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FARM DAIRY EFFLUENT APPLICATION ON AN ARGIUDOLL CULTIVATED WITH ALFALFA (*Medicago sativa* L.): BIOMASS PRODUCTION AND PERSISTENCE

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ABSTRACT

The intensification of dairy production systems has resulted in a higher generation of waste. The aim of this study was to evaluate the impact of different doses of farm dairy effluent (FDE) applied to the soil on aerial biomass production, persistence and chemical composition of alfalfa pastures, in Santa Fe province, Argentina. The study was conducted in four randomized complete blocks with four treatments (FDE doses): 0 m^3 ha^{-1} (T0: without FDE), 82 m^3 ha^{-1} (T1: low rate), 151 m^3 ha^{-1} (T2: medium rate) and 282 m^3 ha^{-1} (T3: high rate). The soil application of FDE increased the total aerial biomass produced (ABP) but reduced the persistence of plants. Total nitrogen (Nt) of alfalfa was significantly higher, but phosphorus (P) concentrations did not vary after the application of FDE to the soil . Ca and Mg concentrations in plants were lower in T3 compared to T0, which can be explained by the imbalance produced in the soil after FDE was applied, presenting high levels of Na, K and NH4-N but low levels of Ca and Mg. Soil application of FDE on alfalfa pastures must be carefully planned in terms of composition and amount to be applied in order to increase biomass production, prevent the decrease in persistence, and control changes in the mineral composition of plants.

Keywords: macronutrients, manure, pasture, soil management.

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INTRODUCTION

Argentine dairy farms are facing a process of concentration and intensification. The average dairy farm has increased in cow productivity and herd size (Lazzarini et al., 2019), while intensification of dairy production systems has resulted in a higher generation of waste in areas with a large concentration of livestock (Lazzarini and Budracco, 2021). Farm dairy effluent (FDE) has a high content of organic matter (OM), with a low content (lower than 12%) of dry matter (DM). It is composed of manure, urine, food scraps, and wastewater generated from cleaning and sanitation operations of milking facilities, presenting highly variable physical, chemical, and biological characteristics (Houlbrooke et al., 2004; Christensen and Sommer, 2013; Whalen et al., 2019).

FDE is commonly stored in open treatment ponds, and subsequently, applied to the soil. The main reason to dispose effluents on soils is to prevent them from being discharged into watercourses or permanently stored in ponds (Morero et al., 2021). However, soil application of effluents may cause negative environmental effects, such as nitrate contamination of groundwater; loss of nitrogen and phosphorus, leading to eutrophication of watercourses; build-up of heavy metals on the soil; increased mobility of organic pollutants; greenhouse gas emissions and odors; and health effects related to the spread of pathogens and estrogens (Houlbrooke et al., 2004; Imhoff et al., 2021). In addition, the excessive amounts of potassium in FDE may cause a nutritional imbalance in plants, deterioration of soil physical properties, and metabolic problems in dairy cows consuming potassium-rich pastures (Bolan et al., 2017).

The use of FDE as organic soil amendment can provide a solution to waste disposal (Gambaudo et al., 2014), and in turn improve soil fertility and land productivity, adding commercial value to a by-product that is often considered as waste (Imhoff et al., 2021). In this sense, proper agronomic management is required to add the suitable amount of nutrients provided by OM (based on crop requirements), and also to prevent negative effects on the environment and pasture performance (Eghball and Barbarick, 2017; Whalen et al., 2019; Ghiberto et al., 2020).

In the central-eastern area of Santa Fe, Argentina, dairy farms are predominantly pasture-based, with cows grazing year-round in a non-irrigated system (Lazzarini et al., 2019). Alfalfa (*Medicago sativa* L.) is the main pasture used in this region (Lazzarini and Baudracco, 2021). Soils show nutritional deficiencies that could be corrected with the application of FDE to the land (Gambaudo et al., 2014; Imhoff et al., 2014), and thus improve forage crop production. However, the use of FDE on alfalfa pastures has shown contradictory responses.

Considering the need for the rational management of FDE, the aim of this study was to evaluate the impact of different doses of FDE applied to the soil on aerial biomass production, persistence and chemical composition of alfalfa pastures.

MATERIALS AND METHODS

The study was conducted on a dairy farm located 1.5 km east of Cavour village, Santa Fe province, Argentina (31°21′59′′ S, 61°00′28′′ W). The study area is located in the region known as "*Pampa Llana santafesina*," which has a flat relief. The soil parent material is loess. The soil is a typic Argiudoll, Rincón de Ávila series (Soil Survey Staff, 2014), with silty loam texture in the A horizon (0-20 cm depth), silty clay loam texture in the BA horizon (20-30 cm depth), and silty clay texture in the B_t horizon (30-55 cm depth). The climate in the region is subhumid-humid mesothermal (c2b′3ra′). The annual average temperature is 18.05°C, with average temperatures of 11.8°C and 25°C in the coldest and hottest months, respectively. Annual average rainfall is 967 mm; the rains are concentrated between October and March, accounting for 69% of the total rainfall record. Annual potential evapotranspiration is 907 mm, while runoff can occur from November to March, when rainfall exceeds evapotranspiration; the slope is greater than 1% (INTA, 1991).

The experiment lasted approximately 20 months. Planting was carried out on July 16, 2014 and the last sampling was conducted on April 1, 2016. Alfalfa (*Medicago sativa* L.) was planted using a no-tillage seeding system, with a 0.175 m spacing between rows. The seeds were not inoculated with nitrogen-fixing bacteria prior to planting and no herbicides or insecticides were required. The preceding crop was soybean. An homogeneous area was chosen within a 30 ha field and plots of 20 m long x 3 m wide were established with a 2-m spacing between them; a randomized complete four-block design was used (Fig. 1), with four treatments (FDE doses): 0 m^3 ha^{-1} (T0: without FDE), 82 m^3 ha^{-1} (T1: low rate), 151 m^3 ha^{-1} (T2: medium rate) and 282 m^3 ha^{-1} (T3: high rate). The values did not turn out to be multiples as expected because doses were applied with a farmer´s manure spreader, which was very simple and did not allow a uniform spread of FDE. The amounts of FDE used are shown in Table 1.

The FDE was treated in a facultative lagoon

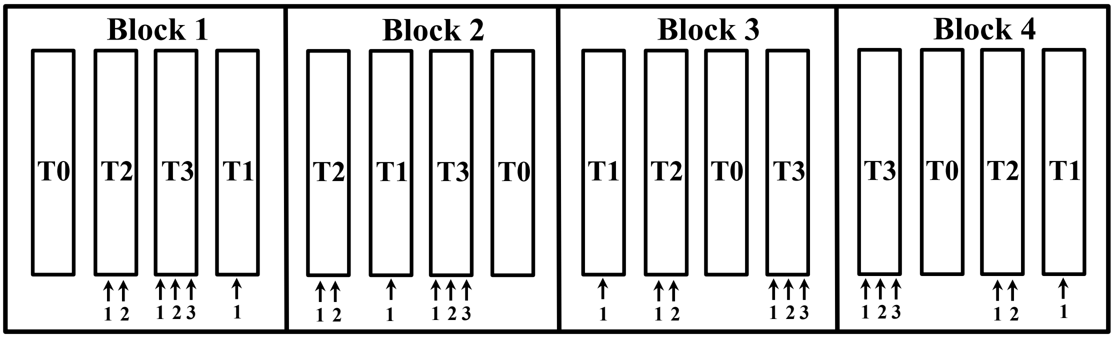

Fig. 1. Experimental design with four treatments: 0 m^3 ha^{-1} (T0: without FDE); 82 m^3 ha^{-1} (T1: low rate); 151 m^3 ha^{-1} (T2: medium rate) and 282 m^3 ha^{-1} (T3: high rate). T2 and T3 were completed by spreading twice and three times in the same place, respectively. Application dates were January 22, 2015 (1); February 23, 2015 (2) and April 1, 2015 (3).

Table 1. Amounts of FDE applied in each treatment.

FDE application	1° (January 22)	2° (February 23)	3° (April 1)	Total
	--------------------	m^3 ha^{-1}	--------------------	
T1	82	0	0	82
T2	74	77	0	151
T3	71	118	93	282

designed for a retention period of 150 days. The effluent was collected from the surface layer, i.e., from the aerobic zone. The FDE was applied on soil using a 6000 L manure spreader with a splash plate applicator by surface broadcasting.

T2 and T3 were completed by spreading twice and three times in the same place, respectively. Consecutive soil applications aiming to complete T2 and T3 were spaced in time to avoid driving on wet fields and to prevent potential plant death from excessive FDE application. Thus, applications were conducted on January 22, 2015 (T1, T2 and T3); February 23, 2015 (T2 and T3); and April 1, 2015 (T3) (Fig. 1). The passing of the manure spreader wheels was restricted to the same paths on successive application dates in order to avoid excessive damage on alfalfa plants within the plots. All measurements were performed outside the wheel tracks.

Prior to FDE application, soil chemical properties were assessed to establish a baseline nutrient status. In each experimental plot, composite soils samples were collected at depths of 0-5 cm, 5-20 cm and 20-30 cm, and the following determinations were made: OM (Walkley and Black), total nitrogen (N_t) (Kjeldahl), nitrate nitrogen (NO3-N) (phenoldisulfonic acid method), available phosphorus (P) (Bray and Kurtz N° I), pH (soil reaction in water, 1:2.5 ratio), electrical conductivity (EC_e) of the saturation paste extract (conductimetry), and sulfate sulfur (SO4-S) (turbidimetry) (Sparks, 2009).

Three FDE sub-samples were collected from the spreader for each plot to verify the actual amount of FDE applied. Then, the samples were sent to the laboratory for analysis. DM, ash, total nitrogen (N_t), ammonium nitrogen (NH4-N) and phosphorus (P) contents were determined by spectrophotometry, while calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K) were determined by atomic absorption (Horwitz and Latimer, 2007). The total amount of applied nutrients per plot was calculated from the applied volume and the concentration of each nutrient.

Pasture persistence and aerial biomass produced (ABP) were determined by measuring the number of plants and ABP in each plot in the flower bud stage. The evaluation was carried out in three fixed microplots of one linear meter (sub-samples) per plot, with a total of 48 samples for each cutting date. After counting, plants were cut at a height of five centimeters above ground level for ABP determination.

All samples were weighed immediately after cutting. A representative aliquot was taken, dried at 60 °C in a forced air oven until constant weight to determine DM content, and then ground to pass through a 1-mm sieve, using a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA). ABP was determined based on DM content, expressed as kg ha^{-1} of DM. The accumulated ABP throughout the year was calculated by adding the production recorded for each month.

The samples of the last cutting date of treatments T0 and T3 were evaluated to determine ash content (Ash) in muffle furnace. In addition, contents of phosphorus (P) and calcium (Ca) were determined by colorimetry, while potassium (K) and sodium (Na) were measured by flame photometry. Total nitrogen content (N_t) was determined using the Kjeldahl method (Horwitz and Latimer, 2007).

Data were analyzed using the InfoStat statistical software (Di Rienzo et al., 2016); the F-test of variance analysis was carried out with the subsequent comparison of means, using the Tukey test, with a 5% significance level after confirming normal distribution with the Shapiro-Wilkes test.

RESULTS AND DISCUSSION

The accumulated rainfall during the 20 months of the experiment (June 2014-April 2016) was 2,368 mm, which exceeded the historical average over the same period (Fig. 2). Atypical events were recorded during the periods of November 2014 to March 2015 and February to April 2016.

The trial was conducted in a soil rich in OM and total nitrogen (N_t), with a low content of nitrate nitrogen (NO3-N) and available sulfur (SO4-S), a good concentration of P on the surface and a slightly acidic pH (Table 2).

The chemical composition of the FDE used in each application is shown in Table 3. DM content and nutrient concentration were highly variable. Therefore, as suggested by Whalen et al. (2019), analyzes of the FDE are required to determine a proper application rate schedule. Once the composition of the FDE is known, the dose can be established based on crop nutrient demand (e.g., N or P) and soil nutrient content to meet the most limiting nutrient requirement.

Regarding OM, the FDE provided more than 6,000 kg ha^{-1} in T1 and T2, and more than 16,700 kg ha^{-1} in T3 (Table 4). The applied amount of N_t was 470 kg ha^{-1} in T3, of which 80 kg ha^{-1} was in the form of NH4-N. It is relevant to highlight that the FDE provided high amounts of Na and K and limited amounts of Ca and Mg to the soil.

Pasture persistence was affected by the soil application of FDE. The number of plants (pl) at 121 days after seeding (DAS), and before FDE application was 125 pl m^{-2} (Table 5). This number was lower than the target value (250 pl m^{-2}) for the central area of the Santa Fe province (Romero et al., 1991). Some of the factors that could have reduced the number of plants before FDE application are the late seeding date, the lack of surface soil moisture, and the preceding crop. The second plant count was carried out 236 DAS and 15 days after the first FDE application. This time, the number of pl m^{-2} in T1 was lower than that recorded in T0, T2 and T3. A significant loss in the number of plants per m^2 was observed

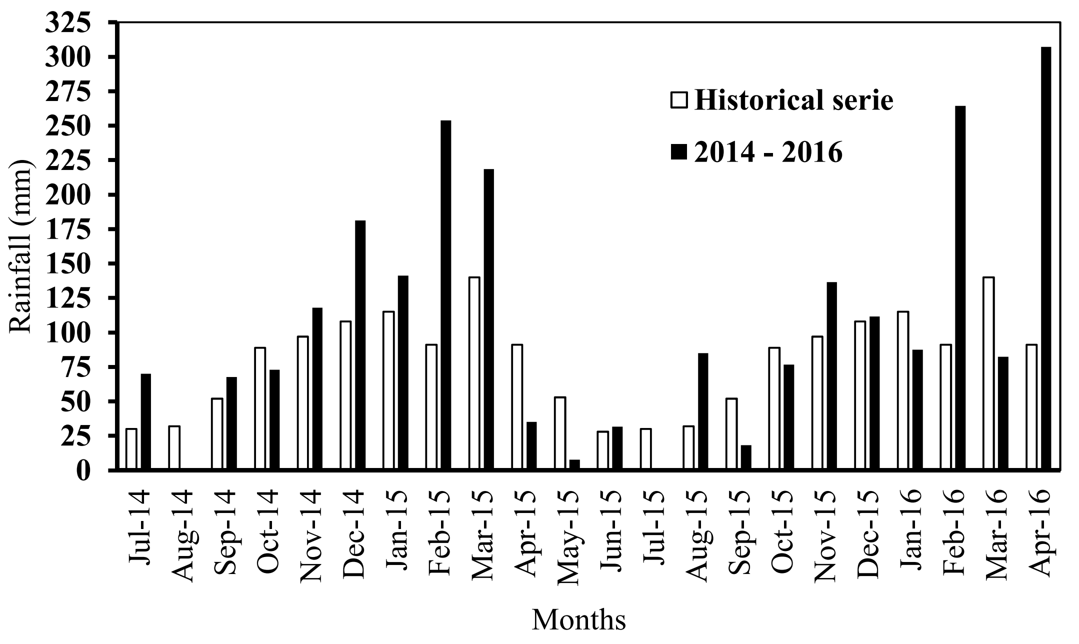

Fig. 2. Average monthly rainfall during the experiment (■) and historical rainfall average values (□).

Table 2. Chemical characteristics of the soil.

Depth cm	pH	EC_e $dS\ m^{-1}$	OM	N_t %	NO3-N	SO4-S $mg\ kg^{-1}$	P
0-5	6.05	0.6	3.53	0.188	3.4	0.4	37.5
5-20	6.01	0.3	2.16	0.114	2.0	2.2	16.0
20-30	6.07	0.3	1.88	0.100	5.0	4.9	14.5

pH: soil reaction in water; EC_e: electrical conductivity of the saturation paste extract; OM: organic matter; N_t: total nitrogen; NO3-N: nitrate nitrogen; SO4-S: sulfate sulfur; P: available phosphorus.

Table 3. Chemical properties of the FDE.

FDE application	DM	Ash	N_t	P	Ca	Mg	Na	K	NH4-N
	-------%-------			---mg L^{-1}---					
1° (January 22, 2015)	7.88	5.84	576.8	400	98	100	417	555	218
2° (February 23, 2015)	0.74	0.38	326.2	500	40	43	533	722	218
3° (April 1, 2015)	12.3	7.48	4197.2	400	80	106	589	1019	504

Table 4. Amounts of nutrients provided to the soil in each application.

Treatment	FDE application	DM	Ash	OM	N_t	P	Ca	Mg	Na	K	NH4-N
		---kg ha^{-1}---									
T1	(1° T1)	6442	376	6066	47	33	8	8	34	45	18
T2	1°	5812	339	5472	43	30	7	7	31	41	16
	2°	574	2	571	25	39	3	3	41	56	17
	(1°+2°T2)	6385	342	6043	68	68	10	11	72	97	33
T3	1°	5615	328	5287	41	29	7	7	30	40	16
	2°	873	3	870	38	59	5	5	63	85	26
	3°	11439	856	10583	390	37	7	10	55	95	47
	(1°+2°+3°T3)	17927	1187	16740	470	125	19	22	147	220	88

Table 5. Number of plants in the alfalfa pasture during the experimental period.

Counting	1	2	3	4	5	6	7	8	9	10	11
Date	**10/15/14**	**2/7/15**	**3/27/15**	**5/6/15**	**6/19/15**	**3/8/15**	**9/28/15**	**11/2/15**	**12/23/15**	**1/29/16**	**1/4/16**
T0	125 a	95 b	81 c	77 d	66 c	62 c	49 c	42 b	41 a	33 b	22 b
T1	123 a	86 a	62 b	58 b	57 b	53 b	42 ab	42 b	35 a	32 ab	21 b
T2	126 a	94 b	67 b	64 c	55 b	51 b	44 b	41 b	36 a	32 ab	21 b
T3	127 a	97 b	52 a	48 a	38 a	38 a	38 a	36 a	35 a	29 a	17 a
MSD	11	7	6	4	5	4	5	5	7	3	3

MSD: minimum significant difference. Mean values followed by the same letters in each column are not significantly different ($P < 0.05$).

between the second and third counts, while significant differences were found between the FDE treatments and the control (Table 5).

The fourth plant count, which was conducted 35 days after the third FDE application, resulted in significant differences between the FDE treatments and the control. Subsequently, there were significant differences in the number of plants from the fifth to the seventh counts between T0 and T1, and between T2 and T3. After the second FDE application, T3 (high rate) was more affected than the other treatments in all the experiment, with a higher number of dead plants.

Plant loss in the FDE treatments could be partially attributed to the phytotoxic effect of NH4-N, which can affect plant growth when it is found at high concentrations as observed in this study. It is well known that regrowth after alfalfa cutting depends on the nutrient reserves accumulated in roots and in the so-called crown of the plants. The FDE was applied after cutting the plants. The high concentration of NH4-N caused the death of buds each time FDE was applied, forcing the plant to regrow again. Thus, this process may have contributed to the reduction of crown reserves, favoring plant weakening and death. Another factor that could contribute to plant death was the high rainfall rate and temporary flooding (anaerobic condition) that occurred from November 2014 to March 2015, causing anoxia conditions. Under this condition, the conversion of NH4-N to NO3-N could have been slower, with the negative effect of ammonium persisting longer. Some studies have demonstrated that FDE may be used safely in alfalfa pastures in arid and semi-arid regions (Lloveras et al., 2004; Martin et al., 2006), where anaerobic conditions do not occur, increasing the release of NH_4^+ and reducing oxygen diffusion. Biomass production also decreased in the months after the application of FDE, mainly in T3, which agrees with the plant number reduction observed. Studies conducted by Lasa et al. (2001) and Horchani et al. (2011) showed that ammonium accumulation in shoots was correlated to growth reduction. Furthermore, Horchani et al. (2011) described that alfalfa is partially tolerant to ammonium in excess, which may explain a less negative effect in T1.

Another factor reducing the number of plants under the FDE treatments could be the surface crust of OM that was observed covering bud regrowth, which can affect the normal development of plants. This crust was caused by the high amount of DM provided by the FDE, especially in the last application in T3 (Table 4). Similar findings were reported by Kelling and Schmitt (2003), who used a topdressing system of liquid dairy manure on alfalfa pastures in a dairy farm and observed a decrease in the number of plants and the development of bare spots appearance in the topdressed plots when high FDE rates were applied.

Despite the decrease in the number of plants, significant differences were found in the total ABP among treatments, either in individuals cuts or in the accumulated ABP (Table 6, Fig. 3). The DM content in alfalfa plants remained around 25%, with no differences among treatments. Our results contrast with those of Martin et al. (2006), who found no effect of the application of dairy manure on the alfalfa biomass after 13 cuts throughout the study. In that research, fresh and composted manure was applied to fertilized plots

Table 6. Aerial biomass production in different cuts.

Plant cut N° Date	1 10/15/14	2 11/25/14	3 1/7/15	4 2/7/15	5 3/27/15	6 5/6/15	7 6/19/15
T0	1047.8 a	1595.5 a	1477.7 ab	1070.4 a	1033.6 a	1564.1 a	471.8 ab
T1	1301.0 b	1633.8 a	1696.3 b	1582.9 bc	1557.7 b	1472.5 ab	564.5 b
T2	1330.3 b	1788.0 a	1248.2 a	1369.3 b	1126.1 a	1187.9 b	369.5 a
T3	1324.7 b	1797.1 a	1584.9 b	1609.4 c	1206.0 a	1199.1 b	536.1 b
MSD	162.9	212.0	254.5	235.2	334.7	333.2	113.8
Plant cut N° Date	**8 8/3/15**	**9 9/28/15**	**10 11/2/15**	**11 12/23/15**	**12 6/29/16**	**13 1/4/16**	**Total**
T0	1069.5 b	927.9 a	994.7 a	1949.6 a	1073.4 b	695.1 a	14971.1 a
T1	1307.4 c	1312.8 b	1504.2 bc	2498.0 ab	1249.5 b	886.2 b	18566.8 b
T2	821.9 a	871.3 a	1272.2 b	2289.9 a	756.6 a	709.8 a	15141.2 a
T3	1236.1 bc	1073.4 ab	1659.9 c	3129.7 b	1320.3 b	784.1 ab	18460.8 b
MSD	215.4	259.2	247.5	646.3	312.6	135.8	1221.6

MSD: minimum significant difference. Mean values followed by the same letters in each column are not significantly different ($P < 0.05$).

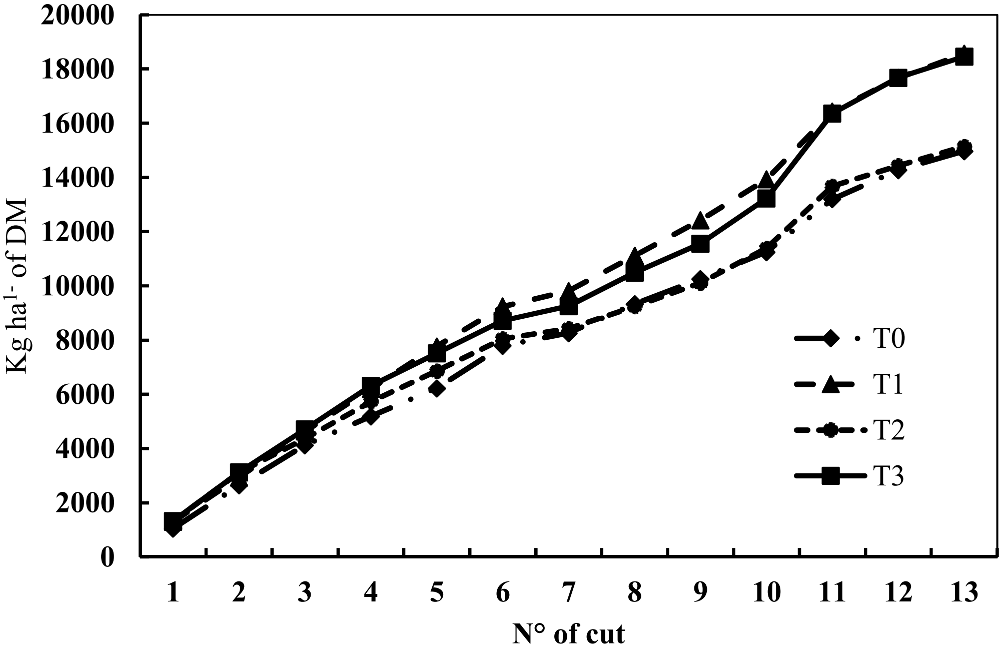

Fig. 3. Accumulated dry matter production throughout the experiment in each treatment: 0 m^3 ha^{-1} (T0: without FDE), 82 m^3 ha^{-1} (T1: low rate), 151 m^3 ha^{-1} (T2: medium rate) and 282 m^3 ha^{-1} (T3: high rate).

after each harvest at a rate intending to replace removed N from the previous cutting.

Commonly, plant number decreases with time, reducing biomass production. Our results indicate that the plant number reduction observed in the FDE treatments was compensated by the presence of more vigorous plants, with a higher number of buds and probably deeper roots. This agrees with previous findings reported by Romero et al. (1995). The decrease in biomass production in T2 from June was somewhat unexpected. In our experiment, the average number of plants until the ninth count was 37 pl m^{-2} in all the treatments. Romero et al. (1991) concluded that 30-70 pl m^{-2} are enough for the Pampas grasslands to achieve maximum forage yields.

Nutrient concentration in alfalfa plants was altered due to the input of the FDE. Alfalfa N_t level was significantly higher under the FDE treatments. The Mg concentration in the pasture decreased 0.03% in T3 compared to T0. A similar behavior was observed in Ca concentration, with a decrease in T3 compared to T0. Conversely, Na and K levels significantly increased in T3 plants compared to T0 plants. P concentrations and ash content remained unchanged after FDE application (Table 7).

The reduction in Ca and Mg concentrations in the alfalfa plants of T3 might may be explained by the high input of K, Na and NH4-N to the soil through the FDE applied. It has been demonstrated that a higher absorption of certain cation can cause a lower uptake of other cations, whereas the presence of high concentrations of K^+, NH_4^+ or Ca^{2+} in the soil, or a combination of such ions, restricts the absorption of Mg^{2+} (Mengel et al., 2001; Bolan et al., 2017). Wilkinson et al. (1999) also reported that net Mg^{2+} translocation rates from roots to shoots decreases when K^+ concentration increases in roots. The reduced Mg^{2+} translocation rate from roots to shoots appears to be the cause of antagonism between K and Mg absorption. From an economic point of view, K and Mg interactions in plants are extremely important to maintain animal health and performance as well as high-quality plant production (Wilkinson et al., 1999). This occurs because unsuitable Mg concentrations in forage create hypomagnesaemia or grass tetany, which is a metabolic disease affecting dairy livestock under intensive management and/or grazing systems (Bolan et al., 2004).

CONCLUSIONS

Soil application of FDE on alfalfa pastures increased the production of total aerial biomass but reduced persistence when applied at a high rate. Plant loss could be explained by the phytotoxic effect of nutrients from FDE, such as

Table 7. Pasture chemical composition.

Treatment	N_t	Ash	Ca	Mg	Na	K	P
		------------------------------g%------------------------------					
T0	4.359 a	11.540 a	1.246 b	0.170 b	0.018 a	2.549 a	0.258 a
T3	4.533 b	12.197 a	1.106 a	0.143 a	0.024 b	2.830 b	0.255 a

Mean values followed by the same letters in each column are not significantly different (P < 0.05).

ammonium, the superficial crust effect of OM on bud regrowth, and the anaerobic conditions occurring in wet periods. In terms of chemical composition, FDE application changed the nutrient composition of the pasture. The obtained results indicate that soil application of FDE on alfalfa pastures should be carefully planned. The composition of FDE must be evaluated prior to application so that the dose to be applied is adequate to promote the increase in biomass, without affecting plant persistence.

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