manganese (Mn) concentration was not significant and ranged from 14.2- to 38.8 mg kg^{-1}, but was below the appropriate concentration range (40.0 to 250.0 mg kg^{-1}). Fe (Figure 4g) and foliar Cu concentration ranged, respectively, from 75.2- to 108.4 mg kg^{-1}, and from 9.0- to 18.0 mg kg^{-1}, and were within the range considered adequate. The foliar Zn concentration increased linearly and ranged from 14.2- to 18.2 mg kg^{-1}, which is considered low, since the ideal range from 20.0- to 50.0 mg kg^{-1} is considered adequate (Figure 4h).

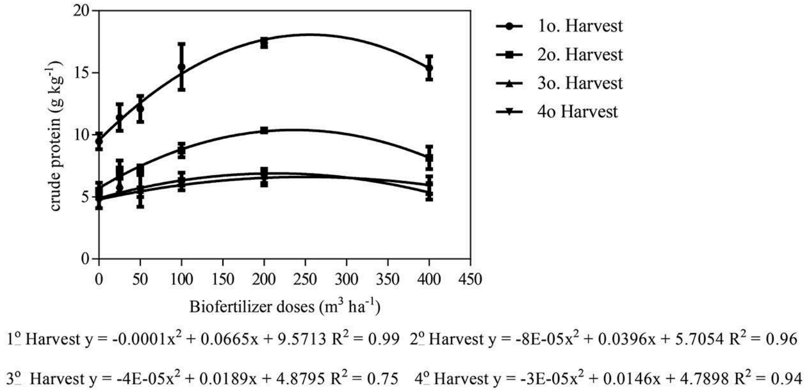

Figure 5. The interaction of crude protein (CP) was determined by harvest dry shoot mass, as a function of harvests and biofertilizer rates in Mulato II *Urochroa* grass biofertilizer. The vertical bars associated with symbols represent the mean standard error (n = 5). All equation models and their coefficients are significant at 1% level.

Nutritive value of forage

The Crude protein (CP) presented significant differences, between the rates and the interaction organic biofertilizer and harvests (Figure 5a). The first growth cycle showed significant quadratic response up to the 333 m^3 ha^{-1} of biofertilizer with an average value of 13.5 g kg^{-1} CP. The second harvest presented a positive and quadratic response of up to 248 m^3 ha^{-1}, but was of lower magnitude, at 7.9 g kg^{-1} CP on average. Third and fourth harvests were similar with quadratic effects up to 243 m^3 ha^{-1} of biofertilizer, with critical values of 6.0 g kg^{-1} CP. For the neutral detergent fiber (NDF) and acid detergent fiber (ADF), there were no significant differences after organic biofertilizer application, with only NDF interacting between rates versus harvests. The NDF values found ranged from 72.8- to 73.6 g kg $^{-1}$, while the ADF levels ranged from 41.6- to 42.3 g kg $^{-1}$.

Discussion

The SDWY and RDWY of Mulato II *Urochloa* grass were positively influenced in a quadratic manner, (Figure 1, 2a and 2b). The effect of organic biofertilizer rates on SDWY of the forage is an indication that the plants responded positively to the application after the harvest and the interval between harvests was sufficient for the plants to reach their maximum yield (Figure 2a). In a study that evaluated N and K rates in *Megathyrsus maximum*, cv. Xaraés, Rodrigues et al. (2006) reported that increases in N and K rates positively influenced SDWY. These authors reported that, in order to obtain greater SDWY, it is indispensable to use higher N and K rates. Similar results were obtained by Lavres Junior and Monteiro (2003), that studying *Megarthysus maximum* cv. Mombaça under N and K rates observed similar behavior. The importance of N and K fertilization for the main yield factors such as dry weight in tropical grasses were also reported by Rodrigues et al. (2006).

Results similar to these were found by Schimidt et al. (2003), when studying the effects of N rates (0, 60, 120, and 180 kg ha^{-1}) as a biofertilizer in *Megarthysus maximum* cv. Tanzania, observed SDWY was higher in the fertilized vessels with the highest biofertilizer rates in the third harvest occurring at 84 days after planting. Lemes et al. (2013) studying the increasing bovine biofertilizer rates of alfalfa observed a linear increase in SDWY and RDWY. Silva Neto et al. (2010) verified that SDWY of *Megarthysus maximum* cv. Marandu increases with biofertilizer rates; however, the higher SDWY was found in the first harvest, as compared to that in the second. Martuscello et al. (2005); Martuscello et al. (2006) also found similar data of SDWY of *Megathyrsus maximum* cv. Massai (Guinea grass) and Xaraés Palisadegrass, respectively, submitted to different N rates. Drumond et al. (2006) also observed that the SDWY of Tifton 85 Bermuda grass was increased with liquid swine manure application.

The literature suggests that plants in N deficient soils tend to increase their RDWY as a way to explore a higher soil volume. Schimidt et al. (2003) found higher SDWY and RDWY with 180 kg ha^{-1} of biofertilizer application. An increase in RDWY of 69% for the dose at the maximum point of organic biofertilizer of 330 m^3 ha^{-1} was observed in relation to the control. Excess biofertilizer available at the highest dose yielded average growth (lower SDWY), possibly because organic N reserves may have stimulated plants to reduce the energy investments required to expand the root system.

The highest OM concentration was found in the treatments that did not receive with the biofertilizer and in those that received initial rates of 25 and 50 m^3 ha^{-1} (Figure 3a). The pots that received the higher biofertilizer rates had a reduction in these levels. This is explained by the increase in the microbial activity motivated by the biofertilizer application (Figure 2a), since this is used by the plant in the synthesis of the SDWY and RDWY. These data were also obtained by Brito et al. (2008), that studied the transformation of OM and N during compost of the solid fraction of bovine manure with and without the straw addition, in static cells and in stirred cells and who concluded that the OM content decreased in all cells.

The pH index of the soil had a quadratic response and showed little variation with the biofertilizer application, which usually occurs with the N fertilizers use. Similar results, that demonstrate this reduction, were found in several studies, as verified by Costa et al. (2008), on the application of ammonium sulfate and urea in Marandu palisadegrass (Primavesi et al. 2005), urea (45% N) and ammonium nitrate (32% N) in Coastcross Bermuda grass and urea in *Zea mays* (Lange et al. 2006). The exchangeable K^+, Ca^{2+} and Mg^{2+} content were considered suitable for the good development of the forage species according to Van Raij et al. (1997) (Figure 3c and 3d). Soil S maximizes fodder yield, especially in areas with low organic matter concentrations (Bonfim Silva and Monteiro 2006). According to Van Raij et al. (1997), the soil S-SO_4^{2-} content found at the end of the experiment was classified as high, at above 10 mg dm^{-3}. It was believed that this result was much higher due to fertilization of the initial correction of experiment with potassium sulfate (K_2SO_4).

The main source of available B for the soil is OM (Van Raij et al. 1997), which shows that the levels of B in the soil confirm the results found, since in this experiment the OM concentration also increased with the biofertilizer application. According to the result found, the B levels in the soil varied at a low level (0.14–0.19 mg dm^{-3}) (Figure 3h). The Mn contents decreased with the biofertilizer rates when compared to the control treatment. According to Van Raij et al. (1997), soil available Mn content were considered as means (1.3- to 5.0 mg dm^{-3}) as a function of treatments.

The N levels in the SDW of Mulato II (Figure 4a), which were below the appropriate range for *Urochroa* grass, should vary between 13 and 20 g kg^{-1}, according to Werner et al. (1997). In pastures fertilized with biofertilizer, the N deficiency in the plant can be explained by the greater immobilization during the decomposition of organic residues and by the longer time required in the OM mineralization rates in short-lived experiments. OM would also be able to reduce the impact of the losses because approximately 40% of N is immobilized in the tissues and roots is recyclable. Of the total N applied, the plant can recover 70% in the medium and long term (Aguiar 2011).

The P concentration in plant is close to the upper critical limit of the range considered adequate for Palisadegrass (0.8- to 3.0 g kg^{-1}) according to Werner et al. (1997) (Figure 4b). In the evaluation of the four harvests, there was an increase in the P concentration found after application of the organic biofertilizer. Contrary results were found by Primavesi et al. (2006), that studied the effect of N rates on Marandu Palisadegrass and concluded that N in the form of urea decreased the P concentration, and when applied in the form of ammonium nitrate, the P concentration did not vary. Reijeneveld et al. (2014) studied the relationships between soil fertility, forage quality and manure composition applied to pasture areas of dairy farms and found positive linear correlations with S-SO_4^{2-}, P, K^+, and Mg^{2+} in soil and pasture.

The K concentration in the SDW increased quadratically (Figure 4c), and these data are in agreement with those found by Werner et al. (1997), which considers adequate the range of nutrients to be 12- to 30 g kg^{-1}. Batista and Monteiro (2010), explain that when N rates higher than 200 mg dm^{-3} are applied, there is a decrease in the K concentration in the SDW caused by the dilution effect. In the present experiment, this dilution effect was not observed.

Calcium concentration had a linear decreasing effect, but was considered adequate (3–6 g kg^{-1}) according to Werner et al. (1997). The Mg concentration was considered in the ideal range (1.5- to 4 g kg^{-1}) according to Werner et al. (1997). S concentration decreased with increasing biofertilizer rates due to the effect of dry weight dilution, and these results are within the optimal range for Palisadegrass, which is 0.8- to 2.5 g kg^{-1} according to Werner et al. (1997) (Figure 4e). Boron concentration decreased linearly and according to Werner et al. (1997), these levels are within the appropriate concentration range (10- to 25 mg kg^{-1}) (Figure 4f).

The Mn concentration ranged from 14- to 39 mg kg^{-1}, and according to Werner et al. (1997), these levels are below the appropriate concentration range (40- to 250 mg kg^{-1}). However, the Fe and Cu concentration of 75.2- to 108.4 mg kg^{-1} and 9.0- to 18.0 mg kg^{-1}, respectively, are within the range considered adequate (Werner et al. 1997). Primavesi et al. (2004) also found divergent results where the Cu concentration increased linearly with the N application in the form of urea and had a quadratic relationship with ammonium nitrate.

Zinc (Zn) concentration was considered low (Figure 4h) because according to Werner et al. (1997), the suitable contents range is from 20- to 50 mg kg^{-1}. Primavesi et al. (2006) found linear and quadratic responses in Marandu Palisadegrass for the sources of urea and ammonium nitrate, respectively, for the Zn concentration, where in relation to the control treatment the contents increased 5.3 and 6.9 mg kg^{-1} for each N source studied.

The CP content presented a quadratic response up to the 333 m^3 ha^{-1} rate. However, Rocha et al. (2002) did not verify the effect on CP levels as a function of N rates applied to grasses of the genus Cynodon. Inyang et al. (2010) studied the nutritive value of Mulato II *Urochroa* grass and found higher CP values (139 g kg^{-1}) in the spring and summer months. Mulato *Urochroa* grass has superior nutritive values compared to those of other warm-season grasses, with CP concentration fluctuating between 90 and 170 g kg^{-1} (Lascano et al. 2006). In addition, Vendramini et al. (2014) studied the nutritive value of 3 new *Urochroa* grass hybrids Mulato II, Cayman, and BR02/1794, which had similar CP concentration (mean: 13.7 g kg^{-1}).

Inyang et al. (2010) harvested Mulato II at 4-week intervals from September to November in South Florida and observed that the CP ranged from 100- to 180 g kg^{-1}. The decrease in the CP concentration at longer harvest frequencies in warm-season grasses in attributed to N dilution effects caused by greater dry weight and associated cell wall deposition. Pequeno et al. (2015) studied the nutritional value of convert HD 364 *Urochroa* grass (= Mulato II) harvested at 28–42-day frequencies in CPs of 147 and 132 g kg^{-1}, respectively, which were superior to those found in this experiment.

Rodrigues et al. (2006) evaluated the CP content in five cultivars of Bermuda grass (Tifton 85, Florakirk, Tifton 68, Florona and Florico) at 14-, 28-, 42-, 56- and 76 day-old plants and found an average of 14.4 g kg^{-1}, which was higher than the values found in this study. The values found in this experiment were considered low for the studied species compared with those in the literature data. Mulato Palisadegrass has shown higher nutritive value compared to other warm season grasses, with average values of CP ranging from 9- to 17 g kg^{-1} and an in vitro digestibility of organic matter from 55- to 62 g kg^{-1} (Lascano et al. 2006). Reijeneveld et al. (2014) did not find an effect of manure application in dairy pastures over 20 years on the quality of the forage that obtained an average NDF content of 72 g kg^{-1}, and the CP content decreased over the years.

The NDF and ADF did not show significant differences. The NDF values were above 70 g kg^{-1}, and are in agreement with those reported by other authors. Rodrigues et al. (2006) that evaluated five cultivars of Bermuda grass and, found an average of 76 g kg^{-1} of NDF found similar results. The NDF concentration reported by Pequeno et al. (2015) in convert HD 364 were lower than those found in the present experiment, and the NDF values were not higher than 600 g kg^{-1}. Orrico Junior et al. (2013) found that the NDF, ADF, cellulose, hemicellulose, and lignin presented negative linear behavior as a function of the increasing N rates. The CP contents and the 'in vitro' dry matter digestibility were linearly related.

Van Soest (1994) emphasized the importance of NDF values to verify the quality of forage plants. The author established that NDF levels greater than 60 g kg^{-1} of dry weight are negatively associated with the voluntary consumption of forage by the animals and consequently, reduce animal performance. From the data obtained in this experiment, Mulato II *Urochroa* grass would present limitations in the voluntary consumption of animals (NDF greater than 60 g kg^{-1}).

Rocha et al. (2002) evaluated N rates in grasses of the genus Cynodon and found average levels of ADF, regardless N rates of 40.38, 40.68 and 39.49 g kg^{-1}, respectively, for Coastcross, Tifton 68 and Tifton 85 Bermuda grass, which were higher values than those found in this study. Soares Filho, Rodrigues, and Perri (2002) studied the ADF levels in seven *Cynodon* cultivars (Tifton 68, Tifton 78, Tifton 85, Florakirk, Florico, Florona, and Coastcross Bermuda grass), obtaining a mean of 40.1 g kg^{-1} in the wet season and 38.9 g kg^{-1} in the dry season, and the values in both seasons are above the average found in this study.

With different organic biofertilizer rates, there was a quadratic response in the accumulated SDWY of the aerial part until the dose of 400 m^3 ha^{-1} was reached. The organic biofertilizer positively influenced soil chemical attributes and foliar concentration. However, only the CP content presented a quadratic response, and the NDF and ADF levels were not affected by the influence of organic biofertilizer applied to the soil.

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References

Aguiar, A. P. A. 2011. *Correction and fertilization of pasture soil [Correção e Adubação do Solo da Pastagem]. Uberaba.* Brazil: UNIUBE.

AOAC (Association of Official Analytical of Chemists). 1990. *Official methods of analysis of the AOAC*, 15 edn. Washington, USA: AOAC.

Argel, P. J., J. W. Miles, J. D. Guiot, H. Cuadrado, and C. E. Lascano. 2007. *Mulato II (Brachiaria hybridida CIAT 36087) cultivar: High quality grass and forage production, resistant to spittlebugs and adapted to acidy tropical soils.* Cali, Colombia: CIAT.

Batista, K., and F. A. Monteiro. 2010. Alterations in potassium, calcium and magnesium concentrations in Marandu palisadegrass fertilized with nitrogen and sulfur rates. *Revista Brasileira Ciência Do Solo* 34:151–61.

Bonfim Silva, E. M., and F. A. Monteiro. 2006. Nitrogen and sulphur for productive characteristics of Signalgrass from degrading pasture area. *Revista Brasileira De Zootecnia* 35:1289–97.

Bonfim Silva, E. M., M. C. Silva, A. F. Schlichting, R. A. Porto, T. J. A. Silva, and M. Koets. 2014. Development and production of grass-convert HD364 subjected to water stress. *Revista Agro@Mbiente On-Line* 8:134–41.

Brito, L. M., A. L. Amaro, I. Mourão, and J. Coutinho. 2008. Transformation of organic matter and nitrogen during composting of the solid fraction of cattle slurry. *Revista Brasileira Ciência Do Solo* 32:1959–68.

Campos, F. B., C. M. B. Nussio, and L. G. Nussio. 2004. *Methods of food analysis [Métodos de Análises de Alimentos].* Piracicaba, Brazil: Fundação de Estudos Agrários Luiz de Queiroz.

CONAMA (Conselho Nacional de Meio Ambiente). 1997. *Resolution 1237/1997 [Resolução 1237/1997]*. Brasília, Brazil: CONAMA.

Costa, K. A. P., V. Faquin, I. P. Oliveira, C. Rodrigues, and E. C. Severiano. 2008. Nitrogen doses and sources in Marandu pasture. I - Changes in soil chemical properties. *Revista Brasileira Ciência Do Solo* 32:1591–99.

Drumond, L. C. D., J. R. Zanini, A. P. A. Aguiar, G. P. Rodrigues, and A. L. T. Fernandes. 2006. Dry matter production of irrigated Tifton 85 forage, with different liquid swine dejection rates. *Revista Engenharia Agrícola* 26:426–33.

Embrapa (Empresa Brasileira de Pesquisa Agropecuária). 2013. *Brazilian soil classification system [Sistema Brasileiro de Classificação de Solos]*. Rio de Janeiro, Brazil: Embrapa Solos.

Inyang, U., J. M. B. Vendramini, L. E. Sollemberger, M. L. A. Silveira, B. Sellers, A. Adesogan, L. Paiva, and A. Lunpha. 2010. Harvest frequency and stubble height affects herbage accumulation, nutritive value, and persistence of 'Mulato II' brachiariagrass. *Forage and Grazinglands* 8:1–7.

Lange, A., J. L. N. Carvalho, C. J. C. Damin, and J. J. Marques. 2006. Changes in soil properties due to application of nitrogen and straw in maize grown under no-till system. *Ciência Rural* 36:460–67.

Lascano, C. E., J. Miles, P. Avila, and G. Ramirez. 2006. *Screening of sexual and apomitic Brachiaria hybrids for digestibility and protein*. Cali, Colombia: CIAT.Annual Report.

Lavres Junior, J., and F. A. Monteiro. 2003. Tillering, leaf area and Mombaçagrass root system in response to nitrogen and potassium combinations. *Revista Brasileira De Zootecnia* 32:1068–75.

Lemes, L. L., C. V. Soares Filho, M. Garcia Neto, and R. Heinrichs. 2013. The chemical properties of soil for alfalfa production after biofertilizer application. *Semina: Ciências Agrárias* 34:2211–18.

Lemes, L. L., C. V. Soares Filho, M. Garcia Neto, and R. Heinrichs. 2016. Biofertilizer in the nutritional quality of alfalfa (*Medicago sativa* L.). *Semina: Ciências Agrárias* 34:2211–18.

Malavolta, E., G. Vitti, and S. A. Oliveira. 1997. *Evaluation of nutritional status of plants; principle and applications [Avaliação do Estado Nutricional de Plantas: Princípios e aplicações]*. Piracicaba, Brazil: Potafós.

Martuscello, J. A., D. M. Fonseca, D. Nascimento Júnior, P. M. Santos, D. N. F. V. Cunha, and L. M. Moreira. 2006. Morphogenetic and structural characteristics responses of Massai grass under nitrogen fertilization and defoliation. *Revista Brasileira De Zootecnia* 35:665–71.

Martuscello, J. A., D. M. Fonseca, D. Nascimento Júnior, P. M. Santos, J. I. Ribeiro Junior, D. N. F. V. Cunha, and L. M. Moreira. 2005. Morphogenetic and structural characteristics of Xaraés grass submitted to nitrogen fertilization and defoliation. *Revista Brasileira De Zootecnia* 34:1475–82.

Moreira, A., N. K. Fageria, G. B. Souza, and A. R. Freitas. 2010. Production, nutritional status and chemical properties of soils with addition of cattle manure, reactive natural phosphate and biotite schist in Massai cultivar. *Revista Brasileira De Zootecnia* 39:1883–88.

Orrico Junior, M. A. P., A. C. A. Orrico, S. R. Centurion, N. S. Sunada, and J. L. Lucas Junior. 2013. Nutritive value of Piatã grass fertilized with different doses of biofertilizers. *Revista Agrarian* 4:312–19.

Pequeno, D. N. L., C. G. S. Pedreira, L. E. Sollenbergerr, A. F. G. Faria, and L. S. Silva. 2015. Forage accumulation and nutritive value of Brachiariagrasses and Tifton 85 Bermudagrass as affected by harvest frequency and irrigation. *Agronomy Journal* 107:1741–17490.

Primavesi, A. C., O. Primavesi, L. A. Corrêa, H. Cantarella, and A. G. Silva. 2005. Cations and anions uptake by coastcross grass fertilized with urea and ammonium nitrate. *Pesquisa Agropecuária Brasileira* 40:247–53.

Primavesi, A. C., O. Primavesi, L. A. Corrêa, H. Cantarella, A. G. Silva, A. R. Freitas, and L. J. Vivaldi. 2004. Nitrogen fertilization in coastcross grass: Effects on nutrient extraction and apparent nitrogen recovery. *Revista Brasileira De Zootecnia* 33:68–78.

Primavesi, A. C., O. Primavesi, L. A. Corrêa, A. G. Silva, and H. Cantarella. 2006. Nutrient content in marandu grass biomass due to nitrogen sources and levels. *Ciência E Agrotecnologia* 30:562–68.

Quaggio, J. A., B. V. Raij, and E. Malavolta. 1985. Alternative use of the SMP – Buffersolution to determine lime of soils. *Communication Soil Science Plant Analysis* 16:245–60.

Reijeneveld, J. A., G. W. Abbink, A. J. Termorshuizen, and O. Oenema. 2014. Relationships between soil fertility, herbage quality and manure composition on grassland-based dairy farms. *Europe Journal Agronomy* 56:9–18.

Rocha, G. P., A. R. Evangelista, J. A. Lima, and B. Rosa. 2002. Nitrogen doses on grasses of the Cynodon genus. *Ciência Animal Brasileira* 3:1–10.

Rodrigues, L. R. A., T. J. D. Rodrigues, R. A. Reis, and C. V. Soares Filho. 2006. Dry matter production and chemical composition of five *Cynodon* cultivars. *Acta Scientiarum Animal Science* 28:251–58.

Schimidt, L. T., P. F. Dias, S. M. Souto, R. O. P. Rossiello, A. M. Zanine, G. L. Macedo Junior, and B. M. Pereira. 2003. Effects of N doses of from slurry and sampling dates in Tanzania grass development. *Revista Ciência Agronômica* 34:5–10.

Silva Neto, S. P., J. E. C. Silva, A. C. Santos, J. G. D. Castro, V. P. Dim, and A. S. Araújo. 2010. Agronomic and nutritional characterstics of Marandugrass from the application of liquid slaughterhouse effluent. *Acta Scientiarum Animal Science* 32:9–17.

Soares Filho, C. V., R. Heinrichs, S. H. V. Perri, and A. C. Correia. 2015. Chemical attributes in soil and production of "*Cynodon dactylon*" cv. Tierra Verde on doses of organic biofertilizer. *Revista Brasileira De Saúde E Produção Animal* 16:33–35.

Soares Filho, C. V., L. R. A. Rodrigues, and S. H. V. Perri. 2002. Production and nutritive value of ten forage grasses in the northwest region of the state of São Paulo. *Acta Scientiarum Agronomy* 24:1377–84.

Van Raij, B., J. C. Andrade, H. Cantarella, and J. A. Quaggio. 2001. *Chemical analysis for fertility evaluation of tropical soils [Análise Química para Avaliação da Fertilidade de Solos Tropicais]*. Campinas, Brazil: Instituto Agronômico de Campinas.

Van Raij, B., H. Cantarella, N. O. Andrade, J. A. Quaggio, and A. M. C. Furlani. 1997. Interpretation of soil analysis results. In *Fertilization and liming recommendations for the State of São Paulo [Recomendações de Adubação e*

Calagem para o Estado de São Paulo], eds B. Van, Raij, H. Cantarella, J. A. Quaggio, and A. M. C. Furlani, 8–13. Campinas, Brazil: IAC.

Van Soest, P. J. 1994. *Nutritional ecology of the ruminant*, 2nd edn. Ithaca, USA: Cornell University Press.

Vendramini, J. M. B., L. E. Sollenberger, A. B. Soares, W. L. Silva, J. M. D. Sanchez, A. L. Valente, A. D. Aguiar, and M. K. Mullenix. 2014. Harvest frequency affects herbage accumulation and nutritive value of Brachiariagrass hybrids in Florida. *Tropical Grasslands* 2:197–206.

Werner, J. C., V. T. Paulino, H. Cantarella, N. O. Andrade, and J. A. Quaggio. 1997. Forages. In *Fertilization and liming recommendations for the State of São Paulo [Recomendações de Adubação e Calagem para o Estado de São Paulo]*, eds B. Van Raij, H. Cantarella, J. A. Quaggio, and A. M. C. Furlani, 263–237. Campinas: IAC.