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Soil Dynamics and Nitrogen Absorption by a Natural Grassland under Cow Urine and Dung Patches in an Andisol in Southern Chile

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Abstract: The objective of this study was to assess N dynamics in the soil, and the response in regard to dry matter yield (DM yield; kg·DM·ha^{-1}), N yield (g·N·m^{-2}) and N concentration (g·N·kg^{-1} DM) under urine patches and dung pats and in the affected zone of the grassland soil over 304 days. The amount of N under the urine and dung patches increased until day 10 and then started to decrease until the end of the experiment, reaching control treatment levels. The DM yield in the urine patch zone and the 0–10 cm zone around the dung pat was higher than in the control treatment throughout the evaluated period, while in the 0–30 cm zone around the urine patch and the 10–20 cm zone around the dung pat the DM yield was negatively affected by the application of excreta. The maximum accumulated N yield was up to two times higher than the control in the 0–10 cm zone of the dung pat, while the mean concentration of N throughout the period was 42% higher than the control for the "urine patch" zone and 47% higher in the 0–10 cm area around the dung pat. The total absorbed N was 19% and 15% for urine and dung excreta, respectively. Further research is needed to explain the variables that might affect the results obtained, and it is necessary to evaluate the botanical composition as a factor that contributes to this effect.

Keywords: dairy cow excreta; N mineralization; N accumulation in grass; multispecies grassland

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1. Introduction

Excretion from grazing animals, mainly through urine and dung deposition, plays a key role in nutrient recycling in pastoral systems [1], with N being the nutrient that constitutes the largest proportion of these excreta [2,3]. In general, the total N excreted is distributed between dung (20–55%) and urine (45–80%) and the approximate amounts of N supplied via urine and dung patches produced by grazing cows are approximately 500 kg·ha^{-1} and 1000 kg·ha^{-1}, respectively, assuming patch areas of 0.35 m^2 for urine (with 2 L per urination) and 0.07 m^2 for dung (with 310 g DM per dung pat), which are typical concentrations of N in urine and dung [3]. Furthermore, the N rate of urine and dung is high enough to cause damage to the leaves and roots of plants where it is deposited [4].

Although the solid fraction of urine is mainly made up of N, potassium, and sulfur, research has focused mainly on N due to its environmental implications. The most important N compound in urine is urea, which makes up more than 70% [5]. According to the type of feeding, when the protein consumption increases, the proportion of N as urea also increases up to 90% in the case of dairy cows [6,7]. The hydrolysis of urea is rapid, and it is hydrolyzed by 80–90% after 48 h of deposition [8]. After the hydrolysis of urea, the nitrification process of NH_4^+ to NO_3^- begins [9]. The dynamics of N in grassland after deposition have been extensively studied in temperate climates [2,10–12]. Several studies have shown that nitrogen uptake in the area affected by a urine patch ranges from 11 to 49% [2,10,12–16].

Most of the N present in dung is organic and insoluble in water, with approximately 45–65% found in protein and related compounds, 5% in nucleic acids, 3% in ammonia, and the remainder consists of partially degraded nucleic acids, bacterial cell walls, and fiber-bound N [17]. For this reason, the decomposition and mineralization of the N contained in dung can last up to 2 years since much of it is immobilized and accumulates in the soil, whereas the changes in the mineral N of the soil are usually short-lived [11]. Vegetation generally does not penetrate through the dung pat and increases in dry matter around the patch are generally offset by the lost area that is covered by dung [2,10–12]. When dung is excreted, it begins to gradually disappear through microbial decomposition of organic matter and through incorporation into the soil by macrofauna [2,18–20], although the rate of disappearance can vary widely depending on the climate and moisture content of the stool and according to the presence of specific soil fauna [2]. It has been observed that the patch can take up to 100 days to fully decompose [21,22].

Although there are numerous studies that describe the localized dynamics of N over time in patches of urine and dung, both in the depth of the affected soil and in the absorption dynamics of the plants, there are no studies of this type in Andisols in southern Chile. Most soils in southern Chile are of volcanic origin and are classified as Andisols [23]. They cover between 50–60% of the arable land of the country (5.4 million ha [24]). Due to their andic properties [25,26], these soils have completely different characteristics than other types of soils worldwide. Andisols have been described as soils with variable charge [25], high air- and water-holding capacity [27] and high hydraulic conductivity [28]. In addition, these soils present low bulk density (<0.9 $g \cdot cm^{-3}$) and elevated levels of organic carbon [29]. For these reasons, the study of the behavior of N from the excreta over time in an Andisol is essential to accurately elucidate the amount of this nutrient that is available for absorption in the grass and the real area or "effective area" of the herbage that is affected by each excreta. The objective of this work was to describe the dynamics of N availability in different layers of the soil profile under urine and dung patches and to determine what part of the N excreted is absorbed by the plants according to the area around the patches that are affected over 10 months.

2. Materials and Methods

2.1. Experimental Site and Design

The experiment was carried out at the "Estación Experimental Agropecuaria Austral" (Austral Agricultural Experimental Station, EEAA), of the "Universidad Austral de Chile" (39°47′ S, 73°14′ W). The mean annual precipitation is 2.472 mm with 75% of it falling from April to September. The ambient temperature is moderate, due to the oceanic influence, with a mean monthly maximum of 15 °C in summer and a minimum of 5 °C in winter [30]. A 30 × 40 m flat area of multispecies grassland was fenced off. The area, which was visually assessed, is composed of several plant species including the predominant *Bromus valdivianus* (approximately 40%), *Trifolium repens* (10%), and other plant species (50%), mainly *Cychorium intybus*, *Hypochoeris radicata*, and *Taraxacum officinale*. The experimental area is typically used for research on grazing and behavior evaluations of different forage species. The *Bromus valdivianus* grassland was established four years before starting this experiment. The meteorological data for the air temperature and daily rainfall were obtained from a meteorological weather station located at the EEAA.

The experimental area was not fertilized or used for grazing for more than 60 days before the beginning of the experiment. Immediately before the application of urine and dung, the herbage was cut to a residue of 5 cm to simulate a typical intensive dairy grazing system in southern Chile.

The experimental design consisted of a complete randomized block with three treatments: urine, dung, and control (no excreta applied). An estimated volume of 2 L of urine (water in the control treatment) was applied in an area of 0.35 m^2 from a 1 m height, and 1.8 kg of dung was deposited in 0.07 m^2 based on Whitehead [3]. Because the sampling was destructive, the experiment considered a unit of urine patch, dung pat, and a control unit for each date. The sectors of each treatment were placed 1.5 m apart from each other (Figure 1).

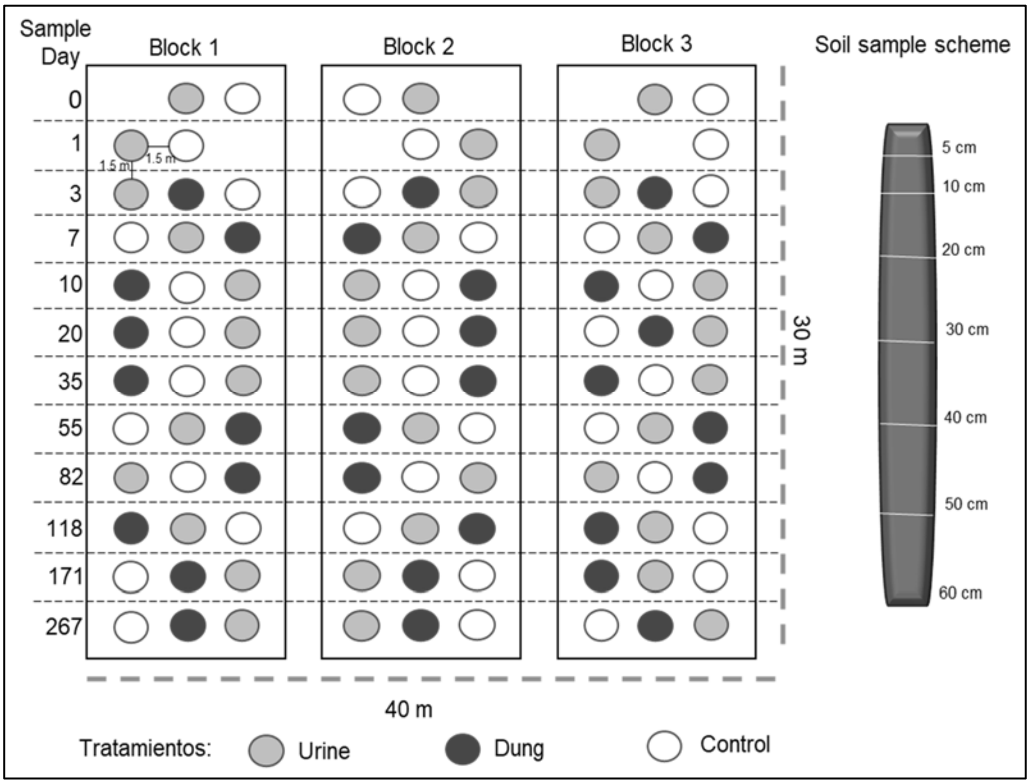

Figure 1. Schematic design for the experiment of soil samples. Each circle indicates the position of each soil sample for urine, dung and control treatments, and a scheme of the soil sample divided by the depth of each layer.

Urine was collected from at least seven cows during milking, and fresh dung was collected immediately after deposition when cows were grazing on the same grassland. Both were obtained one day before being applied in the experimental plots. Dung and urine were collected in separate containers and each excreta was mixed and immediately refrigerated at <4 °C until they were applied in the experimental area. The dung mixtures in the container were covered with polyvinyl plastic to prevent contact with air during refrigeration. Dung patches were placed in a plastic semicircle mesh stretched over the 0.07 m^2 area in order to avoid sample contamination by the dung.

2.2. Total N Determination in Excretes

The pooled urine sample was immediately cooled to <4 °C to avoid volatilization of ammonia (NH_3). On the first day of the experiment, three subsamples of urine were taken to determine the concentration of total N. The dung mixture was refrigerated at <4 °C, and three subsamples were taken to determine the concentration of N. These subsamples were dried and ground to a fine powder (less than 1 mm) to guarantee homogeneity before the analysis of N. Total N was determined through the Kjeldalh digestion method [23]. The total N concentration was 0.685% and 2.73% for urine and dung, respectively, equivalent to 396 and 742 kg·N·ha^{-1}. For the determination of the dry matter, three additional subsamples of the dung mixture were taken, which were then dried in an oven at 105 °C until they reached a constant weight.

2.3. Soil Samples

Soil sampling of the areas affected by excreta began on 4 December 2018 (day 0) and ended on 27 August 2019 (day 267), with a total of 12 samplings, while the sampling of plants took place from 27 December 2018 (day 23) to 8 October 2019 (day 304) with a total of 4 samplings (as defined by Saarijärvi and Virkajärvi [11]). The area was not fertilized during the experimental period of this study. The methodology was based on the works of Saarijärvi and Virkajärvi [11] and Aarons et al. [21].

The first sample was taken 30 min after the application of urine to allow the urine to infiltrate into the soil, corresponding to day 0. The next samples were taken on days 1, 3, 7, 10, 20, 35, 55, 82, 118, 171, and 267. The sampling depths for the urine and control treatments from day 0 to 3 were in the depth layers of 0–5, 5–10, 10–20, 20–30, and 30–40 cm. From day 7, deeper layers (40–50 and 50–60 cm) were also included. In the dung treatment, the dung pat was removed before soil sampling. Soil samples were taken in the area below the plastic mesh. The sampling of the dung treatment began on day 3 with depth layers of 0–5, 5–10, 10–20, and 20–30 cm. From day 7, the 30–40 cm layer was included, and the 40–50 cm layer was included from day 55 (Figure 2). Three subsamples were obtained for each treatment and for each sampling day. Mineral N (NH_4^+ and NO_3^-) was determined through the Kjeldalh digestion method [31]. The dung pats were removed, weighed and analyzed for dry matter and total N concentration.

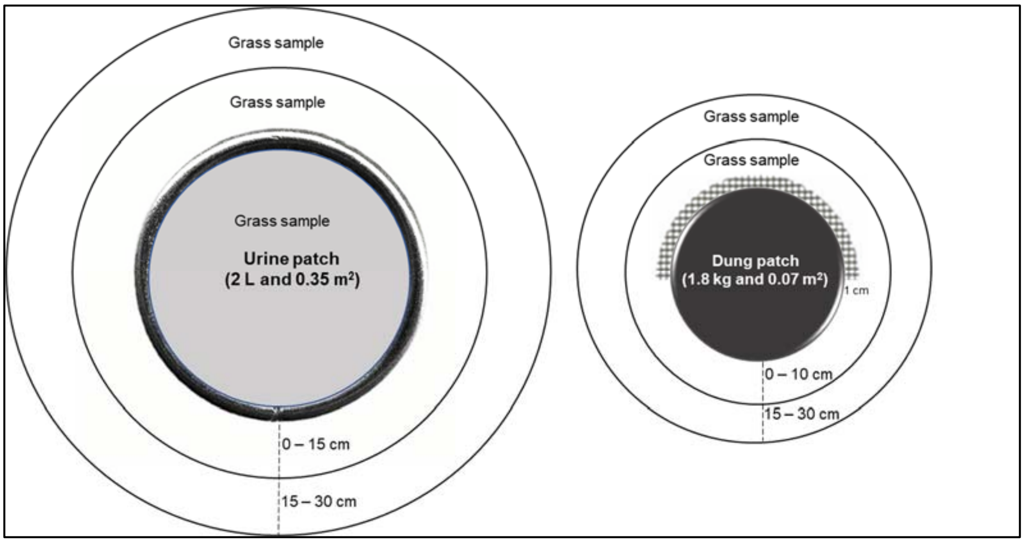

Figure 2. A diagram showing the center portion of an experimental unit, the area to which cattle urine and dung were applied, and each zone of grass sampled is shown for both excretes.

2.4. Herbage Sampling

The herbage was cut at 5 cm in height. The dry matter and N concentration were measured in all treatments on days 23, 49, 199, and 304. In the urine treatment, samples were taken from the center of the ring affected with urine, from the area 0–15 cm and 15–30 cm from the edge of the ring, while herbage samples from dung treatment were taken from the zones 0–10 and 10–20 cm from the edge of the dung pat (Figure 2). To determine the herbage dry matter of the herbage, samples were weighed and then dried in an oven at 40 °C for 4 days. Once dry, the samples were ground to 2 mm in size and the N concentration of each sample was determined according to the methodologies described by Sadzawka et al. [31].

2.5. Statistical Analysis

The experimental design was a randomized complete block design with three treatments (control, dung, and urine). The response variables of each zone analyzed were calculated as the difference for each treatment compared to the control, i.e., changes in DM yield, N content of herbage and N yield caused by dung and urine. The statistical differences between the treatments for each layer and for each day sampled were analyzed by ANOVA. The statistical differences between the control and each zone of urine and dung treatment were analyzed for each day sampled via ANOVA. Horizontal bar graphs were made for each day of soil sampling, presenting the amount of mineralized N for each soil depth layer sampled and for each treatment. GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA) was used for each statistical analysis.

3. Results

3.1. Weather

Air temperature and rainfall recorded during the experimental period are shown in Figure 3. No rainfall occurred during the three first soil samplings, but between one day before day 7 and on day 10, 33 mm of rainfall occurred. In the period from day 20 to day 118, there was almost no rain, except on sampling day 35 when it rained 3 mm. This period corresponded to summer days. For the sampling carried out in the same period (day 0 to 118; 4 December to 1 January 2019) the average temperature was 15.5 °C, with a maximum of 25 °C on 15 February 2019. Before day 171, the rain began to increase, and 12 days before day 171, it rained 36 mm. Ten days before the last sampling, the rainy days were more frequent and abundant, and it rained a total of 67 mm, including the sampling day, which corresponded to the last days of winter and the first days of spring, while the average temperature was 9 °C. From the beginning of the experiment until the first herbage sampling on 27 December 2018, the accumulated rain was 34 mm, mainly in the middle of this period, and on the second herbage sampling day (day 49) the rain was similar, while the average temperature was 16 °C and 15 °C, respectively. Between the second and third herbage samplings, there was 667 mm of rain, and the greatest amount of rain started on day 80 day (1 April 2019), and was equivalent to 91% of the total rainfall in this period, i.e., 26 days before the sampling (6 March 2019), and the average temperature was 10 °C. The accumulated rainfall from the third herbage sampling until day 304 was 909 mm, but a month before the fourth sampling, the rainfall was 10% of the total rainfall in that period, and the average temperature was 9 °C.

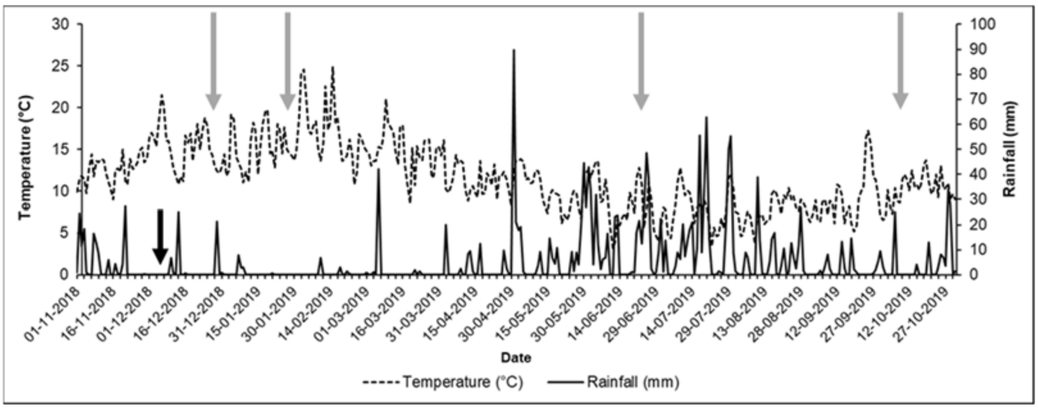

Figure 3. Temperature (°C) and rainfall (mm) for the experimental period. The black arrow indicates the first day of soil sample and gray arrows indicate grass sample dates.

The dung pat disappeared in June 2019. If we consider all the zones studied for the urine patch and dung pat (including the area covered by the dung pat), the accumulated DM yield of the control was 60% higher than that of the urine and 38% higher than that of the dung. However, the N yield was higher in the dung zone and lower in the control. The calculated total absorbed N was 75 kg·N·ha^{-1} from urine and 115 kg·N·ha^{-1} from dung, which is equal to 19% and 15% of the total N amount deposited, respectively.

3.2. Dynamics of N Mineralization in the Soil

When excreta were applied (day 0) the concentration of N in the soil varied from control only on the upper layer of the urine patch (0–5 cm, p = 0.0231) (Figure 4). One day after urine application, the amounts of N in the soil were 3.5 to 3.9 times higher than the control at all depths at the urine patch. On day 3, the amounts of N in the soil were the greatest at urine patches, ranging from 129 to 196 kg·N·ha^{-1}. On the other hand, intermediate values (65 to 111 kg·N·ha^{-1}) for the soil under the dung pats were observed. The lowest values were observed in the control area (Figure 4). By day 7, N under dung pats and urine patches were similar (overall average 104 kg·N·ha^{-1}). Both treatments had higher N amounts than the control area at all depths. However, by day 10, the soil under urine patches surpassed that under the dung pats regarding N, mainly due to an increase in this variable at the deepest strata (from 20 cm depth and below). No variation in depth for the control area was observed. In general, the N amounts under urine patches and dung pats increased until day 10 and then started to decrease until the end of the experiment. From day 55, there was no difference in soil N at any depth until the end of the experiment (overall average values were 34 and 18 kg·N·ha^{-1} at day 55 and 267, respectively). The only exception was at day 171 when the area under dung pats in the upper strata (0–10 cm depth) showed a greater value when compared with urine patches and control.

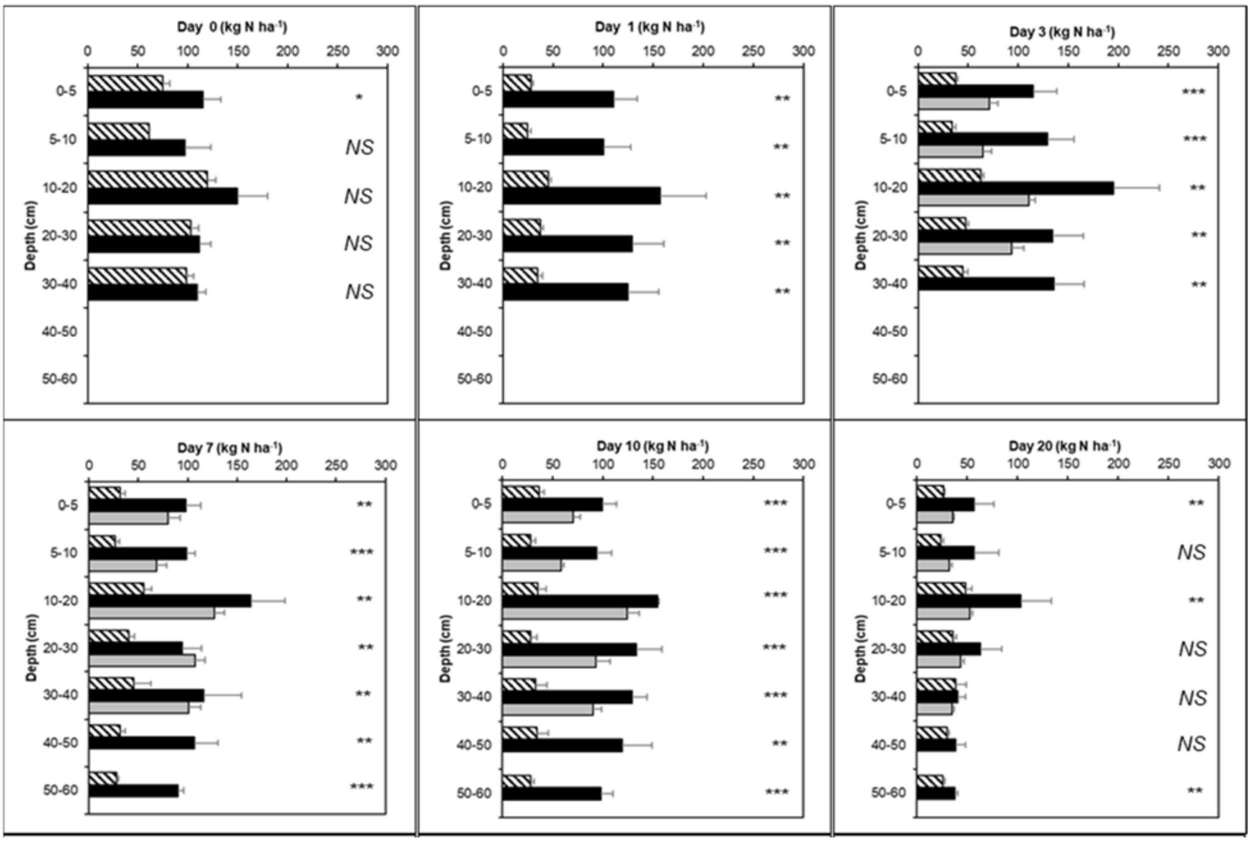

Figure 4. *Cont.*

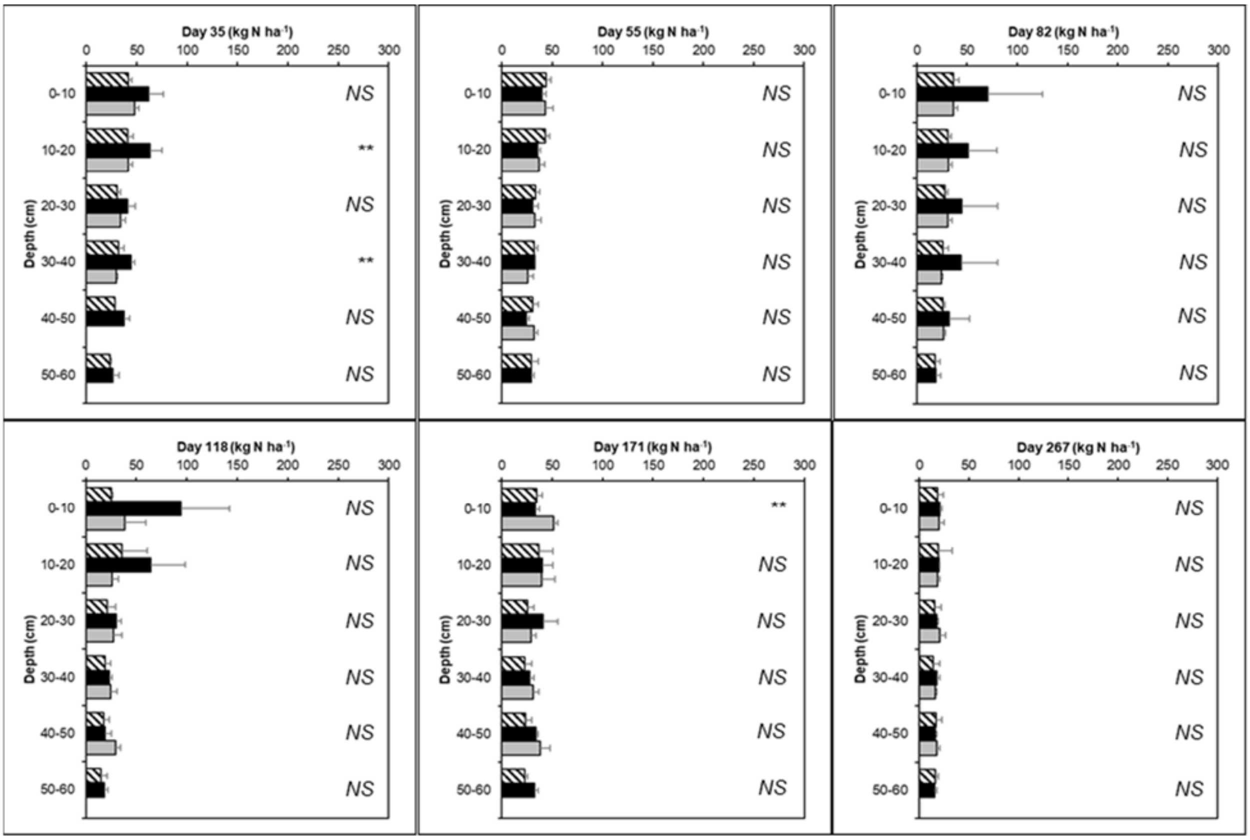

Figure 4. Amounts (kg·N·ha^{-1}) of nitrogen in the soil under the control (slashed area), urine patches (solid black area) and dung pats (gray area) treatments during the experiment. Horizontal bars represent standard errors of the means. Statistical differences are shown for the comparison between the control, urine and dung within each depth and day. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; NS: non-significant.

3.3. DM Yield and N Utilization by Herbage

Dry matter yield did not differ from the control under the urine patches throughout the experiment (Table 1). However, lower values than the control on the two rings were observed at day 23 ($p < 0.01$) in the 15–30 cm zone as well as at day 49 ($p < 0.0001$), and in the 0–15 cm zone at day 199 ($p = 0.0089$). Regarding the area affected by the dung pats, the effect was present in the 10–20 cm zone at day 49 ($p = 0.0015$), in the 0–10 cm zone at day 199 ($p = 0.0252$), and in the 10–20 cm zone at day 304 ($p = 0.0186$; Table 1). Likewise, on day 199 the DM yield was higher in the 0–10 cm zone around the dung pats than in the control, whereas on day 304, the DM yield was lower than the control in the 10–20 cm zone.

Nitrogen yield under the urine patches was affected in the early stage of the experiment, specifically in the patch area at days 23 and 49 ($p < 0.05$), with higher values than the control, and in the outer circle at day 49 ($p = 0.0413$), with a N yield higher than the control (Table 1). Both the inner and outer circles around the dung pats showed higher values of N yield than the control at days 49 ($p = 0.002$) and 199 ($p < 0.05$), respectively, ranging from 130 to 160%.

Nitrogen concentration in the DM was affected by the urine patches, both in the patch area and in the circles at day 23 ($p < 0.01$), with values between 7 to 2.1 higher than the control (Table 1). At day 49, only the 0–15 cm zone and the patch area were 1.5 and 2 times higher ($p < 0.05$) than the control, respectively. Regarding the dung pats, the effect was only present at the end of the experiment, when the inner and outer circle showed an N concentration in the DM that was around 40% higher than the control.

Table 1. Grass dry matter (DM) yield ($kg \cdot ha^{-1}$), nitrogen (N) yield ($g \cdot N \cdot m^{-2}$) and N concentration ($N \cdot g \cdot kg^{-1}$ DM) on/around the excretal patches on each sampling date of the experiment (s.e. = standard error of mean). *p* values for urine and dung are given as differences from the control treatment. Numbers in bold highlight the significant differences ($p < 0.05$).

	Day 23 (27 December 2018)			Day 49 (22 January 2019)			Day 199 (21 June 2019)			Day 304 (8 October 2019)		
	Mean	**s.e.**	***p* Value**	**Mean**	**s.e.**	***p* Value**	**Mean**	**s.e.**	***p* Value**	**Mean**	**s.e.**	***p* Value**
DM yield ($kg \cdot ha^{-1}$)												
Control	1565	154		792	36		1208	41		661	34	
Urine												
Patch area	+103	157	0.6491	+145	195	0.4795	−157	81	0.1139	+129	127	0.3818
0–5 cm	−527	54	**0.0090**	−116	67	0.1595	−249	66	**0.0089**	−101	50	0.1691
15–30 cm	−968	76	**0.0002**	−405	33	**<0.0001**	−181	132	0.2168	−196	144	0.2547
Dung												
0–10 cm	+373	393	0.3975	+367	210	0.1151	+916	346	**0.0252**	−178	113	0.3185
10–20 cm	−342	156	0.1501	−239	42	**0.0015**	+383	187	0.0729	−306	67	**0.0186**
N yield ($g \cdot N \cdot m^{-2}$)												
Control	2.2	0.4		1.0	0.2		2.2	0.4		1.4	0.2	
Urine												
Patch area	+3.2	0.8	**0.0064**	+1.3	0.4	**0.0127**	+0.8	0.4	0.1780	+ 0.5	0.4	0.2886
0–15 cm	+0.7	0.3	0.2064	+0.3	0.2	0.2583	+0.4	0.3	0.4345	−0.3	0.1	0.1572
15–30 cm	−0.8	0.2	0.1203	−0.4	0.1	**0.0413**	−0.5	0.2	0.3592	−0.2	0.4	0.6419
Dung												
0–10 cm	+2.3	1.5	0.1635	+1.3	0.1	**0.0002**	+3.6	1.0	**0.0080**	−0.3	0.2	0.3108
10–20 cm	+0.2	0.2	0.6137	+1.4	0.2	**0.0002**	+1.9	0.8	**0.0463**	+0.1	0.3	0.7800
$g \cdot N \cdot kg^{-1}$ DM												
Control	14.8	2.6		12.6	2.1		18.2	3.6		21.1	1.9	
Urine												
Patch area	+16.8	2.6	**0.001**	+12.4	0.8	**0.0002**	+9.5	1.6	**0.0360**	+2.6	1.4	0.3202
0–15 cm	+12.8	1.9	**0.0026**	+6.5	1.5	**0.0322**	+8.2	1.6	0.0621	−2.0	0.7	0.3706
15–30 cm	+8.9	0.4	**0.0067**	+2.5	2.3	0.4410	−1.0	2.1	0.8202	+2.9	3.9	0.5378
Dung												
0–10 cm	+7.0	3.6	0.1441	+11.2	4.8	0.0584	+9.0	2.3	0.0603	+8.3	1.3	**0.0075**
10–20 cm	−1.7	0.5	0.5531	+0.5	0.5	0.8108	+7.1	2.6	0.1370	+8.8	2.3	**0.0416**

The accumulated DM yield throughout the experiment only differed from the control in the outer and inner circles of the urine patches ($p < 0.001$), showing values between 27% and 44% lower than the control (Table 2). The accumulated N yield showed an opposite trend between the zones. The N yield was around 80% higher than the control in the patch area ($p = 0.0002$) but was 32% lower in the 15–30 cm zone ($p = 0.0074$). On the other hand, the N yield was consistently higher in the area affected by the dung pats, and was 1.5 and 2 times greater than the control area for the outer and inner circles, respectively. The concentration of N in the DM was consistently higher than the control in all the areas affected by the excreta ($p < 0.05$), being on average around one third higher than the control (Table 2).

Table 2. Accumulated grass dry matter (DM) yield (kg·ha^{-1}), nitrogen (N) yield (g·N·m^{-2}) and N concentration (N·g·kg^{-1} DM) on/around the excretal patches throughout the experiment (s.e. = standard error of mean). p values for urine and dung are given as differences from the control treatment. Numbers in bold highlight the significant differences ($p < 0.05$).

	DM Yield (kg·ha^{-1})			N Yield (g·N·m^{-2})			g·N·kg^{-1} DM		
	Mean	s.e.	p Value	Mean	s.e.	p Value	Mean	s.e.	p Value
Control	4232	174		6.9	0.6		67.5	3.3	
Urine									
Patch area	+195	220	0.5050	+5.6	0.8	**0.0002**	+31.8	3.8	**<0.0001**
0–15 cm	−1011	84	**0.0004**	+0.9	0.2	0.2079	+25.5	1.1	**<0.0001**
15–30 cm	−1757	107	**<0.0001**	−2.2	0.2	**0.0074**	+11.5	1.7	**0.0125**
Dung									
0–10 cm	+1473	814	0.1071	+6.8	2.1	**0.0096**	+34.7	5.5	**0.0003**
10–20 cm	−510	165	0.0595	+3.7	0.8	**0.0048**	+14.0	3.5	**0.0162**

4. Discussion

4.1. Mineral N Dynamics in the Soil

The focus of our work was to study the application of excreta in summer and under seasonal weather conditions that characterize the region and also to study how the dynamics of nitrogen vary over time.

The mineralization of N from urea began immediately after urine application, which has already been shown by others [8,11], and can be explained by the high proportion of N as $CO(NH_2)_2$ (~30–60%) [15], which is rapidly hydrolyzed and made available [2]. On the other hand, although on day 0 there were only statistical differences between the urine and the control in the 0–5 cm layer, the results showed that the amount of mineral N was the highest for the control treatment for the entire experiment. On day 0, the soil had a low moisture level since no rain events had occurred during the previous 10 days. In addition, there were high ambient temperatures. Therefore, these results could be due to the rewetting of the dry soil resulting in a flush of respiration by microbiota [32,33], often increasing within an hour and up to five times higher than in soil constantly kept moist [34,35]. This flush usually persists for up to 10 days after rewetting [36–38]. However, the latter did not occur in the control treatment in our experiment. The maximum effect of the urine treatment occurred between day 3 and 10 of the experiment, presumably penetrating to a depth greater than 60 cm. Nevertheless, this mineralized effect began to decline from day 20, reaching values similar to the control from day 35, which was also reported by Saarijärvi and Virkajärvi [11] and Buckthought et al. [39].

Our results showed that there was a rapid mineralization of N under the dung pat between day 3 and 10. After that it quickly fell to control levels until the end of the experiment. In addition, it was estimated that its effect was still present beyond a 40 cm depth in the soil. The fast N mineralization under dung pats was unexpected as the dilution of N in the soil under the dung patch should have been quite slow [40]. Even though there was no rain in that period, it is likely that because the dung presented with an initial percentage of water (90%), there was percolation of the fluids through preferential flows through the macropores that are connected to the soil surface [8,41–43]. Additionally, the rapid presence of mineralized N in the soil between days 3 and 10 is contradictory to other studies, which found that the decomposition and mineralization in dung pats may last up to 2 years and the dynamics of excretal N varies greatly depending on climatic and soil conditions [2,10–12,44].

However, there is often a rapid increase in the amount of microbial biomass in dung pats during the first 5 days after deposition, and during this period, there is a substantial mineralization of organic N [45]. Moreover, dung decomposition occurs most rapidly in moist conditions [18] and is accelerated by the presence of soil fauna, such as earthworms and dung beetles. The effect of soil fauna in burying and fragmenting dung is reflected in the greater reuse of the nutrients by grassland plants when such fauna is present [46]. In our work, the dung pats had visually disappeared in June 2019, which could be explained by the fact that there is more rainfall in the winter period and its intensity was high, which also contributes to its rate of decomposition [47]. It has been suggested that the proportion of N that was not mineralized was probably immobilized as soil microbial biomass [48], stored as urea and emitted as NH_3 [49], as well lixiviated, and with smaller amounts of denitrification in the form of N_2O and N_2 [14,50,51].

4.2. Dynamics of Mineral N Utilization by Grass

Our results showed that the DM yield in the inner patch area of the urine treatment was not different than the control treatment over time, which is contradictory to several similar studies. Increases in DM yield have been observed even with urine applications equivalent to more than 1000 $kg \cdot N \cdot ha^{-1}$ [10,11,14,15,39,52]. However, the DM yield in the 0–15 cm and 15–30 cm zones of urine treatment and in the 10–20 cm zone of dung treatment was unusually lower than the control treatment. Even though the botanical composition was not evaluated in this work, it was observed that in the treatments under the addition of N to the soil via both excreta, the proportion of *Bromus valdivianus* was favored in comparison to the other plants species. However, it has been reported that the effect of urine N on fast-growing species are often visible for about 3 months [53]. Several studies have shown that soil fertility provides the opportunity for fast-growing species to increase in grasslands [54–56], and these changes can occur in the short term when a degraded natural grassland is fertilized [57]. Additionally, climatic and others soil variables may exert selection pressure on plant species, which can determine the variability and diversity of genotypes [58–60].

According to Balocchi and López [61], naturalized grassland is a multispecies community that is fundamentally dominated by perennial grasses, with a variable proportion of broadleaf species and legumes, which characteristically represent less than 5% of the total annual yield of the grassland. The relative abundance of stress-tolerant broadleaf species such as *Hypochaeris radicata* are normally associated with soils under low soil fertility [62], which constrains plant growth, root development and can cause plant toxicity, especially for fast-growing species such as *Bromus valdivianus* [63].

It has been reported that the nutritional quality of a grassland varies according to the diversity of pasture species, which improves as there is a greater presence of better-quality forage species, and fewer species of low nutritional value, such as broadleaf species [64]. The results of our work showed that even though the DM yield was generally lower in the zones around the urine and dung patches, except on day 199 for the 0–10 cm zone of the dung treatment, there was a higher concentration of N ($g \cdot N \cdot kg^{-1}$ DM) with respect to the control treatment throughout of the entire experimental period. This would imply that in the control treatment, it is possible that there was a greater presence of pasture species of low forage value with a higher fiber density, a characteristic that decreases forage quality [56]. In addition, during the summer, when the high temperatures and summer drought are detrimental to the development of the species, broadleaf species invade the pasture, especially when there are low levels of soil fertility [65]. Thus, the addition of N should have positively affected the pasture species responses.

The amount of N absorbed by the herbage in the "urine patch", in the 0–15 cm near the urine patch zone and in the total evaluated zone of the dung treatment was greater than the control. Similarly, increases in N uptake and DM yield under higher N rate conditions have been reported [52,66–68]. The pasture N uptake has also been found to increase linearly with increasing urine N inputs [52]. Our work showed that the maximum cumulative N yield ($g \cdot N \cdot m^{-2}$) and N concentration ($g \cdot N \cdot kg^{-1}$ DM) for both excreta were ~98% and 47% higher than the control, respectively. These values are higher than those reported in other studies, where the absorption of urinary N by pasture was only 25 to 50% compared with the control [10,13,14,69].

Even though the effect of the dung on N mineralization in the soil was only 10 days, the greater absorption of N from the herbage than the control treatment from day 49 in the entire zone evaluated for dung, could be explained due to the slow mineralization of N from organic N of the dung over time, where this nutrient is rapidly absorbed by the herbage [3]. It could also be because the requirements of the pasture may be fulfilled by a high soil mineral N supply because of organic matter mineralization [61,70].

The percentage of N absorption from the urine treatment shown in our experiment is consistent with that reported by other researchers, ranging from 15 to 20% [2,8,11,12,14,71–73]. However, the absorption percentage of the dung treatment in our work was higher than that shown in other studies, varying between 0 and 8% [10,11,73].

5. Conclusions

The similar effect of the dung and urine treatments through the depth of the soil and over time would suggest that in the Andisols of southern Chile under natural grasslands, there are many macropores that could induce preferential flows and that could affect nitrogen mobility. However, the effect was short-lived, presumably due to uptake by the pasture and losses due to immobilization by the soil, volatilization, denitrification and leaching However, our results were based on the specific prevailing weather conditions described, thus we consider that it would be relevant to carry out further research that assess the dynamics of nitrogen with the application of excreta at different times throughout the year.

The negative effect outside the urine patch on the DM yield compared to the control treatment with the application of urinary N could be attributed to changes in the botanical composition, and because of pasture species of high forage value with a low fiber content, such as *Bromus*. However, the application of N from urine and dung would favor the growth of fast-growing species such as *Bromus valdivianus*, which was observed visually in all the evaluated zones of both excreta. The accumulated absorption of N in the zones affected by urine and dung treatments was up to double that of the control treatment.

This is the first work of this kind carried out in a multispecies natural grassland on volcanic soils in southern Chile, and further research is needed to provide a more detailed explanation of the variables that might influence the effects of applying N from bovine excreta on the pasture characteristics, and to evaluate the botanical composition as an explaining factor.

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