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Research Paper

The effects of bovine urine application on two soil nitrogen compounds and growth of three forage grasses in the Colombian Piedmont plains

Efecto de la aplicación de orina bovina en dos compuestos nitrogenados del suelo y el crecimiento de tres pastos en el piedemonte de los Llanos Orientales de Colombia

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Abstract

The effects of application of bovine urine on biomass and nitrogen (N) accumulation in 3 tropical grasses (*Urochloa decumbens* cv. Basilisk, *U. humidicola* cv. Humidicola and *Megathyrsus maximus* cv. Mombasa), and on available N concentrations in soil (NH_4^+-N, NO_3^--N) were studied using a randomized complete block design with 3 replicates. There were significant interactions between species and urine application over time in terms of herbage accumulation and N concentration (P<0.01), with significant differences in the concentrations of N available in the soil (P<0.01). Soil temperature and precipitation had important effects on the concentrations of both soil ions. Application of bovine urine increased dry matter accumulation of all grasses in the short term and of *U. decumbens* over the whole year. Application of urine increased soil N levels, but for *U. humidicola* and *M. maximus* the effects were transient. It is necessary to continue with longer-term studies in the Piedmont plains to determine the effects of livestock grazing on the biogeochemical cycles, environmental impacts and natural mitigation options that the ecosystem offers, e.g. CO_2 sequestration, biological nitrification inhibitors and organic matter decomposition.

Keywords: Ammonium, herbage accumulation, *Megathyrsus maximus*, nitrates, nitrogen, *Urochloa* spp.

Resumen

En un Oxisol del piedemonte llanero colombiano se estudiaron los efectos de la aplicación de orina de bovinos en la acumulación de biomasa aérea y nitrógeno (N) en las gramíneas tropicales *Urochloa decumbens* cv. Basilisk, *U. humidicola* cv. Humidicola and *Megathyrsus maximus* cv. Mombasa, así como en las concentraciones de N disponible en el suelo (N-NH_4^+, N-NO_3^-). Para el efecto se dispuso de un diseño de bloques al azar con tres repeticiones. Para la producción de materia seca y concentración de N se observaron, a través del tiempo, interacciones significativas (P<0.01) entre especies y la aplicación de orina, con significancia estadística en las concentraciones de N disponible en el suelo (P<0.01). La temperatura del suelo y la precipitación fueron factores importantes asociados con las concentraciones de ambos iones de N en el suelo. La aplicación de orina bovina incrementó la acumulación de materia seca en los tres pastos a corto término y en *U. decumbens* a través del año. Igualmente se incrementaron los niveles de N en el suelo, pero para *U. humidicola* and *M. maximus* el efecto fue transitorio. Se sugiere continuar con estudios a largo plazo para determinar efectos del pastoreo de bovinos sobre los ciclos biogeoquímicos, su impacto ambiental y opciones de mitigación naturales que el ecosistema ofrece, como son secuestro de CO_2, los inhibidores biológicos de la nitrificación y la descomposición de materia orgánica.

Palabras clave: Amonio, *Megathyrsus maximus*, nitratos, nitrógeno, producción de biomasa, *Urochloa* spp.

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Introduction

The Colombian Piedmont plains are a transition zone located between the slopes of the eastern Andes mountain range and the eastern plains (Llanos Orientales) in Colombia. With an area of 2,010,000 ha (7.6% of the Colombian Orinoquia region), they occupy a strip of land parallel to the mountain range. In general, the plains are known for their low soil fertility, aluminum toxicity and predominant acidity with high clay content, aluminum saturation and low concentrations of available phosphorus and interchangeable bases (IGAC 2004). These properties limit plant growth and only species with physiological adaptations can survive (Rao 2001).

The warm temperatures and humid environment enable the whole territory to be used for cropping and livestock production, mainly under grazing with Zebu (*Bos indicus*) cattle. Pastures are composed of native and increasingly planted species, such as *Urochloa decumbens* (Stapf) R.D. Webster (syn. *Brachiaria decumbens* Stapf), *U. humidicola* (Rendle) Morrone & Zuloaga (syn. *B. humidicola* (Rendle) Schweick., including cv. Llanero, previously considered as *B. dictyoneura*) and *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs (syn. *Panicum maximum* Jacq.) plus some legumes, all of which are adapted to the edaphoclimatic conditions of the area (Bernal 1994). Organic matter concentration in soils is low and producers do not usually apply chemical fertilizers to their grasslands, mainly due to costs. Cattle are relied upon to disperse nutrients to the soil through their excreta. Di and Cameron (2012) reported that "under a dairy cow urine patch, the N-loading rate from a single urination can be as high as 1,000 kg N/ha", which should benefit grass production.

Nitrogen (N) is the highest nutrient requirement for grasses and is absorbed through the roots in the form of nitrate (NO_3^-) and ammonium (NH_4^+) ions. In general, the plants prefer the former over the latter, mainly due to its greater solubility in water and because NH_4^+ ions in high concentrations can be toxic (Whitehead 2000). Many factors can alter the concentration of the ions mentioned above; precipitation, temperature and humidity are among the abiotic factors that influence immobilization and mineralization of N, mediated by microbial populations (Saggar et al. 2004; Dubeux Jr et al. 2007; Cameron et al. 2013).

The objective of the present investigation was to evaluate the effects of bovine urine on biomass and N production in 3 regionally important grasses, together with the concentrations of available N in the soil.

Materials and Methods

Study site

The study was performed at AGROSAVIA "La Libertad" Research Center (4°03'33.2" N, 74°27'27.1" W; 412 masl). The average temperature is 26.5 °C and mean annual precipitation is 2,800 mm (Rincón et al. 2010). The climate classification corresponds to Tropical Humid Forest (Holdridge 1978; IGAC 2004).

Environmental variables

The soil within the studied area was classified as an oxisol (Mejia 1996) with the following physico-chemical characteristics: bulk density (cylinder method) – 1.23 g/cm^3; pH (suspension soil:water, 1:1 v/v) – 4.4; organic carbon (Walkley-Black) – 1.95%; total nitrogen (organic carbon × 0.0862) – 0.14%; phosphorus (Bray II) – 8.2 mg/kg; potassium, calcium and magnesium (extraction with NH_4^+ 1 M acetate, pH 7) – 0.08, 1.43 and 0.29 meq/100 g, respectively; exchangeable acidity (extraction with 1 M KCl) – 2 meq/100 g; effective cation exchange capacity (exchangeable bases + exchangeable acidity) – 3.83 meq/100 g; and 32, 25 and 43% sand, silt and clay, respectively (McKean 1993).

Temperature and water volume in the soil were measured daily with a Decagon® datalogger, to calculate the water-filled pore space (WFPS), using the formula recommended by USDA (2014). Particle density was assumed as 2.65 g/cm^3 (Lin et al. 2013). Atmospheric temperature, precipitation and evaporation were recorded daily at the meteorological station of the research center.

Experimental methodology

A randomized complete block design was adopted with 18 plots of 2 × 2.5 m, in a factorial arrangement with repeated measures in time. The factors were 3 regional grass species: signalgrass (*Urochloa decumbens*) cv. Basilisk (CIAT 606), koroniviagrass (*U. humidicola*) cv. Humidicola (CIAT 679) and guineagrass (*M. maximus*) cv. Mombasa (BRA-006645) × 2 levels of bovine urine application (with and without) with 3 replicates. Urine was applied at the beginning of the rainy and dry seasons, respectively (May 2014 and February 2015). The grasses were established 2 years prior to the application of the urine treatments, without any management prior to the experiment.

The N applications were separated into dry and rainy seasons (DS and RS, respectively). However, the split data did not comply with the statistical assumptions for analysis of variance. In consequence, the data were analyzed together to strengthen the model, containing the experimental variation within the grass species to reach normality and homoscedasticity.

Estimation of the amount of bovine urine to apply

In RS and DS separately, urine was collected from 10 Zebu cows [418 ± 17.4 kg live weight (LW)], grazing a *U. decumbens* pasture.

To determine the amount of urine to be applied, a small amount of urine was collected at the beginning of each season and preserved with 5 mL of 5% H_2SO_4 per 100 mL urine. Thereafter, concentrations of N (Kjeldahl) and creatinine (commercial kit) in the urine were determined. The amount of urine excreted per animal/day was calculated using the following formula (Valadares et al. 1999):

VU = W × (Ccreatinine/Mcreatinine)
where:
VU = Urine volume (L/d);
W = Live weight (kg);
Ccreatinine = Creatinine coefficient (mg/kg LW/d); and
Mcreatinine = Creatinine concentration in the sample (mg/L).

Creatinine coefficient value was assumed as 17.3 mg/kg LW/d (Rennó et al. 2000).

Assuming a theoretical stocking rate of 2 animals/ha in *Urochloa* pastures, plus 9 months for RS and 3 months for DS and a standard area of 1 ha, the total volume of urine voided in each season was calculated using the following formula:

U = (V × SR × Period)/A

where:
U = Amount of urine/season (L/m^2);
V = Volume of urine/animal/d (L);
SR = Stocking rate (animals/ha);
Period = Season length (days); and
A = Standard area (m^2).

Urine was collected from cows by massaging the vulva and stored at 2 °C without acid preservation, to obtain the necessary volumes needed for the plots.

Urine was sprinkled on the pasture at the start of each season (13 May 2014 and 2 February 2015) in the plots randomly allocated to the urine treatment. Application rates were 1.37 and 0.77 L urine/m^2 for RS and DS, corresponding to 7.8 and 6.1 g N/m^2 (78 and 61 kg N/ha). The lower amounts of urine/nitrogen applied in the DS were a combination of a higher concentration of N in the urine (0.57 and 0.8% N in RS and DS, respectively) and the shorter duration of DS.

Control plots were established to simulate an area without animal effects; they received no N application, i.e. no urine.

Herbage accumulation and N content

Every 28 days, herbage mass was determined by harvesting 2 samples per plot, using a 0.5 × 0.5 m quadrat and leaving 10 cm stubble height. Each sample was dried at 60 °C for 48 h to calculate dry matter (DM) yield. Samples were then ground in a Wiley mill to pass a 1 mm stainless steel screen for determination of N concentration by the Kjeldahl method. N content was calculated by multiplying the DM yield by the N concentration in each sample. Following sampling, additional material within each experimental unit was cut and removed from the area, leaving the same stubble height in all plots.

Due to logistical issues in management of the research center, sampling periods were restricted to May–December 2014 (RS) and February–April 2015 (DS).

Available nitrogen in soil

During the first month after urine was applied, soils in each experimental plot were sampled weekly by collecting and pooling 8 soil cores to 20 cm depth. Samples were dried at 50 °C and passed through a 2 mm sieve. Concentrations of ammonium (NH_4^+-N) and nitrate (NO_3^--N) nitrogen were determined by extraction using 1 M KCl, with reduction of ammonium for the calculation of NO_3^--N (AOAC 1998). The analyses were performed in the Laboratory of Soil and Water of the Universidad Nacional de Colombia (Bogotá).

Statistical analyses

The data did not comply with the statistical assumptions for linear models due to the presence of outliers, so outliers were eliminated by confidence intervals with one standard deviation. Significant effects of the treatments were determined by analysis of variance and post-hoc HSD Tukey test.

To determine the level of association between the climatic variables and N available in soil, a linear regression model was used, after verification of statistical assumptions. For the construction of the model, all variables were evaluated through backward elimination, forward selection and stepwise regression, selecting those factors with lower scores of Akaike Information Criterion (AIC) (Winner 2018).This criterion uses model fit and the number of parameters as criteria. In comparing models, the model with the smallest AIC is considered optimal, computing the value with the following regression equation (Kaps and Lamberson 2017):

$\mathrm{AIC} = n \log(SS_{RES}/n) + 2 \times p$
where:
SS_{RES} = Residual sum of squares;
n = Number of observations; and
p = Number of parameters in the model.

Finally, Pearson's correlation test was performed among those predictors with the greatest adjustment to their respective response variable.

All data were analyzed using the RStudio® software, with a level of significance of $P<0.05$.

Results

Environmental variables

Water balance (precipitation and evaporation) and air temperature are presented in Figure 1. These revealed a high rainfall incidence during April–June 2014 and November–December 2014, with a relatively dry period in January–March 2015. Average relative humidity was 86%.

During both sampling periods, average soil temperatures were 26.5 ± 0.7 and 28.6 ± 0.7 °C for RS and DS, respectively. Similarly, average water-filled pore space (WFPS) values were 51.8 ± 4.3 and 49.5 ± 6.0% in the same periods of time (Figure 2).

Herbage accumulation and N content

The growth of pasture followed a seasonal pattern with highest yields during the rainy season and lowest production in the dry season (Table 1). The response in forage accumulation to application of urine was not uniform across species over the total period, with *U. decumbens* showing a 33% increase ($P<0.05$) in growth compared with its Control, while the remaining species showed no responses (Table 1). However, during the periods immediately following urine application, i.e. harvests in June 2014 and April 2015, DM accumulation averaged over the 3 species increased by 20.8% (June) and 80.8% (April). In the intervening period DM yields each month did not vary significantly between urine and Control treatments.

Similarly, application of bovine urine significantly increased N content overall in *U. decumbens* ($P<0.05$), with no significant responses in *U. humidicola* and *M. maximus* (Table 2). As for DM accumulation, during the month following application of urine to the grasses, N uptake by plants treated with urine was 79% higher than by Control in June 2014 and 58% higher than by Control in April 2015 (Table 2).

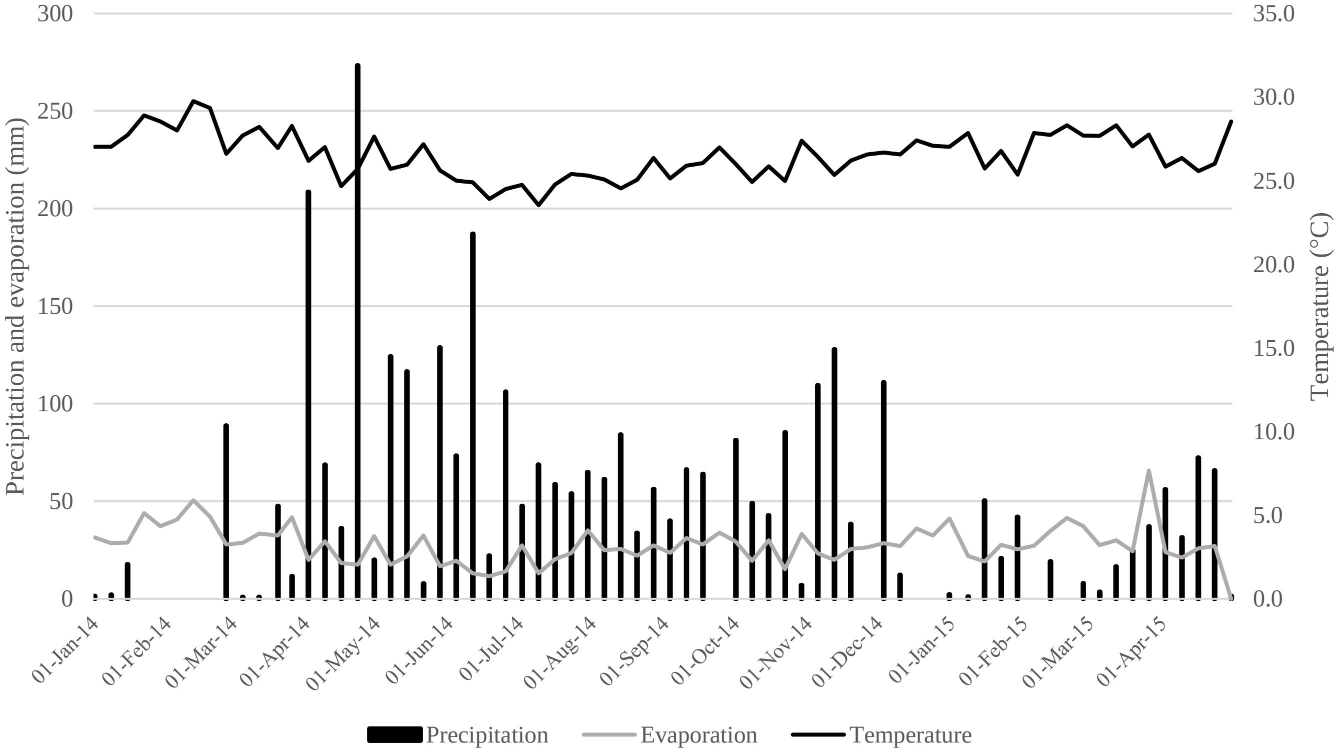

Figure 1. Accumulated precipitation, evaporation and average atmospheric temperature in AGROSAVIA "La Libertad" during the study period.

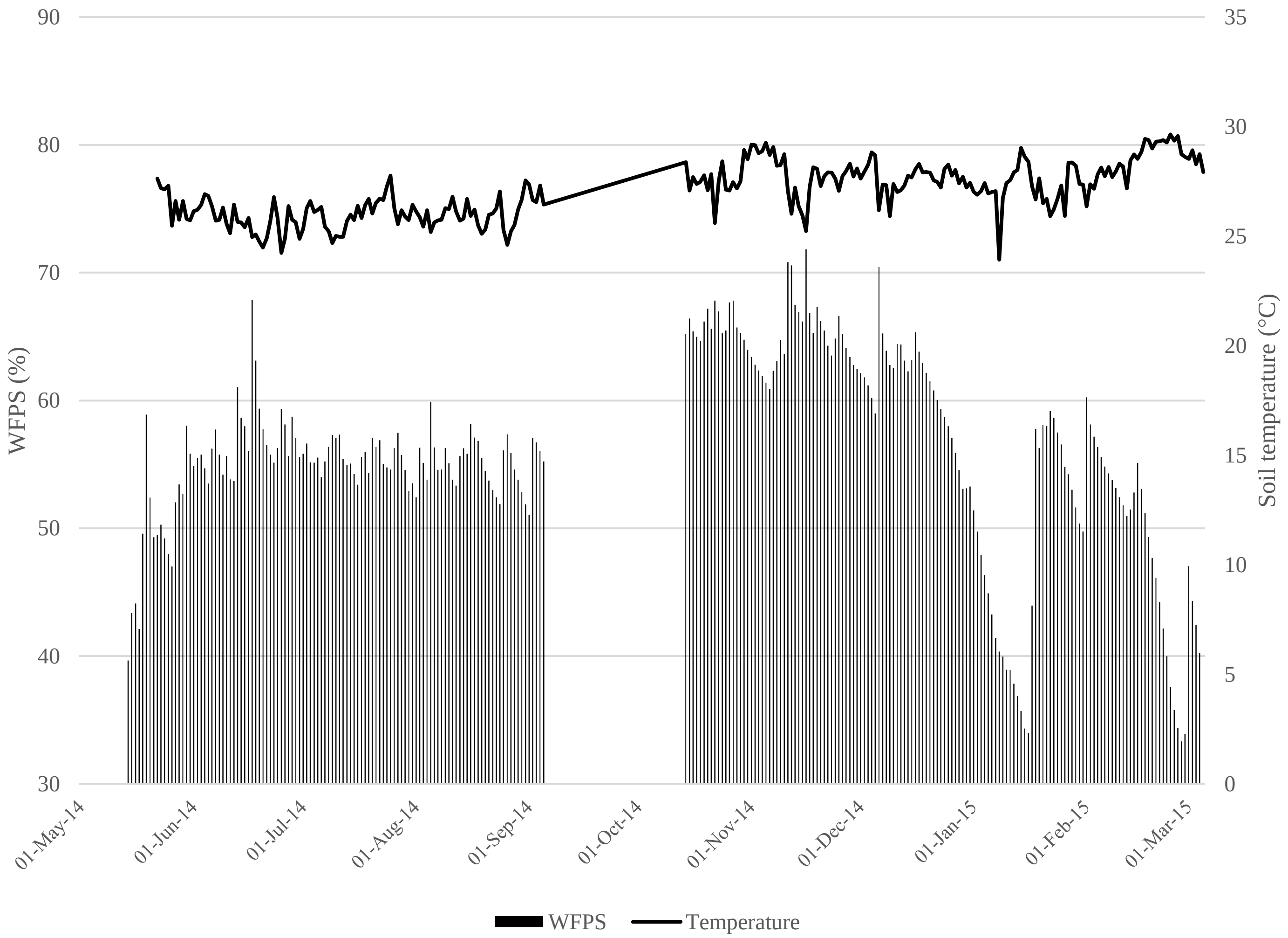

Figure 2. Changes in soil moisture level (WFPS) and temperature during sampling period for ammonium and nitrate concentrations. Data from September to October 2014 were not recorded because of datalogger damage.

Table 1. Effects of species and urine application on monthly herbage accumulation (t DM/ha) during 2014–2015.

Month	*Urochloa decumbens*		*Urochloa humidicola*		*Megathyrsus maximus*	
	Control	Urine	Control	Urine	Control	Urine
Jun	1.03	1.20	1.43	1.67	1.09	1.42
Jul	0.96	1.75	1.27	1.35	1.90	1.68
Aug	1.45	1.78	1.88	1.68	1.89	1.46
Sep	1.49	1.09	1.67	1.59	0.84	1.05
Oct[1]	1.65	1.98	2.42	2.45	2.15	1.37
Nov	1.20	1.54	1.43	1.57	1.26	1.02
Dec	1.00	1.36	2.02	1.36	1.10	0.73
Feb	0.30	0.53	1.40	1.29	0.61	0.67
Apr	1.23	2.49	0.99	1.59	0.95	1.65
Mean ± s.e.	1.14 ± 0.1a	1.52 ± 0.1b	1.61 ± 0.1a	1.60 ± 0.1a	1.31± 0.1a	1.23 ± 0.1a
Mean ± s.e.	1.3 ± 0.01B		1.6 ± 0.07A		1.2 ± 0.08B	

Means within species followed by the same lower-case letters are not different (P≥0.05).
Overall means for species followed by the same upper-case letters are not different (P≥0.05).
[1] The harvest in October 2014 was made 36 days after the previous defoliation event, due to logistical issues at the research center.

Table 2. Effects of urine application in May 2014 and February 2015 on N content (kg N/ha) of 3 tropical grasses.

Month	*Urochloa decumbens*		*Urochloa humidicola*		*Megathyrsus maximus*	
	Control	Urine	Control	Urine	Control	Urine
Jun 14	14.6	22.3	13.5	25.7	22.3	41.9
Jul 14	17.4	33.8	21.8	20.2	25.6	35.5
Aug 14	23.1	30.8	21.5	23.4	37.1	30.5
Sep 14	30.1	20.8	28.6	26.7	21.0	25.6
Oct 14	26.9	35.9	26.6	25.9	42.9	28.7
Nov 14	21.6	27.0	20.7	21.4	26.9	22.5
Dec 14	16.5	22.2	22.1	19.7	27.8	17.1
Feb 15	5.8	11.3	14.5	24.9	12.4	13.5
Apr 15	17.2	29.9	11.1	20.5	20.6	26.6
Mean ± s.e.	19.2 ± 1.9a	26.0 ± 2.3b	19.8 ± 2.0a	22.9 ± 1.5a	26.3 ± 2.9a	26.3 ± 3.0a
Mean ± s.e.	22.6 ± 1.3AB		21.3 ± 1.0B		26.3 ± 1.7A	

Means within species followed by the same lower-case letter are not different ($P \geq 0.05$).
Overall means for species followed by the same upper-case letter are not different ($P \geq 0.05$).

Soil concentrations of NH_4^+-N and NO_3^--N

Table 3 shows the concentrations of the two N forms during the month following treatment application. A peak in concentration of NH_4^+-N was registered following urine application in May 2014 with declining levels during the following month. The second application showed a lesser effect on concentration during DS.

In the first week after urine application in RS, the NO_3^--N concentrations were minimal, and displayed a substantial increase later in the season. However, during the DS, concentrations remained relatively stable during all samplings regardless of treatment (Table 3).

Seven variables were evaluated in building the explanatory model: rainfall, air and soil temperatures, relative humidity, WFPS and concentrations of NO_3^--N and NH_4^+-N. All initial models presented Akaike Information Criterion (AIC) scores greater than 30, which were reduced once different methodologies were applied (Table 4). Soil temperature and NO_3^--N concentration had the greatest effects on NH_4^+-N concentration in soil, while rainfall and soil temperature were the factors that best predicted changes in NO_3^--N concentration in the soil.

Table 4. Best adjusted models for soil NH_4^+-N and NO_3^--N concentrations as response variables.

Method	Best model[1]	AIC[2] score	
		Initial model	Final model
Backward	$NH_4^+ = ST + NO_3^-$	32.0	25.0
	$NO_3^- = Rain + AT + RH + ST$	35.1	32.2
Forward	$NH_4^+ = ST + NO_3^-$	30.7	25.0
	$NO_3^- = Rain + ST$	38.2	30.9
Stepwise	$NH_4^+ = ST + NO_3^-$	30.7	25.0
	$NO_3^- = Rain + ST$	38.2	30.9

[1] AT = Air temperature; ST = Soil temperature; RH = Relative humidity; Rain = Rainfall;
[2] AIC = Akaike Information Criterion.

Table 3. Effects of urine application on concentrations of NH_4^+-N and NO_3^--N (mg/kg) in soil, during the month following application (2014–2015).

Day	NH_4^+-N			NO_3^--N		
	Control	Urine	Mean ± s.e.	Control	Urine	Mean ± s.e.
May 16	17.7	33.3	23.9 ± 3.5a	0.6	1.3	0.9 ± 0.6b
May 22	8.0	16.0	11.2 ± 2.2de	20.9	21.2	21.1 ± 6.0a
May 29	6.2	9.2	7.7 ± 1.5de	23.4	8.3	17.4 ± 9.5ab
Jun 5	6.6	7.5	7.1 ± 0.7e	25.9	10.3	18.1 ± 7.8ab
Feb 4	14.7	17.7	16.2 ± 1.0bc	7.6	6.8	7.2 ± 2.1ab
Feb 12	16.0	18.6	17.3 ± 1.3b	8.1	8.2	8.1 ± 3.2ab
Feb 19	8.9	12.2	10.5 ± 0.9de	6.3	4.0	5.2 ± 2.2ab
Feb 25	10.6	14.0	12.3 ± 0.9cd	0.5	7.7	4.1 ± 2.4ab
Mean ± s.e.	11.7 ± 1.0A	15.3 ± 1.0B		11.6 ± 3.3A	8.5 ± 1.8A	

Overall means within columns followed by the same lower-case letter are not different ($P \geq 0.05$).
Means within N soil ions followed by the same upper-case letter are not different ($P \geq 0.05$).

Discussion

Herbage accumulation and N content

The highest herbage accumulation in all pastures was registered in October 2014. During this time, humidity, precipitation and temperature favored DM production, as opposed to the following month, when temporary waterlogging in some plots reduced growth rates. Conversely, the lowest herbage accumulation was registered in all plots during February 2015. This month is part of the DS in the Piedmont plains, when little rainfall is received (Figure 1), resulting in lower soil moisture levels (Figure 2) but elevated temperature. Water is vital in plant metabolic processes and supplies electrons for the reduction of CO_2 in photosynthesis. Likewise, its elimination through transpiration is linked to the stomatic conductance of the leaves; to avoid water loss, a lower moisture content within the plant decreases the stomatal opening, reducing the amount of CO_2 taken in by the leaf for photosynthesis. All this is reflected in reduced plant growth (Berlyn and Cho 1999).

Observing the total N content for the study period, Table 2 shows important differences between plant species, with the highest values for *M. maximus* and *U. decumbens*. Due to its larger foliar area, type of growth and genetic characteristics, *M. maximus* normally presents a higher rate of photosynthesis (Silva 2004) and efficiency in N use (Pérez 2014) than other tropical grasses, meaning a high herbage accumulation and crude protein concentration (Fernández et al. 2004). In this study, *U. humidicola* produced more DM than *M. maximus* (Table 1), which may be explained by its growth habit. *U. humidicola* grows by stolons, with a higher DM compared with their leaves but lower digestibility (Vergara and Araujo 2006). Moreover, *Urochloa* grasses have morphological adaptations to promote better utilization of N available in soils with low fertility, such as increased amount and length of roots or lower leaf expansion per unit dry weight (Rao et al. 1995). These factors decrease the dependence of forages on high N concentrations in the soil, presenting lower concentrations of N in their tissues in consequence (Rao 2001). The effect is more pronounced in *U. humidicola*, probably through its ability to synthesize biological nitrification inhibitors from its roots (Subbarao et al. 2009).

When analyzing responses to treatments over time, significant differences in N content were observed between the urine treatments and their controls in the first month after urine application (Table 2). The regional recommendation is to apply 50–70 kg N/ha to *Urochloa* pastures for grazing, as a compromise between biomass production and nutritional value of the forage (Rincón et al. 2010). During our experiment, we applied the equivalent of 61 and 78 kg N/ha in DS and RS, respectively, which would have generated the significant response in N concentration during these first samplings.

While herbage accumulation also increased, the responses were not large enough to detect significant differences (Table 1). The absence of differences in the following months was possibly due to the transformation and use of the N applied within the soil through ammonification and nitrification or by its escape from the system by volatilization or denitrification (Orozco 1999). Although the second application of urine occurred in February 2015, none of the pastures showed effects in their productive variables in the following month, and responses were delayed until April 2015. This could happen due to weather conditions during the DS, with few precipitation events (Figure 1) and decreased soil moisture (Figure 2) in January and February 2015. Reduced levels of water in the soil solution suppress activity of the microbial populations responsible for making N available for plants (Cameron et al. 2013), which could result in loss of N by ammonia volatilization (Jantalia et al. 2012) or its inorganic preservation by the low soil pH and presence of clays. The immobilization of N by the soil during the DS could preserve this element until the return of the rains, when it is mineralized by the microbial populations, making it available for the plants (Baggs et al. 2010).

Concentrations of ammonium and nitrate N in the soil

According to Table 3, NH_4^+-N was always present in the soil, since both treatments showed concentrations greater than 6 mg/kg. With total N concentration of 0.14%, this soil could be considered as low in organic matter. However, it is known that soil microorganisms present adaptive capacities in such situations, fulfilling their function as regulators of the N cycle in the soil (Dubeux Jr et al. 2007; Chirinda 2015). Additionally, the application of urine could increase the concentrations of NH_4^+-N in the soil during the RS, decreasing over time as the N source was transformed into other compounds.

The marked increase in NO_3^--N observed in the RS after treatment application (Table 3) was possibly due to the interaction of the biochemical processes of ammonification and nitrification (Orozco 1999). Likewise, Baggs et al. (2010) suggested that lower moisture levels in the soil could stimulate nitrate ammonification, which would also explain the lower nitrate levels during the DS.

It is noticeable how the concentrations of NH_4^+-N and NO_3^--N differed from the baseline levels. Due to their reductive conditions, oxisol soils could stimulate ammonification over nitrification, favoring the production of ammonia and its subsequent loss due to volatilization (Cameron et al. 2013).

Urochloa grasses showed a higher N content (Table 2) during the RS, which could be explained by a delay of nitrification until the second month after application of bovine urine. Although tropical grasses are capable of absorbing small amounts of N in NH_4^+-N form without exhibiting intoxication (Moser et al. 2004), they prefer to absorb this element as NO_3^--N, which is more soluble and easily transportable in the roots (Azcón-Bieto and Talon 2008). Its higher concentration in the soil would then imply greater absorption, resulting in a greater deposition of N components within the plant.

Variation in NH_4^+-N and NO_3^--N concentrations was noted in the control plots, despite urine not being applied on these experimental units. This phenomenon requires further investigation, but Day and Detling (1990) suggested that forage responses on urine patches relative to unaffected areas may be associated with increased soil N availability and root N concentration. The impact of urine beyond the area of application is aided by the large proportion of water in the urine and the mobility and availability of the nutrients it contains (White-Leech et al. 2013), reaching areas 3 times or more the area to which it was deposited (Haynes and Williams 1993).

Available N values reported in this study differ from those reported from other investigations. Conducting an experiment to determine ammonifying microbial populations, Verhamme et al. (2011) reported concentrations of approximately 190 and 70 g/kg of NH_4^+-N and NO_3^--N, respectively, in a soil in Scotland, while Salazar-Sosa et al. (2009) published values of 50–100 mg/kg nitrates in soils cultivated with a mixture of corn and soy in Mexico. Similar results were shown by Trejo-Escareño et al. (2013), evaluating the effects of application of bovine feces in a corn crop. Those studies suggest that the values reported here can be considered low, although this greatly depends on the management given to the soil and the external application of nutrients.

As fundamental components of many biochemical processes in the soil, the concentrations of NH_4^+-N and NO_3^--N are very variable, being affected by several environmental factors (Whitehead 2000). According to linear model building in this study, the environmental variables that had greatest influence on NH_4^+-N were soil temperature and NO_3^--N (Table 4), with high correlations between the response variables (Table 5). The high negative correlation between NO_3^--N and NH_4^+-N may be due to the inverse behavior shown by both compounds during the sampling period (Table 3), where ammonium is an initial component for nitrification, resulting in nitrate as the final product (Ardakani et al. 1974; Whitehead 1995).

Table 5. Correlation coefficients between response variables and predictors of the best-fitted models (%).

Response	Factor	ρ_{xy}
NH_4^+-N	ST	-77.4
	NO_3^-	-57.8
NO_3^--N	ST	67.0
	Rain	36.2

ST = Soil temperature; Rain = Rainfall.

We conclude that applying urine from cows fed with tropical forages increased herbage accumulation in *U. decumbens* but not in *U. humidicola* and *M. maximus* in the Colombian Piedmont plains. However, it did produce temporary increases in N content in the grasses immediately following application. This supports the concept that grazing animals play a beneficial role in dispersing nutrients in pastures. However, the effects seem to be limited in duration possibly because of the loss of N through volatilization. Likewise, the edaphoclimatic conditions and regional agricultural management limit the concentrations of N available in the soil, showing the validity of using forages adapted to these areas.

Finally, it is necessary to determine the relationships between the concentrations of available N in the soil and in the grasses in the region, to understand better the impact of agricultural practices on the N cycle in pastoral systems.

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