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Domestic Wastewater for Forage Cultivation in Cerrado Soil

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Abstract

Fertigation of agricultural crops that are not directly used in human food, with domestic wastewater is a viable alternative for the sustainable use of water resources. The development of agricultural practices that provide high productivity with the sustainability of agroecosystems has been a great challenge. Thus, our aims were to use of domestic wastewater in the planting of *Brachiaria brizantha* cv Marandu, as an alternative for animal feed production in Cerrado soils, and to study the physical-chemical and microbiological impacts of the fertigation. These impacts were evaluated, respectively, by physical-chemical indicators content and diversity of nitrogen fixing bacteria (NFB) and arbuscular mycorrhizal fungi (AMF) in the DGGE profile. The NPK contents of the wastewater were used to determine the five fertigation managements (M1 to M5). M1 and M2 managements had no wastewater and M3 to M5 contained 20, 40 and 60% of NPK from the wastewater. The managements in a completely randomized design with 20 plots and 4 replicates were distributed. Soil samplings prior to fertigation and at the end of the experiment were performed. Leaf biomass productivity was determined in three different grass cuts. After fertigation, changes in physical-chemical indicators and in the viable microbial cells counts were observed. The NPK of wastewater increased the abundance of NFBs and AMFs. Leaf biomass productivity per hectare was directly proportional to NPK concentration. In addition, wastewater did not alter the nutritional composition of Marandu grass. Therefore, the fertigation with domestic wastewater showed to be a viable and promising alternative for reuse of this water in Cerrado soil for animal feed production.

Keywords: fertigation, arbuscular mycorrhizal fungi, nitrogen fixing bacteria, *Brachiaria brizantha* cv Marandu, animal feed

1. Introduction

The water crisis and new discussions on the sustainable use of water should contribute to intensify studies of wastewater reuse in agriculture. Thus, the use of domestic wastewater to irrigate crops, which are not used directly in human food, can be a viable alternative (Silva et al., 2016).

Brazil has the greatest biodiversity of the planet (Dias-Filho, 2014). The Cerrado is the second largest Brazilian biome. It has grassland, savannic and forest physiognomies that dominate the Brazilian Midwest (Sano et al., 2008). This biome is a biodiversity hotspot, because of its species richness and high endemism level (Myers et al., 2000).

In the 1970s, the Cerrado was subject to large financial investments for pastures formation (Andrade et al., 2005). In this period, the replacement of fat grass by more productive grasses, mainly, *Brachiaria* (*B. decumbens*, *B. humidicola* and B. *brizantha*) was done. In addition, the new agricultural areas formation and the abandonment of degraded areas have favored the growth and adaptation of invasive plant species (Dias-Filho, 2014). Thus, the development of agricultural practices that provide high biomass yields with sustainability of agroecosystems has been a great challenge.

Our objectives were to use of domestic wastewater in the planting of *Brachiaria brizantha* cv Marandu, as an alternative for animal feed production in Cerrado soils, and to study the physical-chemical and microbiological impacts of the fertigation.

The nitrogen fixing bacteria (NFB) and arbuscular mycorrhizal fungi (AMF) may be a good parameter to evaluate the biological alterations in soil after fertigation (Silva et al., 2017, Carvalho et al., 2018). Furthermore, these microbial groups are the main microorganisms indicative of soil fertility (Souza et al., 2011, Thongtha et al., 2014).

2. Method

2.1 Experimental Area

The experiment was carried out on the campus of CEULP/ULBRA (Palmas, Tocantins, Brazil) located at an altitude of 254 m, 10°12′46″ S, and 48°21′.37″ W.

At this experimental area of 180 m^2, the *Brachiaria brizantha* cv Marandu growth was performed. The domestic wastewater of CEULP/ULBRA was used in the water and nutritional demands of the grass.

2.2 Soil Preparation and Liming

The soil type of the experimental area was yellow red Latosol (Embrapa, 1999).

Prior the experiment, we cleaned the area with the removal of trees and branches. The vegetative cover was also removed by a plow.

The liming for forage cultivation was performed by the base saturation method (Teixeira et al., 2017). The base saturation was of 40%. The Embrapa (1999) describes from 40 to 45% for this grass. This required the application of 1.73 tons of limestone per hectare.

The dolomitic limestone contained 80% calcium oxide (CaO), 18% magnesium oxide, and 100% of neutralizing power (NP) in relation to calcium carbonate and the actual total neutralization power (ATNP).

2.3 Fertigation Management and Experimental Design

The experiment consisted of five fertigation managements (M1 to M5) and four replicates (R1 to R4) (Figure 1, Table 1).

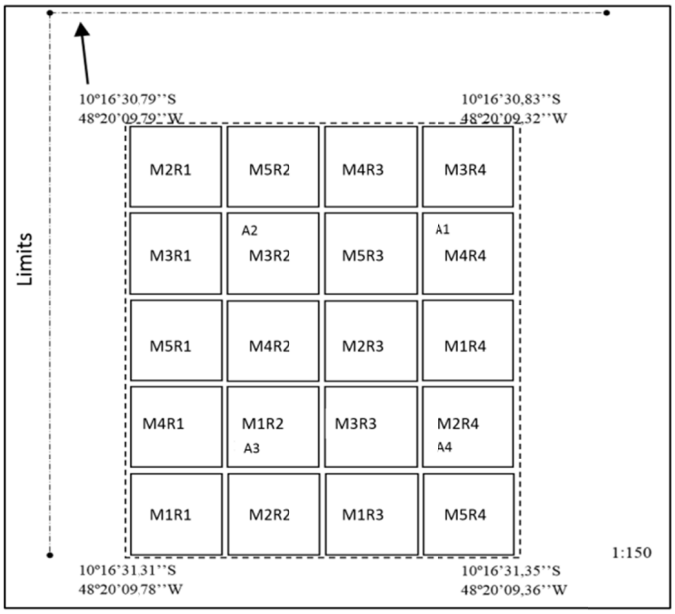

Figure 1. Fertigation managements (M1 to M5) in the planting of *Brachiaria brizantha* cv. Marandu in soil Cerrado. The first soil samples were obtained in the A1 to A4 points, before the start of the fertigation. For each management had four replicates (R1 to R4)

Fertigation managements were randomly distributed in the experimental area (Figure 1). This figure also showed the sampling points of the soil (A1 to A4). The physical-chemical analyses and microbial diversity were performed in these soil samples.

Different doses of nitrogen (N), phosphorus (P), and K potassium (K) were used for managements (Table 1). The M1 management had no NPK. The M2 had only commercial NPK (comNPK). The NPK source from M3 to M5 was comNPK and wastewater NPK (wastewaterNPK).

All managements had liming and water from an artesian well. The water volume of each management was in the ratio between the water balance of Palmas-TO city and NPK content (Table 1).

Table 1. Fertigation managements with or without domestic wastewater that were used in the planting of *Brachiaria brizantha* cv Marandu in Cerrado soil

Fertigation managements	Fertilizer (%)	
	Commercial NPK	Wastewater NPK
M1	0	0
M2	100	0
M3	80	20
M4	60	40
M5	40	60

Note. NPK: nitrogen, potassium, and phosphorus.

2.4 Planting and Management of Grass

B. brizantha due to resistance to long dry periods, good adaptation in Cerrado soil, high regrowth capacity and tolerance to the leafhopper was used (Soares Filho et al., 2002).

The seeds were obtained on the market of Palmas-TO city. They had 60.3% of purity, 80% of germination rate and of 48.24% cultural value. Thus, in the planting, 1.75 kg/ha of seeds were used. Embrapa (1984) recommends 1.5 to 2 kg of viable seeds per hectare. The planting took place on June 24, 2015, with the seeds applied to the haul.

In the plantation area were performed 20 plots with nine m^2 (3 m × 3 m). The management of the plots was determined by lottery. The vertical dimension of the plots was 1.00 m, because of the maximum depth of soil samples.

The effective root depth of 43 cm was used to determine the total water blade for the grass.

In fertilization, phosphorus (P) and potassium (K) contents were applied according to Alcântara and Bufarah (1999). In this step, 627.66 kg of superphosphate ha^{-1} and 119.37 kg ha^{-1} of potassium chloride were applied. The urea was placed as a nitrogen source in a dosage of 150 kg ha^{-1} (Lopes et al., 2013).

2.5 Total Water Capacity in the Soil

The total water capacity (TWC) in the soil depends on the effective roots depth (Z). In grazing, the TWC is of 30 to 100 cm (Klar, 1991). TWC for *B. brizantha* cv. Marandu, in middle texture soil, with pressures from 1/5 to 15 atm was of 43.1357 cm (Cunha et al., 2010).

Water availability factor in the soil for forage is of 0.3 to 0.7 (Bernardo et al., 2008). Thus, the actual water capacity (AWC) in the soil of 25.8 mm was calculated, respectively, with 51.6 mm and 0.5 of TWC and water availability factor.

2.6 Irrigation Management of Crop

The climate of the region is humid and subhumid (C2wA"a") with a water deficit in the winter, annual evapotranspiration between 1.5 and 1.60 mm, mean annual temperature of 27.5 °C, and relative humidity of 80% (INMET, 2013).

The wastewater application was on August 12, 2015, when the grass was about 20 cm of height. In this assay, thirty fertigations were done in the morning and afternoon periods. These applications were done with a watering can (10 liter) on the leaves.

About 6000 students, teachers and visitors from the CEULP/ULBRA campus produce the domestic wastewater. For transportation and storage of this wastewater was used a pump (2 hp), 250 meters of hose of 50 mm in diameter and a water box of 1000 liters.

The comNPK was ground and diluted in the water of an artesian well or in the wastewater, because of the small amount.

2.7 Sampling and Characterization of the Wastewater

The physical, chemical and microbiological analyses were using three samples (0.5 L) of the wastewater (Apha, 2005). The electrical conductivity and pH were determined, respectively, by instrumental and potentiometric methods. The iodometric and oxidimetric methods were used, respectively, for the biochemical oxygen demand (BOD) and the chemical oxygen demand (COD). The total solids, nitrogen, and minerals were determined in analytical balance, Kjedahl method, and spectrophotometry. Total and faecal coliform counts were determined by the most likely number (MPN) method.

2.8 Sampling and Characterization of the Soil

The soil sampling was done in two periods with a Dutch auger (Raij, 2001). The first one was carried out in April 2015, before planting the grass and applying the wastewater, in four points (A1 to A4) and in four soil depths (0-10, 10-20, 20-30 and 90-100 cm) (Figure 1). These samples were identified as A1SD1 to A1SD4, A2SD1 to A2SD4, A3SD1 to A3SD4, and A4SD1 to A4SD4. After the randomization of the experiment, the A1 point was within the M4 management. A2 was in M3 management and the A3 and A4 points were, respectively, in the M1 and M2 managements (Figure 1, Table 1).

The second sampling was performed at the end of the assay, in February 2016. Twelve samples were obtained for each fertigation management (M1 to M5), with four replicates and three soil depths (0-10, 10-20 and, 20- 30 cm).

Twenty grams of soil were placed in dark plastic bags and added in a Styrofoam box containing dry ice. These samples were used for physical, chemical and microbiological analyses. The physical-chemical indicators of soil were determined according to Standard Methods (APHA, 2005).

2.9 Analysis of the Soil Microbiota

Viable microbial cells and DGGE profile were performed as described in Carvalho et al. (2018).

2.10 Sampling and Characterization of the Plant

The plant cuts were carried out on November 13, 2015, January 4, 2016 and February 23, 2016.

The first cut occurred after 140 days of sowing the grass seeds. The planting occurred in the dry season. So, the germination was observed after 30 days. The second and third cuts occurred, respectively, after 50 days and 100 days of the first cut. The cuts were performed at a height of about 10 cm from the soil.

After the plant cuts with a pruning shears, the green mass was determined in an analytical balance. After the green mass determination, the samples were placed in an oven at 65 ^{o}C, with forced ventilation for 72 hours. After cooling to room temperature (25±5 ^{o}C), the dry matter in the air (DMA) was determined in analytical balance.

The DMAs were submitted to milling in a Willey mill to determine the nutrients (N, P, K, Ca, Mg, S, Zn, Cu, Fe, Mn, Na, Co, Mo and B), etheric extract, acid detergent fiber (ADF) and neutral detergent fiber (NDF).

After the nitroperchloric digestion of DMAs (Tedesco et al., 1985), the elements contents were determined by the Kjeldahl method (nitrogen), spectrophotometry (phosphorus), flame emission photometry (potassium and sodium), and plasma emission spectrophotometry (other minerals).

The actual dry matter (ADM) content was determined in an oven at 105 °C until the stabilization of its weight using DMA. The ash content was determined in muffle at 5500C using the MSE.

The ash content was determined in muffle at 550 ^{o}C.

All these analyses were performed according to the methodology of the Association of Official Analytical Chemists (1990) and Embrapa (1999).

2.11 Statistical Analyses

The assay was performed in a completely randomized design (CRD) with five fertigation managements and four replicates.

Statistical analyses of soil and grass samples were performed, respectively, by CRD and CRD in a 5 × 3 factorial scheme. The factorial was the five doses of wastewater (Table 1) and the three cuts. The comparison between the viable microorganisms counts was done by analysis of variance (ANOVA) and Tukey's test. The comparison

between dry matter yields were performed by ANOVA and a regression analysis. These analyses were performed using the free trial version of the Minitab 17 software (2016) at the 5% level.

The clusters and main component of the soil and plant samples were performed in Sigma Plot Software (2016).

The DGGE profiles were analyzed by the unweighted pair group method with arithmetic mean (UPGMA) and Jaccard similarity index in the Bionumerics software (Version 5.1). The similar bands were those with 0.5% of significance level by the post-hoc Bonferroni test.

3. Results and Discussions

3.1 Wastewater Characterization

The chemical composition of the domestic wastewater had higher N, P and K levels than nutrients that shows its potential in the fertigation (Table 2). However, the management of this wastewater over the soil is important, because of high ammonia level (Table 2). This compound has a high evaporation rate in the soil.

Table 2. Physical and chemical indicators and coliform counts of wastewater

Indicators	Amount	Units
Electric conductivity	634	$\mu S\ cm^{-1}$
pH	8.10	-
Biochemical oxygen demand (BOD)	86.80	$mg\ L^{-1}$
Chemical oxygen demand (COD)	213.30	
Total Solids	298.00	
Total nitrogen	74.58	
Organic nitrogen	29.62	
Nitrite	1.23	
Nitrate	3.42	
Ammonia	40.31	
Chlorine	4.56	
Cadmium	7.43	
Copper	0.43	
Iron	3.45	
Aluminum	0.54	
Manganese	0.03	
Magnesium	0.02	
Sulfur	0.92	
Calcium	0.22	
Phosphorus	7.43	
Sodium	5.83	
Potassium	18.60	
Fecal coliforms	1.85×10^{-7}	NMP/100 mL
Total coliforms	2.50×10^{-6}	

The total N, P and K contents (Table 2) were used to determine the wastewater proportion of the fertigation managements (Table 1).

The domestic wastewater composition can vary according to the collection site, climate and the economic and social situation of the population (Kong et al., 2015).

The N content of the wastewater was more than 70 mg L^{-1} (Table 2). The P and total nitrogen contents of crude domestic sewage were, respectively, 4 to 12 and 20 to 70 mg L^{-1} (Metcalf & Eddy, 2003).

The K content of the wastewater of the CEULP/ULBRA was lower than 30 mg L^{-1}. This value was also obtained in Pescod (1992).

The BOD and COD were lower than the values described by Metcalf and Eddy (2003), and Araújo et al. (2010) which were 110 and 800 mg L^{-1} for raw sewage. However, the BOD/COD ratio of our wastewater was similar to the obtained for domestic sewage by Simões et al. (2013).

The sodium content was lower than 40 mg L^{-1} described by Von Sperling (2006).

Our domestic wastewater had medium level of salinization of the soil (Table 2). The salinization risk of the soil could be low (Electric conductivity, EC < 0.25 μS.cm^{-1}), medium (250 < EC < 750), high (750 < EC < 2250) and very high (EC > 2250) (Bernardo et al., 2008). In addition, aluminum also has low concentrations reducing the possibility of contamination of the soil (Table 2).

The basic pH of the wastewater and liming in the soil contributed to the acidity correction (Table 2). Seed germination and seedling growth depend on the acidity of the soil.

The wastewater had of total and fecal coliforms counts smaller than the amount observed in other domestic effluents (Araújo et al., 2010). However, this count was similar to the obtained in domestic sewage from a septic tank (Fonseca, 2007).

3.2 Physical-Chemical Indicators Characterization of Soil Before Fertigation

The sand, silt, and clay amount did not show differences ($p < 0.05$) between the soil depths (Table 3). Thus, this soil is sandy-loam with middle texture.

Only the pH, base saturation and clay not reduced its values with soil depth (Table 3). In the surface, the pH was more acid than at depths above 20 cm. This result may be due to the higher organic matter and minerals in the 0-10 cm than other depths. In addition, there was a positive linear correlation ($R^2 = 0.8795$) between base saturation and pH in soil depths. According to Ronquim (2010), the pH of the Cerrado soil is acid and depends on the sampling points, time and soil depth. Soils from six no-till crops also had acid pH more on the surface than other depth (Nicolodi et al., 2008).

In 0-10 cm, the sampling points had different distributions of the physical-chemical indicators, with few overlapping of them (Figure 2). These results show the heterogeneous character of the soil. The unequal distribution of nutrients in the soil has also been reported in other studies (Pavinato & Rosolem, 2008; Nicolodi et al., 2008; Ronquim, 2010).

Table 3. Physical-chemical indicators of soil samples before planting *Brachiaria brizantha* cv Marandu

AvA Soil Indicators		Sampling points*															
		A_1				A_2				A_3				A_4			
		Profundidade															
		SD_1	SD_2	SD_3	SD_4	SD_1	SD_2	SD_3	SD_4	SD_1	SD_2	SD_3	SD_4	SD_1	SD_2	SD_3	SD_4
pH ($CaCl_2$)		4.2	4.3	4.3	5.1	4.5	4.5	4.6	5.1	4.4	4.5	4.5	5.1	4.2	4.2	4.2	5.1
Sand		44	42	40	37	37	40	32	37	30	37	40	28	32	30	37	37
Clay	%	45	48	52	55	55	52	60	55	63	55	52	65	60	63	55	55
Silt		11	10	8	8	8	8	8	8	7	8	8	7	8	7	8	8
Ca		1	1	0.7	0.6	1.1	0.8	0.7	0.7	0.7	0.9	0.6	0.8	0.9	0.8	0.8	0.6
Mg		0.8	0.7	0.5	0.4	0.8	0.5	0.4	0.4	0.6	0.7	0.4	0.6	0.6	0.6	0.6	0.4
Al	cmolc/dm^3	0.9	0.6	0.4	0.1	0.2	0	0	0	0.3	0.3	0.3	0.1	0.4	0.5	0.4	0
H+Al		9.8	8	8	2.8	5.2	5.5	5	2.5	7.2	6.8	5.2	2.8	8.4	9.3	8	2.9
K		0.07	0.06	0.04	0.03	0.05	0.03	0.02	0.04	0.04	0.04	0.05	0.04	0.04	0.05	0.05	0.02
Cation exchange capacity		11.67	9.76	9.24	3.83	7.15	6.83	6.12	3.64	8.54	8.44	6.25	4.24	9.94	10.75	9.45	3.92
Organic matter	g/dm^3	40	43	30	8	14	14	13	9	30	29	18	9	30	22	26	11
Base saturation	%	16.02	18.03	13.42	26.89	27.27	19.47	18.3	31.32	15.69	19.43	16.8	33.96	15.49	13.49	15.34	26.02
Al saturation		32.49	25.42	24.39	8.85	9.3	0	0	0	18.29	15.46	22.22	6.49	20.62	25.64	21.62	0
Na		2.00	2.00	2.0	2.0	2.00	2.00	3.00	3.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Zn		0.20	2.90	1.30	2.10	0.30	2.30	0.70	2.40	0.20	1.00	1.80	0.90	0.80	0.40	2.30	10.0
B		0.23	0.19	0.14	0.28	0.23	0.19	0.23	0.19	0.23	0.28	0.19	0.23	0.14	0.28	0.19	0.23
Cu	mg/dm^3	0.30	0.20	0.20	0.30	0.20	0.20	0.30	0.30	0.30	0.20	0.50	0.30	0.60	0.60	0.20	0.20
Fe		120.00	62.80	38.40	30.20	41.90	44.20	35.90	31.00	53.90	51.40	37.10	27.40	61.60	63.10	58.50	29.60
Mn		5.40	3.60	2.70	1.00	2.20	2.20	0.20	0.30	2.90	1.80	2.00	1.20	1.70	0.90	1.10	0.30
K		26	24	14	11	21	12	9	15	14	15	19	14	17	18	19	7
P (Melich)		1.5	1.5	1.2	0.8	1.2	0.8	0.8	1.5	1.2	1.5	1.2	1.2	1.5	1.5	1.5	1.2

Note. * The values in this table represent the average of four replicates. These values were compared by analysis of variance (Anova) followed by the Tukey test at 5% probability. The results of these statistical analyses are presented in the text by $p < 0.05$.

We observed an overlap in the organic matter, Mg and K in the A3 and A4 points. The A1 point was more similar to A2 than A3 and A4. These clusters show the importance of randomization of the managements (Figure 2).

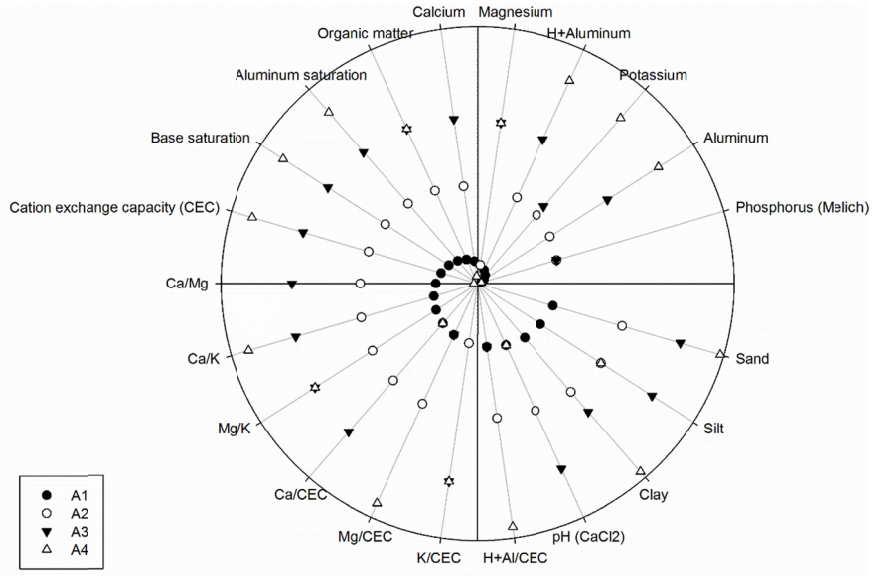

Figure 2. Physical-chemical indicators in the sampling points (A1 to A4) before fertirrigation. These values were of soil samples of 0 to 10 cm soil depth

3.3 Physical-Chemical Indicators Characterization of Soil After Fertigation

We observed a significant increase ($p < 0.05$) in base saturation after fertigation (Tables 4A and 4B). There was no significant difference ($p < 0.05$) in phosphorus content. Changes in soil chemical characteristics were observed, after several years of irrigation with wastewater, because the very slow soil dynamics (Koura et al., 2002). However, changes in the levels of phosphorus, organic matter, magnesium, and calcium were observed after a cycle of agricultural cultivation using sewage (Fonseca, 2001; Azevedo & Oliveira, 2005; Duarte et al., 2008). Thus, changes in the physical-chemical properties of the soil depend of time, crop, soil type, and effluent. Moreover, in our assay no significant changes were observed in the clay, sand, and silt percentage after the fertigation (Tables 4A and 4B). Furthermore, the soil remained acid after fertigation. Duarte et al. (2008) also did not observe a significant difference in soil pH before and after the application of wastewater.

Table 4A. Physical-chemical indicators of soil samples after the fertirrigation of *Brachiaria brizantha* cv Marandu in Cerrado soil

Indicators		Fertigation managements								
		M1			M2			M3		
		Soil Depth (cm)								
		0-10	10-20	20-30	0-10	10-20	20-30	0-10	10-20	20-30
pH ($CaCl_2$)		5.00±0.71	4.65±0.43	4.55±0.24	4.57±0.68	4.3±0.22	4.25±0.13	4.82±0.43	4.57±0.31	4.42±0.10
Clay		36.5±9.85	40.25±5.62	46.5±1.73	36±4.00	39.25±2.06	44.75±6.95	30.5±4.04	34±6.00	40.25±10.69
Sand	%	41.5±8.96	39±6.00	33±2.00	45.5±7.14	40.75±5.62	37.25±8.77	50±2.00	46±10.68	38.5±10.21
Silt		22±2.16	20.75±2.06	20.5±1.73	18.5±4.04	20±4.24	18±3.56	19.5±3.32	20±6.98	21.25±2.99
Ca		2.85±1.82	1.9±1.42	0.85±0.48	1.525±1.46	0.75±0.37	0.45±0.10	2.15±1.31	1.275±1.02	0.575±0.33
Mg		1.275±1.21	0.625±0.43	0.375±0.22	0.7±0.87	0.375±0.29	0.2±0.00	1.1±0.77	0.575±0.49	0.3±0.22
Al	cmolc/dm³	0.075±0.15	0.075±0.15	0.025±0.05	0.15±0.13	0.275±0.13	0.175±0.10	0.025±0.05	0.075±0.05	0.075±0.05
H+Al		5.15±2.54	5.75±1.95	5.25±1.12	5.4±1.81	6.175±1.07	6.3±1.15	5.3±1.41	4.9±1.10	4.9±1.16
K		0.053±0.02	0.041±0.01	0.034±0.01	0.037±0.00	0.041±0.01	0.05±0.02	0.037±0.00	0.0348±0.00	0.031±0.01
CEC		9.325±0.88	8.3±0.85	6.475±0.80	7.625±1.34	7.325±0.72	6.975±1.07	8.55±0.77	6.75±1.52	5.775±1.46
Organic Matter	g/kg	2.70±0.00	2.70±0.57	1.975±0.2	2.5±0.40	2.6±0.20	2.15±0.17	2.8±0.50	2.8±0.60	2.325±0.29
Base saturation	%	44.43±26.40	31.45±19.22	919.75±9.79	27.8±24.88	16.13±9.37	10.35±2.97	37.23±20.12	26.3±15.87	15.43±7.41
Al saturation		5.45±10.90	7.125±14.25	3.375±6.75	11.3±9.03	22.3±12.18	19.65±9.53	0.775±1.55	6.2±4.28	11.33±9.66
Na		1.5±0.58	2±0.82	1.5±0.58	1.25±0.50	1.5±0.58	1.5±0.58	1.25±0.50	2±0.82	1.5±0.58
Zn		3.375±3.39	0.775±0.31	0.775±0.79	0.975±0.13	0.875±0.21	0.875±0.10	1.1±0.18	1.15±0.06	1.15±0.47
B		0.275±0.05	0.2±0.08	0.25±0.06	0.25±0.06	0.225±0.10	0.225±0.05	0.25±0.06	0.275±0.05	0.2±0.08
Cu	mg/dm³	0.1±0.00	0.15±0.06	0.1±0.00	0.175±0.05	0.125±0.05	0.125±0.05	0.125±0.05	0.175±0.05	0.1±0.00
Fe		46±6.48	41.25±9.98	26±4.69	34.75±4.86	33.5±3.87	25.5±4.43	42±8.29	34±13.74	24.25±11.59
Mn		6.25±2.75	3.75±2.06	2±1.15	2.75±0.96	4.25±1.50	3±1.63	17.75±21.70	11±13.04	2.5±1.00
K		20.5±6.61	16±3.27	13±2.00	14.5±1.91	16±3.65	19.5±8.70	14.5±1.00	13.5±1.91	12±2.83
P (Melich I)		1.5±0.58	1.25±0.50	1±0.00	2.5±1.00	1.5±0.58	2.25±0.96	1.75±0.96	1.5±0.58	1.5±0.58

Note. * The values in the table represent the average of four replicates. These values were compared by analysis of variance (Anova) followed by the Tukey test at 5% probability ($p < 0.05$).

Table 4B. Physical-chemical indicators of soil samples after the fertirrigation of *Brachiaria brizantha* cv Marandu in cerrado soil

Indicators		Fertigation managements					
		M4			M5		
		Soil depth (cm)					
		0-10	10-20	20-30	0-10	10-20	20-30
pH ($CaCl_2$)		5.00±0.87	4.8±0.78	4.625±0.50	5.00±0.62	5.00±0.6	4.85±0.66
Clay		27±0.00	35.75±3.95	42±4.24	32.25±5.85	38.5±1	44.75±6.02
Sand	%	50±3.46	40.75±5.62	35±2.00	41.75±6.45	33.25±3.4	32±7.53
Silt		23±3.46	23.5±1.73	23±4.69	26±2.16	28.25±3	23.25±6.99
Ca		2.7±2.35	1.65±1.65	0.975±1.02	1.875±1.81	2.375±1.7	1.825±1.66
Mg		0.975±1.03	0.5±0.38	0.45±0.38	0.675±0.57	0.775±0.5	0.55±0.45
Al	cmolc/dm³	0.1±0.14	0.05±0.06	0.125±0.10	0.05±0.10	0.025±0.1	0.075±0.10
H+Al		3.35±2.06	3.6±1.88	3.05±1.66	2.75±1.26	2.925±1.3	2.575±0.79
K		0.042±0.01	0.069±0.07	0.04±0.01	0.042±0.01	0.0398±0	0.036±0.01
CEC		7.05±1.77	5.8±1.43	4.5±1.49	5.325±2.11	6.1±0.6	4.95±1.63
Organic Matter	g/kg	31±3.27	28±3.83	26±3.83	32±2.00	27±5.7	24.25±4.72
Base saturation	%	49.05±36.25	37.05±28.84	32.05±24.40	42.83±25.12	50.45±26	43.625±24.81
Al saturation		8.275±12.44	5.975±6.90	14±11.65	2.875±5.75	2.175±4.4	4.5±7.14
Na		1.25±0.50	1.25±0.50	1.5±0.58	1.25±0.50	1.25±0.5	1.25±0.50
Zn		1.2±0.41	1.15±0.10	0.925±0.34	4.3±2.83	3.775±2.6	4.9±1.30
B		0.225±0.05	0.25±0.06	0.175±0.05	0.3±0.00	0.225±0.1	0.2±0.00
Cu	mg/dm³	0.15±0.06	0.125±0.05	0.1±0.00	0.1±0.00	0.125±0.1	0.125±0.05
Fe		36±3.74	34±8.12	24.75±3.59	35.75±7.14	30.75±4.3	22.5±4.43
Mn		20.25±14.03	7±3.46	10±12.08	7.75±2.63	8±0	6.25±2.99
K		16.5±5.26	27±28.77	15.5±4.73	16.5±3.42	15.5±4.1	14±2.83
P (Melich I)		2.75±0.96	2.25±1.26	1.5±0.58	2.75±0.50	1.75±16	2.25±0.50

Note. * The values in the table represent the average of four replicates. These values were compared by analysis of variance (Anova) followed by the Tukey test at 5% probability ($p < 0.05$).

The organic matter contents were higher in the fertigation management than in the management without wastewater (Table 4). Thus, infiltration of the effluent into the deeper layers may have occurred. The organic matter is an important factor for soil fertility (Alderson et al., 2015; Malafaia et al., 2016). However, there was no significant difference ($p < 0.05$) in phosphorus and sodium concentrations between managements with or without wastewater (Table 4). These results are important, because it shows a low risk of salinization and eutrophication of the soil by the domestic wastewater.

3.4 Microbiological Analyses

3.4.1 Counting of Viable Microorganisms Before Fertigation

The domestic wastewater did not have significant ($p < 0.05$) counts of fungi and actinomycete (Table 5). However, we observed a viable cell count of bacteria in this wastewater that may be of the coliform group. According to Dionísio (2006), the bacteria were the main microorganisms found in the effluents of the sewage treatment plant of Curitiba/PR/Brazil. Bacteria has distinct ecological niches. In domestic wastewater and in soil, there is, respectively, a predominance of coliforms and rhizobacteria (Wartiainen et al., 2008).

Table 5. Viable microbial cells counts from the soil before planting of *Brachiaria brizantha* cv Marandu and wastewater utilization

Samples	Actinomycete	Bacteria	Fungos
	---------- Log (CFU g^{-1}) ----------		
Wastewater	-[a]	7.29±0.03	-[a]
A1SD1	6.21±0.01	7.44±0.01	5.15±0.03
A1SD2	6.02±0.01	7.37±0.01	4.92±0.01
A1SD3	5.64±0.01	6.95±0.01	-[a]
A1SD4	-[a]	6.69±0.03	-[a]
A2SD1	6.25±0.01	7.45±0.01	5.04±0.04
A2SD2	6.10±0.01	7.37±0.01	4.86±0.02
A2SD3	5.50±0.03	6.97±0.01	-[a]
A2SD4	-[a]	6.68±0.04	-[a]
A3SD1	6.24±0.01	7.44±0.01	5.05±0.01
A3SD2	6.08±0.01	7.36±0.01	4.79±0.02
A3SD3	5.49±0.04	6.96±0.01	-[a]
A3SD4	-[a]	6.67±0.05	-[a]
A4SD1	6.28±0.01	7.43±0.01	5.10±0.06
A4SD2	6.14±0.01	7.36±0.01	4.89±0.01
A4SD3	5.55±0.03	6.93±0.01	-[a]
A4SD4	-[a]	6.65±0.04	-[a]

Note. a: values below 25 colonies. CFU: Colony forming unit. A1SD1: sampling point 1 and soil depth 1 (0-10 cm), A2SD2: sampling point 2 and soil depth 2 (10-20 cm), A3SD3: sampling point 3 and soil depth 3 (20-30 cm), and A4SD4: sampling point 4 and soil depth 4 (90-100 cm). The values in this table represent the mean of four replicates plus or minus one standard deviation. These values were compared by analysis of variance (Anova) and Tukey test at 5% probability ($p < 0.05$).

The soil depth and the microbial group influenced the counts of viable microorganisms before fertigation (Table 5). Regardless of depth, the bacterial community was larger than of filamentous fungi. This prevalence of bacterial community in relation to fungi was also observed in the soil under native vegetation in the southern region of Brazil (Rech et al., 2013). According to these authors, the soil microorganism count varies with the techniques, soil depth and culture medium. However, we observed no difference ($p < 0.05$) in the counts of viable bacteria between the sampling points and depths of 0 to 10 cm and 10 to 20 cm (Table 5). Therefore, the technique and/or culture medium influenced the microbial cells counts more than depth.

The bacterial and fungal cell counts prior to fertigation are similar to the results obtained in other studies with soil samples (Rech et al., 2013).

Only the bacterial community was observed at all depths (Table 5). Therefore, their great dispersion in the soil is due to present aerobic, anaerobic, and nitrogen fixation species (Dunbar et al., 2002, Vázquez et al., 2000, Zehr

et al., 2003). In addition, the microbial activity of six clayey soils was higher in the surface than in the depth of 60 cm showing that the bacteria are present in several soil depths (Vale Júnior, 2011).

Actinomycete colonies were not observed only at depths of 90 to 100 cm (Table 5). The absence of microbial colonies in this soil depth may be due to the nutrient and oxygen limitation. Fungal colonies were observed in 20 to 30 cm and 90 to 100 cm. Furthermore, the communities of these microorganisms are higher in the rhizosphere than other soil depths (Smith et al., 2010, Neeraj, 2011, Silva et al., 2002, 2011).

3.4.2 Viable Microbial Cells Counts After Fertigation

A reduction in microbial cell count with the depth and Bacterial cell count greater than Actinomycete and Fungi were observed in soil samples before and after fertigation (Tables 5 and 6). This result may be due to the unicellular growth of the Bacterial (Madigan et al., 2010).

Table 6. Viable microbial cells counts after the planting of *Brachiaria brizantha* cv Marandu

Fertigation managements	Soil depth	Actinomycete	Bacteria	Fungos
	-------- cm -------	---------- Log (CFU g^{-1}) ----------		
M1	0-10	7.13 ±0.03	8.80 ±0.09	6.64 ±0.01
	10-20	6.83 ±0.03	8.20 ±0.07	5.52 ±0.01
	20-30	6.21 ±0.04	7.55 ±0.04	2.01 ±0.01
M2	0-10	7.23 ±0.02	8.40 ±0.02	6.24 ±0.03
	10-20	6.79±0.01	7.95 ±0.07	5.35 ±0.07
	20-30	6.17±0.02	7.20±0.01	2.12 ±0.01
M3	0-10	6.89 ±0.03	8.32±0.09	5.85 ±0.04
	10-20	6.64 ±0.06	7.93 ±0.05	5.12 ±0.02
	20-30	5.96 ±0.03	7.15±0.06	1.96±0.01
M4	0-10	7.11±0.01	8.36±0.02	6.95±0.04
	10-20	6.82 ±0.03	8.01 ±0.03	5.34±0.03
	20-30	5.87±0.07	7.22 ±0.02	1.86±0.05
M5	0-10	7.36±0.09	8.35±0.03	6.84±0.01
	10-20	7.01±0.06	7.85 ±0.03	5.90±0.02
	20-30	6.10±0.03	7.13±0.02	2.55 ±0.07

Note. CFU: Colony forming unit. The values in this table represent the mean of four replicates plus or minus one standard deviation. These values were compared by analysis of variance (Anova) followed by the Tukey test at 5% probability ($p < 0.05$).

Viable microbial cells counts after fertigation were higher than before planting of *B. brizantha* (Tables 5 and 6). Simões et al. (2013) observed a linear increase in microbial respiration with the wastewater dose applied in the planting of castor bean.

Regardless of fertigation, there was a significant increase ($p < 0.05$) in the viable microbial cells counts after planting of *B. brizantha* (Tables 5 and 6). Thus, the wastewater did not change the viable microbial cell count (Table 5). However, the fungal colonies in the depth of 20-30 cm were observed after fertigation that may be due to the presence of roots and availability of water and nutrients (Table 6). The fungi form mycorrhizal interactions with the roots (Pozo & Azcon-Aguilar, 1997; Oehl et al., 2011).

3.4.3 Microbial Diversity by DGGE Profile Before of Fertigation

The amplification of the *nif H* gene in soil depth of 90 to 100 cm was not observed (Figure 3). Although, viable bacterial cells had been obtained in this depth.

The DGGE band profile showed a large variety of NFB, with the higher number of bands in 0 to 10 cm and 10-20 cm than other depths (Figure 3). This result can be due to the high amount of BFN cells in the rhizosphere (Silva et al., 2011). Furthermore, Da Silva (2012) showed great diversity of *nif H* gene in Cerrado soil by DGGE. This same author identified a predominance of this *nif H* gene in Actinomycete.

Similar to the observed in the soil physicochemical analyses, two main groups were observed in the UPGMA dendogram (Figure 3). The A1 point has 69% of similarity with point A2, A3 and A4 points has 89% of similarity. Therefore, the distance between sampling points influenced the bacterial diversity and shows once

again the importance of randomization of the managements (Figure 1). The sampling site and climatic season influences the diversity of the *16S rRNA* gene (Campelo, 2008). However, there were no significant differences in the BFN diversity at depths studied (Figure 3).

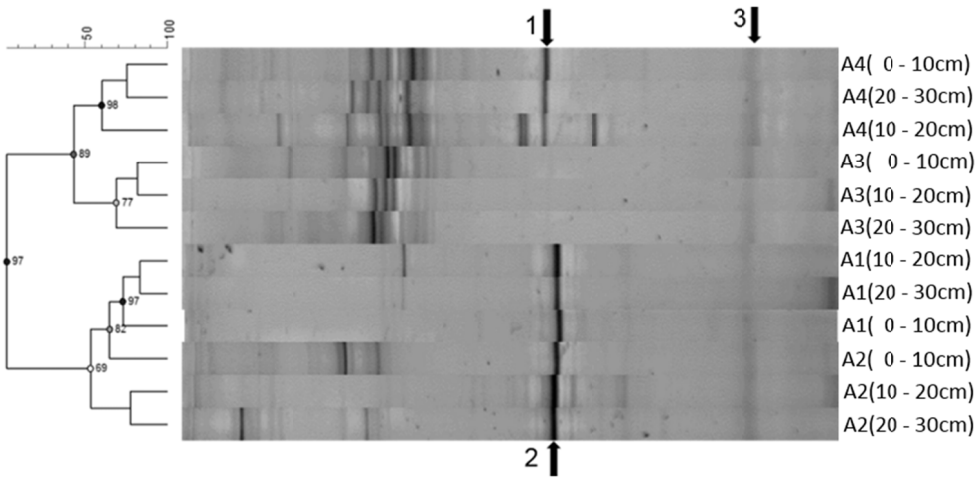

Figure 3. UPGMA dendogram and profile of *nif H* gene bands from soil samples obtained before the fertigation

In Figure 3, the arrows 1 and 2 are the bands with highest intensities in the gel. This group of diazotrophic bacteria is distributed in large numbers in A1, A2, and A4 points. The arrow 3 shows the bands with low intensity. The distribution of this BFN is throughout the studied area.

3.4.4 Microbial Diversity by DGGE Profile After Fertigation

We observed an increase in the bands intensity after the fertigation (Figures 3 and 4). In addition, the diversity and abundance of BNFs were different between the depths and fertigation managements (Figure 4). This result confirms the increase in viable microbial cell counts after fertigation (Tables 5 and 6, Figure 4). The analyses of the *16S rRNA* gene showed a large variation in the soil microbial community, after the application of sewage sludge doses (Val-Moraes, 2008).

The clusters of *Nif H* gene with fertilization managements were observed in the soil depth higher than 0-10 cm (Figure 4). This result shows that wastewaterNPK has a greater impact in the surface than other soil depth or the wastewater has low infiltration rate in soil.

The cluster of M1 management in the in the center of the gel and low bands intensity of M2 management show the effects of wastewaterNPK on microbial community (Table 2 and Figure 3). According Reis Júnior et al. (2011), the limitation of nitrogen can increase the BNFs activity.

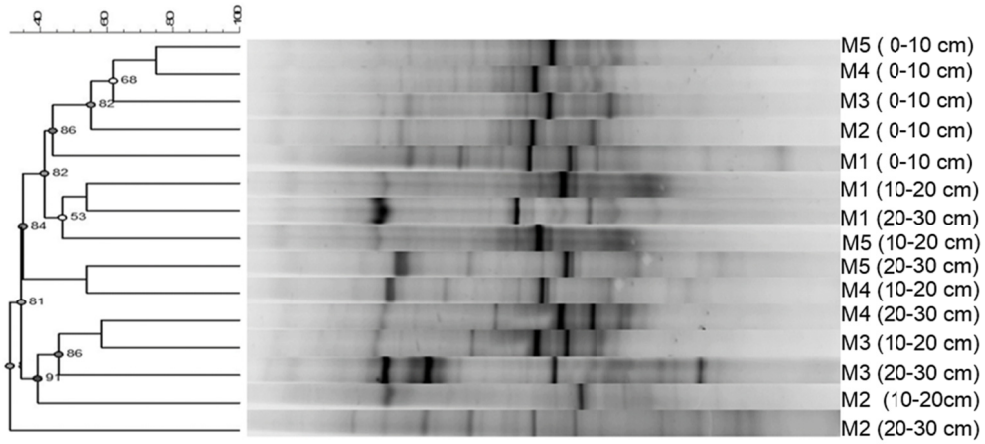

Figure 4. UPGMA dendogram and profile of *nif H* gene bands from soil samples obtained after the fertigation

The M1 management had profiles of *nif H* gene bands similar to the M2 management (Figure 4). These fertigation managements not had wastewater. The M4 management also has profiles of *nif H* gene bands similar to M5 (Table 2 and Figure 4). M4 and M5 were the fertigation management with higherst wastewater levels. In 0-10 cm, the M3 management has 82% similarity with M5/M4. However, M3 management is closer to M2 in the other depth. M3 management has 80% of comNPK and 20% of wastewaterNPK (Table 2). Thus, the wastewater influenced more BNFs community at surface than other soil depths. In addition, the impact of wastewater on BNFs in other soil depths was observed in dose greater than 40% (Figure 4).

After the fertigation, we not observed the bands of the arrow 3 of Figure 3 (Figure 4). However, the bands of arrows 1 and 2 had same intensity before and after fertigation (Figures 3 and 4). Therefore, the changes of the BNF community after fertigation was due to the different periods of sampling, soil management before planting of grass, and soil moisture than the addition of wastewater. This result shows the potential use of domestic wastewater in agriculture, especially in forage planting in the soil in the Cerrado.

Before of fertigation, the DGGE bands profile shows a heterogeneous distribution of fungi in sampling points and in soil at depths when compared to the bands profile of the *nif H* gene (Figures 3 and 5). The amount and intensity of these bands also show a great diversity and concentration of these fungi in the studied area (Figure 5). The fungi help plant in the absorption of water and nutrients, especially phosphorus (Souza et al., 2011, Thongtha et al., 2014). Moreover, Moreira et al. (2007) also verified by the DGGE profile a high diversity of AMFs in different soils. According to them, these soils had high rate of seed germination and seedling growth, due to the richness of AMF. The high intensity of the bands may be due to the viable fungal cells (Table 6) and spores. Thus, the reduction in the quantity and intensity of these bands may show alterations in the FMA community after the application of wastewater.

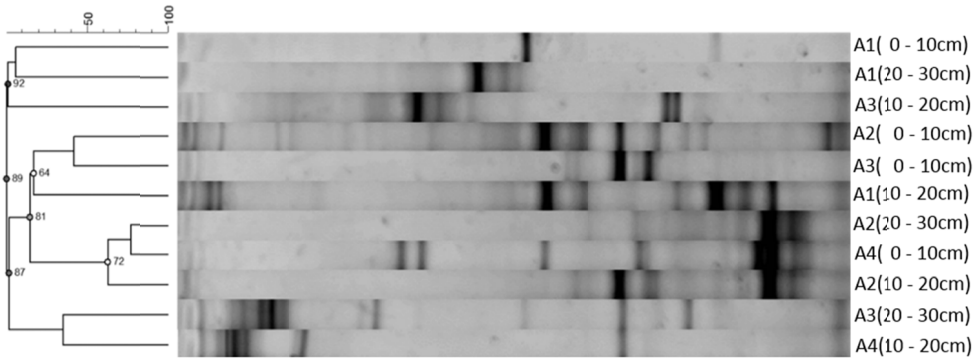

Figure 5. UPGMA dendogram and profile of *18S rDNA* gene bands from soil samples obtained before the fertigation

We not observed the amplification of *18S rDNA* gene in soil depth of 90 to 100 cm (Figure 5). This result confirms the absence of viable fungal cells at this depth (Table 6).

Unlike the *nif H* gene profile, the UPGMA dendogram of the *18S rRNA* gene did not pool the sampling points and depths (Figure 5). This confirms the heterogeneous distribution of AMFs in the studied area, before the soil management, planting of grass and the use of wastewater.

After fertigation, we observed an increase in the number of bands in M2 to M5 managements (Figures 5 and 6). Furthermore, in the fertilization management without NPK (M1) has lower number and intensity of bands than in management with NPK. These results show the influence of NPK on the AMFs diversity.

In 0-10 and 10-20 cm, the fertilization managements (M2 to M5) had a cluster (Figure 6). Similar to the observed in the BFNs profile after fertigation, the AMFs profile of the M3 management shares similarity with wastewaterNPK and comNPK treatments (Figures 4 and 6).

The NPK influenced the AMF community at depth of 10-20 cm (Figure 6). However, the cluster between the fertilization managements in the depth of 20-30 cm confirms that there was no infiltration of wastewater in the deeper soil layers.

There is no similarity by the UPGMA dendogram and DGGE profiles between M1 management and managements with wastewaterNPK (M3 to M5), (Figure 6). Furthermore, the intensity of the bands in these

fertigation managements was larger than the M1. Thus, the wastewater had a positive influence on the abundance of AMF. According to Machado et al. (2011), the water and nutrient content increases the germination rate of AMF spores. Santos et al. (2009) showed also that the use of sewage sludge as fertilizers stimulates microbial activity.

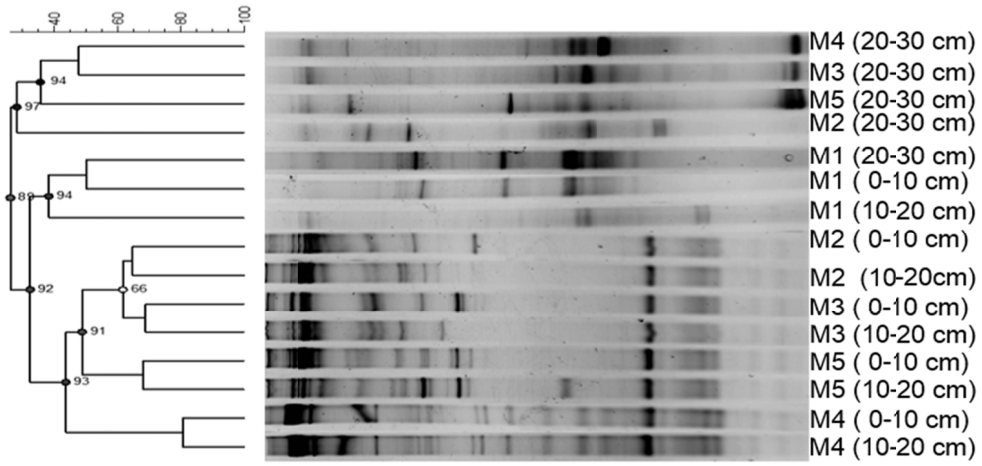

Figure 6. UPGMA dendogram and profile of *18S rDNA* gene bands from soil samples obtained after the fertigation

3.5 Grass Samples Characterization

In this study, three cuts of leaf biomass were done. We do not observe flower and seed production during cuts.

The first cut had the lower leaf dry mass production than other cuts. We did not obtain significant difference ($p < 0.05$) in the dry mass between second and third (Figures 7).

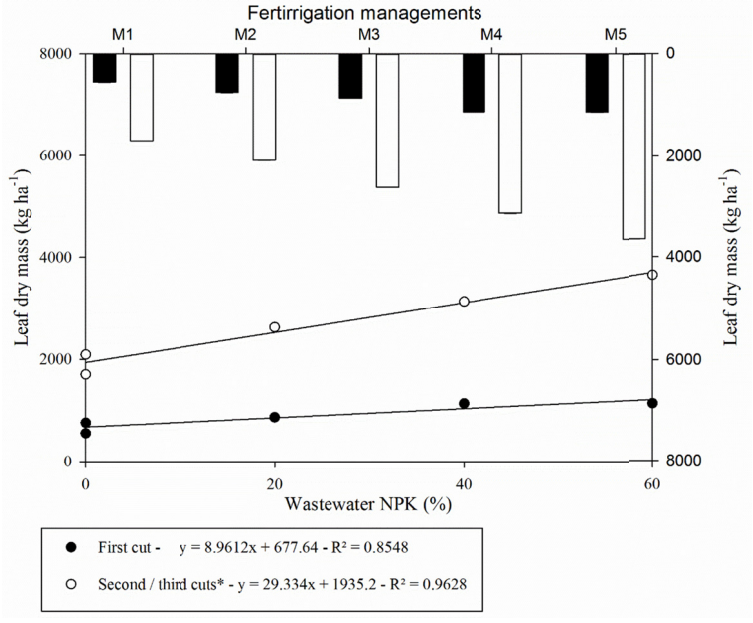

Figure 7. Leaf dry mass productivity of *Brachiaria brizantha* cv Marandu cultivated with different fertirrigation managements (M1 to M5)

Fertigation managements (M3 to M5) had higher leaf dry mass production per hectare (ha) than the managements without wastewater (Figure 7).

In the first cut, the dry mass was the average of each fertigation management (Figure 7). In the other cuts, these values were the mean of both cuts, as there was no significant difference between them ($p < 0.05$) (Figure 7).

The dry mass productivity of *B. brizantha* was directly proportional to the wastewaterNPK (Figure 7). Furthermore, wastewaterNPK had higher leaf dry mass productivity than comNPK (Figure 8). Therefore, comNPK is less available for plant than the wastewaterNPK.

Linear equations are important for leaf dry mass productivity studies using NPK wastewater concentrations. The positive linear effect on leaf dry mass with nitrogen dose was also observed in the planting of Marandu grass (Alexandino et al., 2003) and *Brachiaria decumbens* cv. Basilisk (Maranhão et al., 2010).

The dry mass productivity of the second cut was similar to the third cut (Figure 7). This result shows the regrowth of the Marandu grass with the fertigation that can contribute to reduce the frequency of planting of this grass. The leaf dry mass increases with reduced cut-off interval and regrowth decreases with the cuts (Maranhão et al., 2010; Alexandino et al., 2003).

The iron, cobalt, and etheric extract content of the leaf biomass did not differ significantly ($p < 0.05$) between the second and third cuts (Table 7).

In the last cut, we observed a significant reduction ($p < 0.05$) in nitrogen, crude protein, potassium, zinc, copper, and manganese content (Table 7). However, there is a significant increase ($p < 0.05$) in phosphorus, calcium, magnesium, sulfur and, ADF content (Table 7). Therefore, the cuts had an influence on the nutritional composition of Marandu grass.

The calcium and NDF levels were different between without management (M1) and managements with NPK (M2 to M5) (Table 7). These managements had higher phosphorus, magnesium, and NDF content than the M1 management. Therefore, the domestic wastewater did not alter the nutritional composition of Marandu grass (Table 7).

The crude protein content, in the fertigation managements, was similar to the obtained by Serafim (2010). This author shows a variation of 11.31 to 13.81% in the crude protein content with the use of swine wastewater in the Marandu grass growth.

NDF is the main nutritional component of animal feed (Benett et al. 2008). According to these authors, a forage of better consumption is those with low NDF contents. Thus, only M1 management had a low quality forage.

Table 7. Nutritional composition of *Brachiaria brizantha* cv Marandu

Cuts	N	P	K	Ca	Mg	S	Zn	Cu	Fe	Mn	Na	Co	Mo	CP	EE	ADF	NDF	Ash
	g kg^{-1}	mg kg^{-1}	%															
1	21.76A	1.03B	17.89A	2.43B	1.89B	1.29B	25.35A	9.00A	392.30A	48.45A	113.50B	0.13A	0.44C	13.59A	1.50A	29.60B	55.17B	6.01A
2	20.38A	1.39A	11.78B	2.43B	3.09A	1.27B	25.25A	5.80B	434.40A	28.10B	124.65A	0.17A	0.58A	12.72A	1.36A	31.39AB	61.58A	5.82A
3	18.10B	1.51A	10.16B	3.00A	3.13A	1.51A	16.15B	3.00C	366.60A	25.80B	112.50B	0.10A	0.48B	11.31B	1.48A	33.285A	57.95B	5.74A
Fertigation managements																		
M1	22.10a	1.05b	16.03a	2.29b	1.85b	1.22a	26.25a	5.58a	336.90a	38.33a	114.83a	0.12a	0.50a	13.81a	1.50a	29.37b	53.94b	6.1a
M2	20.38ab	1.25ab	12.07a	2.78a	2.04ab	1.38a	22.92a	7.00a	358.80 a	32.17a	116.64a	0.12a	0.49a	12.73ab	1.47a	31.92ab	59.36a	5.3ab
M3	18.26 b	1.37a	12.17a	2.53ab	2.93a	1.38a	20.01a	5.01a	350.10 a	36.25a	116.58a	0.12a	0.51a	11.41b	1.51a	31.93ab	59.68a	4.1b
M4	19.64ab	1.41a	13.08a	2.74ab	3.10a	1.34a	19.75a	6.01a	320.50a	31.83a	118.25a	0.18a	0.51a	12.24ab	1.36a	32.70a	59.08a	4.7b
M5	20.08ab	1.46a	13.05a	2.75ab	3.22a	1.45a	22.33a	6.08a	322.40a	32.00a	118.08a	0.12a	0.49a	12.50ab	1.36a	31.20ab	59.11a	4.7b

Note. CP: Crude protein, EE: Etheric extract, ADF: Acid detergent fiber, NDF: Neutral detergent fiber. The upper and lower case letters in the columns indicate, respectively, the statistical comparison between cuts and treatments. Thus, the same letters in the same column indicate that there was no significant difference by the Tukey test at 5% significance (see annexes). Statistical analyses were carried out using the free trial version of the Minitab 17 software (2016) available at http://www.onthehub.com/minitab.

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FDA levels are related to the lignin content and digestibility. Therefore, the biomass of third cut had the higher FDA than to the other cuts that may be due to the aging of the plant.

The etheric extract levels did not exceed the limit of 6% of the diet of ruminants (Souza et al., 2009). These levels are lower than those reported by Mari (2003). This author obtained values etheric extract in Marandu grass from 2.7 to 1.9% of dry mass.

The phosphorus concentration in leaf biomass was of 1.03 to 1.51 kg^{-1} (Table 7). This concentration was similar to the fodder plants (Malavolta, 1987).

The aging of the grass influenced significantly ($p < 0.05$) in the Cu concentration (Table 7). We observed a decrease in Cu concentration with time. Concentration of this nutrient was of 3.00 to 9.00 mg kg^{-1}. According to Malavolta (1987), the Cu level in grass is of 6.0 mg kg^{-1}.

The Fe level was high in leaf biomass, because the levels of this element in grass are of 180 to 250 mg kg^{-1} (Malavolta, 1987). However, the iron requirement for ruminants is of 30 to 100 mg kg^{-1} (McDowell, 1999). Thus, our forage would meet this requirement.

The ash content did not differ with the cuts ($p < 0.05$). However, the managements with wastewaterNPK had lower ash content than other treatments (Table 7). The ash content obtained in this study is similar to the observed in Marandu grass cultivated at different times of the year in the state of Piauí/Brazil (Rodrigues Júnior et al., 2015).

4. Conclusions

In this study, we had the following conclusions:

- Fertigation is a viable alternative for the use of domestic wastewater in agriculture in the Cerrado soil, which may represent a reduction in the wastewater amount discarded in the water bodies.
- Fertigation management with up to 60% NPK from wastewater also validates this alternative;
- Changes in soil physical and chemical properties after fertigation depend of the time, the type of crop, and the characteristics of the soil and effluent used;
- The use of fertilizers from domestic wastewater contributes positively with the abundance of NFB and AMF in the soil;
- NPK of wastewater is more available to the plant than commercial NPK;
- Fertigation with domestic wastewater has a low risk of salinization and eutrophication of the soil.

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