EFFECT OF APPLICATION TIMES OF UREASE INHIBITOR (AGROTAIN®) ON NH_3 EMISSIONS FROM URINE PATCHES

Jimena Rodriguez[1], Surinder Saggar[2], Peter Berben[2], Thilak Palmada[2], Nicolas Lopez-Villalobos[3] and Pranoy Pal[2]

[1]Soil & Earth Sciences Group, Institute of Agriculture & Environment, Massey University, Palmerston North 4442, New Zealand
[2]Ecosystems & Global Change, Landcare Research, Palmerston North, New Zealand
[3]Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand

jimeroge@gmail.com

Abstract

In grazed pastures about 80% of urine N in the form of urea is rapidly hydrolysed and is subjected to ammonia (NH_3) losses. The use of urease inhibitors (UI) has been used as a mitigation tool to decrease the rate of NH_3 volatilization from fertilizer urea and animal urine. In previous New Zealand trials the UI effect in reducing NH_3 emission from urine has been measured by applying urine mixed with the UI to the pasture soil thus increasing the chance to better inhibit the urease enzyme. However, these trials do not represent a realistic grazing scenario where urine deposition is highly unlikely to be mixed with Agrotain.

Therefore, to determine the effect of the UI - Agrotain® (marketed by Ballance Agri-nutrients) in reducing NH_3 losses from urine deposition by grazing animals, a field experiment was carried out at Massey University dairy farm # 4 by spraying UI before or after urine application. The treatments were: a control (without urine and Agrotain®), urine alone at 530 kg N ha^{-1} and urine plus Agrotain®. The UI was applied to the chambers and soil plots 5 (UAgr-5) and 3(UAgr-3) days prior to urine deposition, on the same day (UAgr0) and 1 (UAgr1), 3 (UAgr3) and 5 (UAgr5) after urine deposition in autumn. Following treatment application, $NH_{3(g)}$ volatilization was measured using acid traps, and soil mineral N (NH_4^+-N and NO_3^--N) and pH were measured from soil plots at different intervals during 30 days. The application of the UI prior to urine deposition significantly reduced NH_3 losses by 27.6% and 17.5% in the UAgr-5 and UAgr-3 treatments, respectively. These reductions corresponded to reductions in soil NH_4^+-N concentration and soil pH in comparison with urine alone or with treatments where Agrotain® was applied after urine deposition. Application of Agrotain® on the same day that urine reduced NH_3 losses by 9.6% but it was not statistically significant from treatments when Agrotain® was applied after urine deposition. The application of Agrotain® after urine deposition had no effect on NH_3 losses from urine.

Introduction

In New Zealand, pastoral agriculture is the dominant land use and animals are grazed all year round. Animal excreta (urine and dung) from grazing animals make up to 50% of the total N decoupled and recycled in grazed pastures (Saggar et al., 2004). In grazed pasture systems excretal N deposition ranges from 20 to 80 g m^{-2} in dung patches and 50 to 200 g m^{-2} in urine patches (Bolan et al., 2004; Saggar et al., 2009a) which is well above the N requirements of pastures. Approximately 80% of urine N is in the form of urea (Bolan et al., 2004). The urea N in urine is rapidly hydrolysed by the urease enzyme in soil to ammonium (NH_4^+-N). Under

alkaline condition ammonium is converted to NH_3 which is volatilized. Ammonia itself is not a greenhouse gas but when re-deposited on land, acts as an indirect source of nitrous oxide (N_2O). Ammonia emissions may cause eutrophication and acidification of water and soils where it is deposited (Misselbrook et al., 2013; Sanz-Cobena et al., 2012; Zaman & Blennerhassett, 2010) and also represent agronomic losses. Therefore, many approaches to mitigating NH_3 loss have been investigated in New Zealand.

Loss of N as $NH_{3(g)}$ from urine patches ranges between 7% and 14% of the total N applied as urea (Menneer et al., 2008; Singh et al., 2013; Zaman & Blennerhassett, 2010; Zaman & Nguyen, 2012; Zaman et al., 2009; 2013). However, higher figures were also reported in New Zealand. Laubach et al. (2012) found NH_3 losses as high as 25.7% of N applied in urine but overall emission rates were compatible with an annually –averaged emission value of 10% (Sherlock et al., 2008). These figures provide a compelling argument to reduce N losses from animal excretal inputs.

Urease inhibitors (UI) nBTPT [N-(n-butyl) thiophosphoric triamide] sold under the trade name Agrotain® and applied at 0.025% w/w to urine or fertiliser urea has been shown to reduce NH_3 emissions (Abalos et al., 2012; Menneer et al., 2008; Pereira et al., 2013; Sanz-Cobena et al., 2008; 2012; Singh et al., 2013; Watson et al., 2008; Zaman & Blennerhassett, 2010; Zaman & Nguyen, 2012; Zaman et al., 2009; 2013). In theory, UI slow down the conversion of urea ($(NH_2)_2CO$) into NH_4^+-N so that less NH_4^+-N is available for conversion into NH_3 which is susceptible to be volatilized (Bolan et al., 2004).

New Zealand and overseas research suggests that UI reduce about 45% NH_3 emissions from N-fertilisers (Saggar et al., 2009b; 2011; 2013). Saggar et al. (2013) have also developed a method to account for such reductions, and recommended a specific value of 0.055 for $Frac_{GASF}$ (for fertilisers) but did not discuss the effect of UIs on reducing $Frac_{GASM}$ (from animal urine deposited during grazing) due to the lack of quantitative information.

The value of UI for mitigating NH_3 losses will depend on their rate of biodegradation and persistence in soils. Studies suggest that generally UI is likely to last in soils up to 2 weeks, the period during which NH_3 is emitted from urea-N (Manunza et al., 1999).

However, more information is required to investigate the mode of application of the UI to urine patches, and also the optimum time of application of the inhibitor. In all the trials reported previously, urine was mixed with the UI before the application into the soil, increasing the chance to better inhibit the urease enzyme, which is not a realistic grazing scenario. Therefore, the main objective of this trial was to study the inhibitory effect of Agrotain® on NH_3 losses from urine deposition when it is sprayed into a pasture soil before or after the deposition of animal urine.

The specific objectives were:

- To determine the optimum time for Agrotain® application before and after urine deposition to obtain maximum reduction in NH_3 emission
- To understand the effect of Agrotain® on the transformations of mineral N
- To assess the effect in soil pH with the addition of Agrotain®

Materials and methods

Site description

The experiment was set up in dairy farm # 4 at Massey University, Palmerston North. The pasture site was a mix of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). Physical and chemical characteristics of the soil are shown in Table 1.

Table 1. Physical and Chemical Characteristics of the Tokomaru Silt Loam soil

	pH	**CEC (me 100g^{-1})**	**Bulk Density (g cm^{-3})**	**Total C (%)**	**Total N (%)**	**NH_4^+-N (mg kg^{-1} soil)**	**NO_3^--N mg kg^{-1} soil)**
Tokomaru silt loam	5.8	22	1.1-1.3	3.2- 3.6	0.26- 0.27	72.48	4.41

The experimental area was fenced off a year before the experiment started to avoid N deposition from grazing cows and to minimize the effect of previous dung and urine patches, and reduce the inherent variability.

Experimental design

The experiment was laid out in a completely randomized block design with eight treatments, replicated six times resulting in 48 sampling plots for soil and NH_3 volatilization measurements. Treatments comprised of a 'urine only' application (at 530 kg N ha^{-1}), urine plus Agrotain® (at 0.025% weight of Agrotain®/weight of urine-N; 132.5g Agrotain® ha^{-1}) applied 5 and 3 days before urine deposition (denoted as UAgr-5 and UAgr-3, respectively), on same day (UAgr0), and on days 1, 3 and 5 following urine application (denoted as UAgr1, UAgr3, UAgr5, respectively). The experiment also had an untreated control.

Each treated plot (0.5 m x 0.5 m separated by a 0.5 m buffer) comprised of a soil sampling area and a gas measurement chamber area. The sampled soil was analyzed for soil mineral-N (NH_4^+-N and NO_3^--N) and soil pH described below.

On day 1 after treatment application, NH_3 emissions from U, UAgr1, UAgr3 and UAgr5 were essentially similar and were averaged.

In those treatments where Agrotain® was applied before urine (UAgr-3 and UAgr-5), the grass was not mown until day 0 when urine was deposited. Therefore, before urine application on day 0, the grass was mown to simulate the grazing event by the animals in the chambers and soil plots.

Chambers and soil plots were covered the first week to avoid any rainfall events; therefore during the first week rainfall did not influence NH_3 losses.

Urine was collected from Friesian cows while they were milked. After urine collection, it was transferred to 20 L containers, and stored below 4^{o}C to avoid urea hydrolysis until the application in the field. Four urine samples of 100 mL each were taken to analyze total N and C, NH_4^+-N, urea-N and pH. The urine from the all containers was transferred to a 200 L container before the field application.

Ammonia emission measurement

Ammonia volatilization in this experiment was measured using the dynamic chamber method (Fig. 1) (Kissel et al., 1977) that comprised of a volatilization chamber, an acid trap to capture the ammonia and a manifold consisting of 6 air valves to regulate the flow rate inside the chambers. PVC chambers (0.15 m diameter, 0.04 m total height) with a transparent top (to allow photosynthesis) were inserted into the soil to a depth of 0.01 m that gave a headspace volume of 0.5 m^3. The chamber had a vent on the chamber's vertical surface that was connected to an acid trap (250 mL, 0.025 M H_2SO_4) using a tube which were connected to the manifold through to a vacuum cleaner. Air from the chambers was sucked at a constant flow rate (at 6 L min^{-1}, monitored daily) and was passed through the acid trap. Sub-samples of the H_2SO_4 solution in the acid traps were analyzed for NH_4^+-N concentrations and were performed as described below. Samples were taken every day for the first 12 days and then on days 15, 18, 21, 24, 27 and 30.

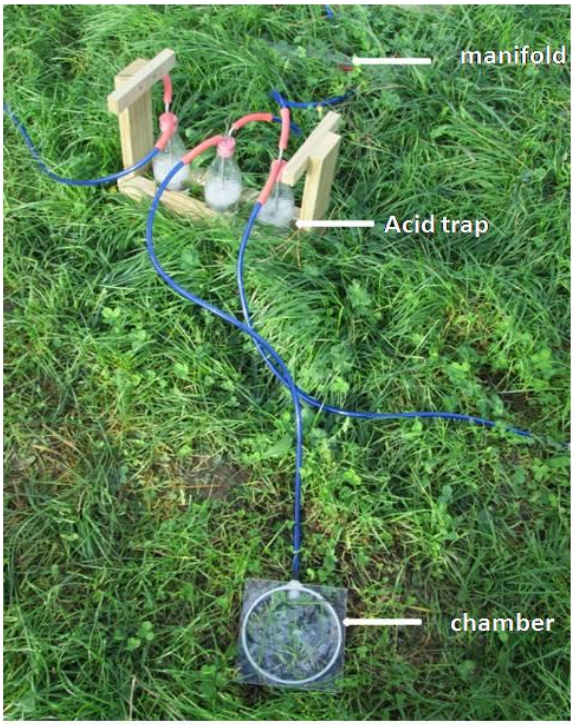

Figure 1. Equipment used to measure NH_3 losses from urine applied to the chambers.

Soil sampling and analyses

Before the application of treatments, six randomly selected soil sampling plots (3 cores each) were collected. Following treatment application, soil samples were collected from the 48 plots adjacent to the gas trapping chambers. These plots were sampled nine times following urine application, on days 1, 3, 5, 9, 12, 15, 18, 21 and 30. At each sampling, three soil cores of 25 mm diameter and 100 mm depth were taken from each plot and bulked to produce one sample.

Before soil analysis, soil samples were sieved (2 mm) to remove plant roots. A sub-sample of 5 g of field moist soil was extracted with 50 mL of 2 M potassium chloride (KCl) solution by shaking for 1 h. The extract was analyzed for nitrate (NO_3^-) and ammonium (NH_4^+) concentrations colorimetrically using Technicon AutoAnalyzer (Blakemore, 1987).

Soil pH was measured at a 1:2.5, soil: water ratio using a pH meter [(pHM83, Autocal pH meter); (Blakemore, 1987)].

A field moist soil sample per plot was weighed and then dried at 105^{o}C for 24h. After drying, these samples were weighed again and the gravimetric water content was calculated.

Statistical analysis

Gaseous emissions and soil parameters (mineral N and soil pH) were analyzed using the MIXED procedure of SAS (Statistical Analysis System, version 9.3; SAS Institute Inc., Cary, NC, USA). The model included the fixed effects of treatment (control, and Agrotain® application before, on the same day and after urine deposition), day of measurement and their interaction and the random effect of the acid traps and soil plots to account for repeated measures on the same experimental unit. The variance between days was homogeneous, but it was heterogeneity between treatments and therefore this was considered in the model. Using the Akaike's information criterion, a compound symmetry error structure was determined as the most appropriate residual covariance structure for repeated measures over time within treatments. Least squares means and their standard errors (S.E.) were obtained for each treatment for days 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 18, 21, 24, 27 and 30 in NH_3 volatilization, and days 1, 3, 5, 9, 12, 15, 18, 21, 24 and 30 in soil parameters analyses.

Results and discussion

Urine composition

The chemical composition of urine applied to the treatments is given in Table 2. It had a pH of 7.6 ± 0.40 and a total C concentration of 11.50 ± 0.16 g L^{-1}. The total N concentration was 4.95 g L^{-1} ± 0.22, of which 3.65 ± 0.22 g L^{-1} was urea component (73.7%). The high pH of urine (7.8) is optimum for urease activity and may result in rapid urea hydrolysis (Cabrera et al., 1991; Singh et al., 2013; Singh & Nye, 1984). Although the value of total N (4.95 g L^{-1}) is below the data reported in other studies, urea-N component (73.7% of N applied) is in the range of data previously published (Pereira et al., 2013; Zaman & Blennerhassett, 2010; Zaman & Nguyen, 2012) where urea N ranged from 70% to 92% of the total N applied.

Table 2. Chemical Composition of Urine

Urine chemical composition	**Values**
NH_4^+-N (mg/L)	267 mg L^{-1} ± 55.08
Urea-N (g/L)	3.65 g L^{-1} ± 0.36
Total N	4.95 g L^{-1} ± 0.22
Total C	11.5 g L^{-1} ± 0.16
pH	7.6 ± 0.40

Data are mean ± sd (n = 4).

Meteorological data

The mean daily temperature during the experiment and over the last 20 years is reported in Fig. 2a. The average daily temperature during the experiment ranged between 8.4^{o}C and 18.9^{o}C, and for the first 3 weeks of the experimental period it was higher than the 20 year average temperatures.

During the measurement period a comparison was made between soil temperature inside and outside of the chambers. Soil temperature inside the chambers was between 3.68 – 26.07 °C and the corresponding values outside chamber were between 5.51 and 28.5 °C. These similar temperature inside and the outside is attributed to continuous inflow of air at 6 L min^{-1}. It appears that this air flow also regulated the air temperature within the chambers which may not have affected the NH_3 emissions.

During the experimental period, a total of 58 mm rainfall was recorded (Fig. 2b), with most rainfall occurring during the first 11 days of the experiment. Rainfall for the subsequent period was negligible. As the chambers were covered during the initial 5 days this rainfall had limited effect on NH_3 emission during the first week when most of the emissions occurred.

Average rainfall during the experimental period was 1.9 mm with the highest rainfall of 13.2 mm recorded on day 6 (Figure 2b). Almost no rains occurred after day 11.

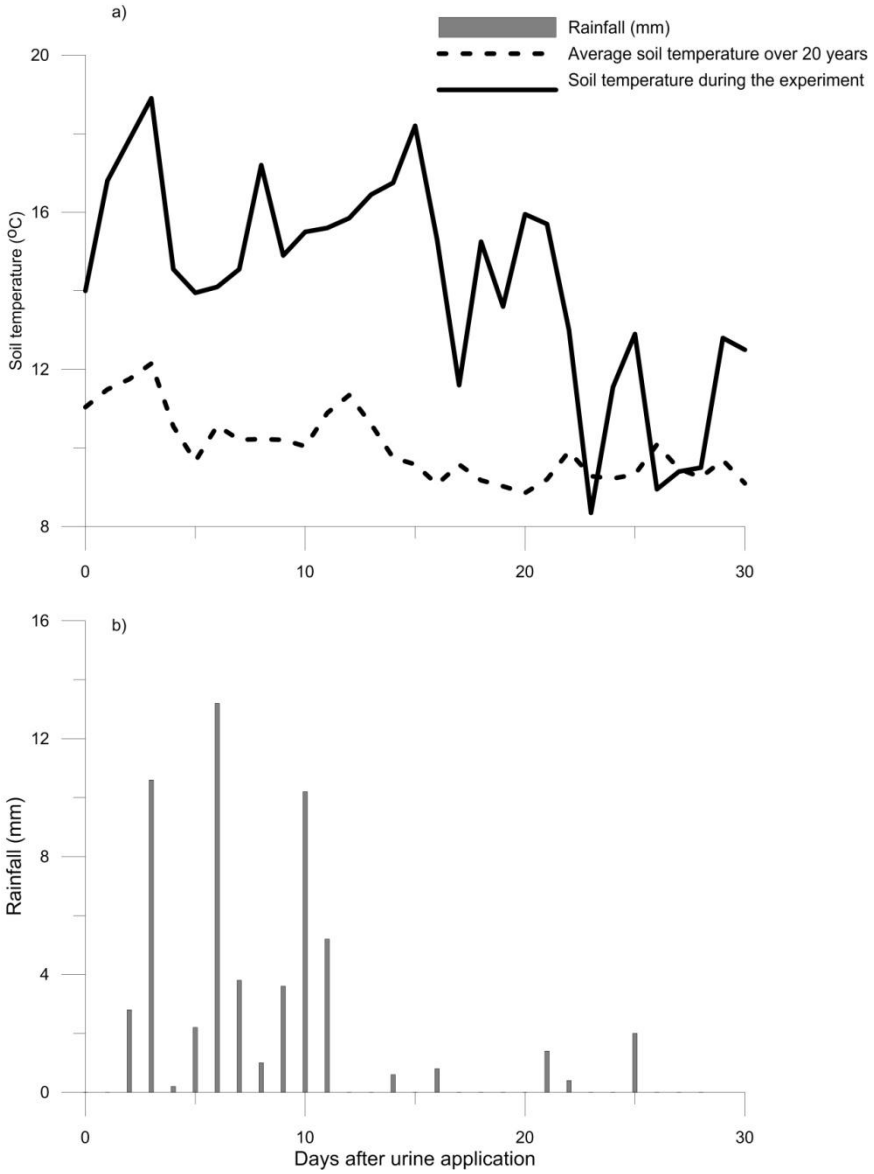

Figure 2. Meteorological data obtained from a meteorological station near the site of the experiment over the experimental period.

Ammonia emissions

Large NH_3 emissions were observed immediately after the application of urine at the rate of 530 kg N ha^{-1} followed by a sharp decline in the remaining measurements. The emissions reached the background levels within 15 days (Fig. 3)

Total NH_3-N emitted from urine cattle (530 kg N ha^{-1}) was 78.08 kg N ha^{-1} (14.7% of the urine-N) (Fig. 4) which is within the range reported in previous studies (Menneer et al., 2008; Zaman et al., 2013; Sherlock et al., 2008). Menneer et al. (2008) reported a 14% of urine-N loss as NH_3 when urine was applied at 775 kg N ha^{-1}.

The application time of the inhibitor had a significant effect on the amount of NH_3 volatilized from the different treatments. The highest amount of NH_3 flux of 36.31 ± 2.18 kg NH_3-N ha^{-1} (mean ± sd) was measured within 24 h from urine only treatments and those which did not receive Agrotain® at the time of urine application (UAgr1, UAgr3 and UAgr5) (Fig. 4). In these treatments, 46.5% of the urine-N was lost as NH_3 during the first 24 hours due to rapid urea hydrolysis (not measured). The high NH_3 emitted on the first day in the current experiment is in agreement with results found by Zaman et al. (2009) and Singh et al. (2003) who observed that most of the NH_3 was lost on the first day of urine deposition in urine only or urine with a nitrification inhibitor.

In the treatments (UAgr0, UAgr-3 and UAgr-5) where Agrotain® was applied the same day or 3 and 5 days before urine application, NH_3 losses were significantly reduced ($P < 0.0001$) within 24 h, compared to urine, UAgr1, UAgr3 and UAgr5. The amount of NH_3 emitted during the first 24 h was 28.82 ± 2.91, 27.77 ± 3.12 and 23.05 ± 2.32 kg NH_3-N ha^{-1} d^{-1}, for UAgr0, UAgr-3 and UAgr-5 (Fig. 4), respectively. This resulted in reducing emissions during the first 24 hours by 20.7 ± 8.0%, 23.5 ± 8.6% and 36.5 ± 6.4%. Over 30 days, NH_3 losses were reduced by 9.6 ± 7.4%, 17.5 ± 11.1% and 27.3 ± 5.5% for the UAgr0, UAgr-3 and UAgr-5, respectively. Zaman and Nguyen (2012) also observed that applying the inhibitor 5 days prior to urine deposition, NH_3 losses were reduced by 38% and 28% in autumn and spring, respectively. However, they reported a higher reduction than in the present experiment because they mixed the inhibitor with urine previous to apply to the soil. Although Agrotain® application on the same day that urine reduced NH_3 losses, it was not statistically different from treatments when Agrotain® was added after urine application.

Therefore, application of Agrotain® 5 days prior to urine was the most effective treatment (27.3%) which was statistically different from UAgr0. It was probably because applied Agrotain® was able to move down into soil profile, and interact with the urease delaying the urea hydrolysis.

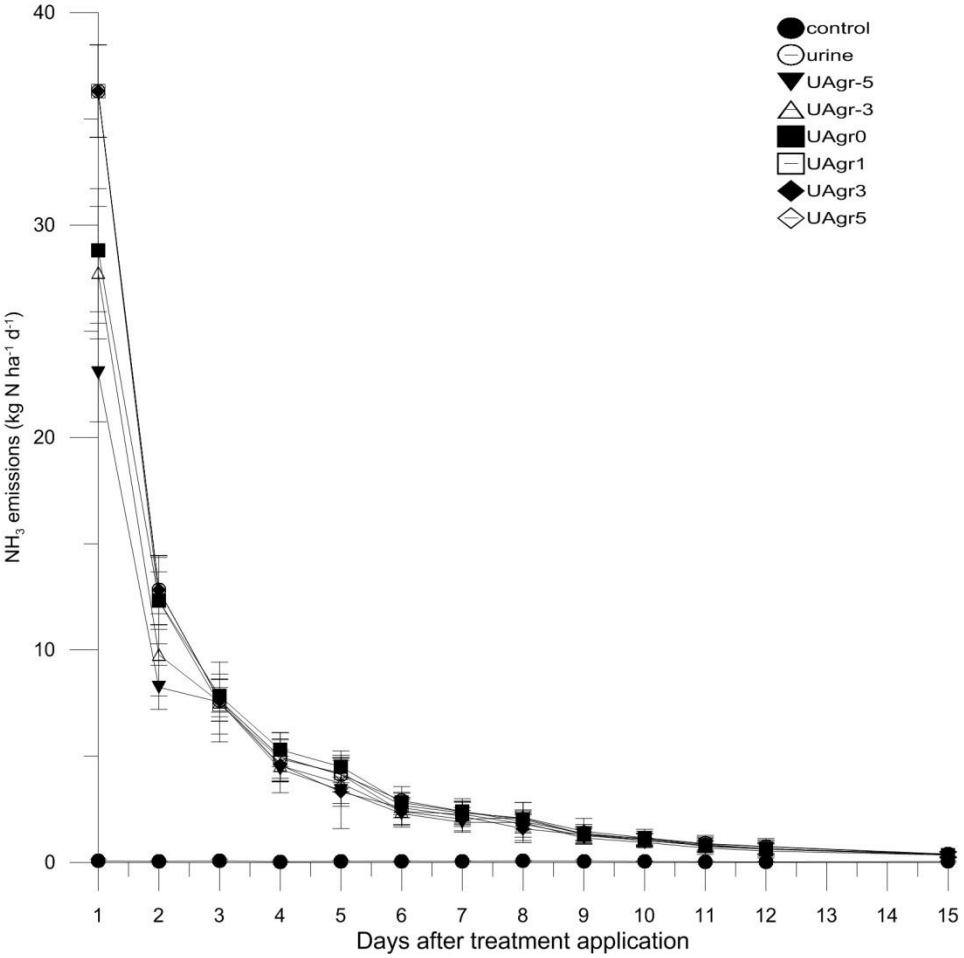

Figure 3. Ammonia volatilization following ruminant urine deposition before, on the same day, and after Agrotain® application in autumn. Data are mean ± sd (n = 6).

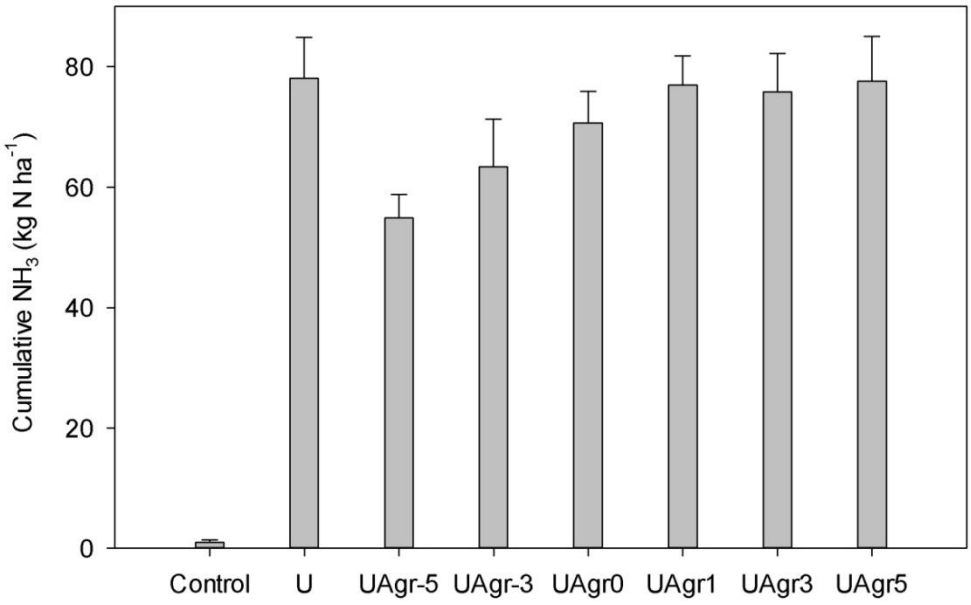

Figure 4. Cumulative NH_3 emissions following urine deposition before, on the same day, and after Agrotain® application in autumn. Data are mean ± sd (n = 6).

Soil pH

Urine application resulted in a sharp increase in soil pH ($P < 0.000$) in all treatments in comparison with the Control treatment (Fig. 5). Following this initial rise, soil pH rapidly declined in all treatments receiving urine and, after day 9, the pH values were smaller than that exhibited by Control treatment.

The soil pH was 6.26 in control treatments and after 24 hours of urine application increased to 6.68 in the urine, UAgr1, UAgr3, and UAgr5 treatments. Soil pH dropped gradually in these treatments until the end of the experiment.

Application of Agrotain® in the UAgr0, UAgr-3 and, UAgr-5 treatments reduced the initial rise of soil pH by 0.09, 0.05, and 0.12 units, respectively. However, there was no significant difference on day 1 between treatments with Agrotain® application. The application of Agrotain® on the same day as the urine, delayed the peak of soil pH by 5 days, where it reached a maximum value of 6.72.

Similar results were observed by Singh et al. (2013) and Zaman and Nguyen (2012). However, the increase in soil pH after urine application in the present study was lower than 1 pH unit reported in those studies. This lower increase in soil pH observed in this study may reflect the lower amount of urine-N hydrolyzed. The reason of the low increase in soil pH after urine deposition could also be the soil buffering capacity which is the ability of the soil to resist changes in the pH (Ferguson et al., 1984).

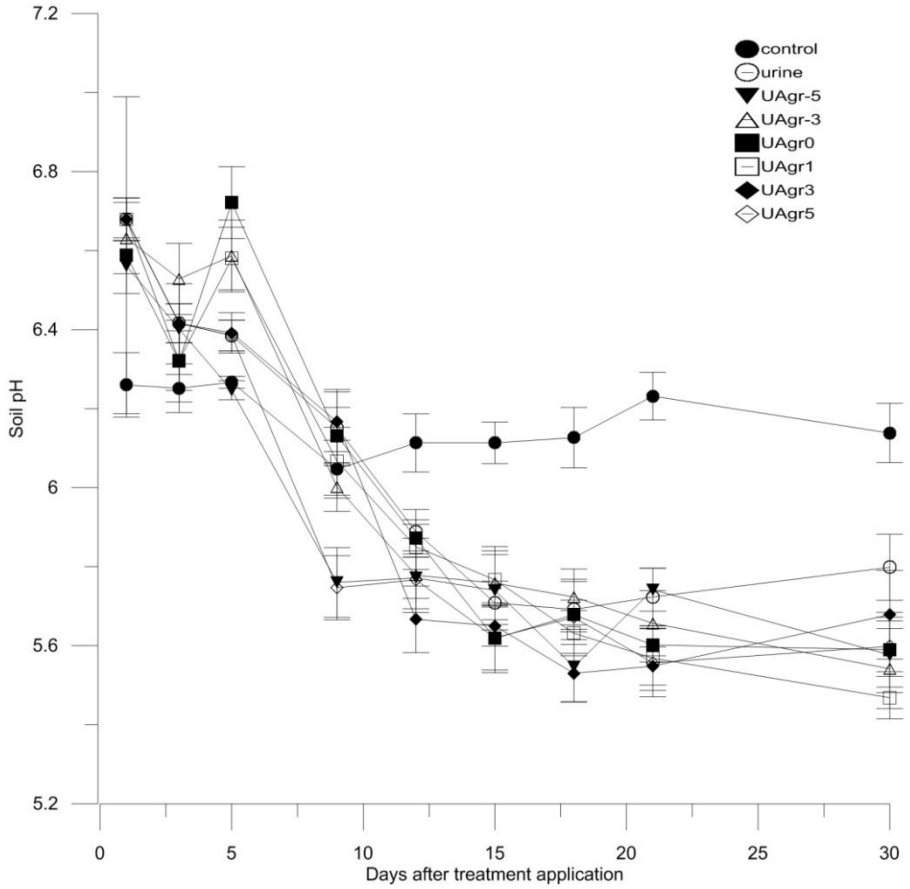

Figure 5. Soil pH at 0-10 cm depth following urine deposition before, on the same day, and after Agrotain® application in autumn. Data are mean ± sd (n = 6).

Mineral N

Application of urine not only resulted in a sharp increase in NH_3 losses, but also resulted in an increase in soil NH_4^+- N concentration within 24 h due to the hydrolysis process (not measured) (Fig. 6a). This process also realized OH^- to the soil resulting also in an increase in soil pH. The initial increase of soil NH_4^+-N concentration was followed by a subsequent decline during the remaining measurements. Soil NH_4^+-N concentration in all treatments was closed to background level after 21 days of the experiment (Fig. 6a).

In the urine only, UAgr1, UAgr3 and UAgr5 treatments, exchangeable NH_4^+-N in the top soil layer reached a maximum value of 305.32 ± 9.35 mg NH_4^+-N kg^{-1} soil after 24 h.

Application of Agrotain® prior to urine deposition (UAgr-3 and UAgr-5) was effective in significantly ($P < 0.0001$) reduce concentration of NH_4^+-N compared to urine, UAgr1, UAgr3 and UAgr5 treatments. In UAgr0, soil NH_4^+-N was reduced but not significantly different from urine, UAgr1, UAgr3 and UAgr5 treatments. After 24 h, NH_4^+-N concentration was 207.19 ± 12.89, 213.10 ± 18.03 and 226.34 ± 109.29 mg NH_4^+-N kg^{-1} soil in UAgr-5, UAgr-3 and UAgr0, respectively.

Therefore, the addition of Agrotain® before urine application resulted in a reduction in NH_3 losses which was also supported by a decrease in soil NH_4^+-N concentration and soil pH. The low concentration of NH_4^+-N in soil could be attributed to a slow rate of urea hydrolysis by the inhibitor. These results are in agreement with that of Zaman and Nguyen (2012) where Agrotain® was applied 5 days prior to urine.

After the initial increase, both soil NH_4^+-N and pH decreased over the experiment. The decrease in soil pH could be explained because NH_4^+-N is transformed into NO_3^--N by the nitrification process or because NH_4^+-N is transformed to NH_3. Both processes release H^+ to the soil, lowering soil pH (Bolan et al., 2004; Haynes & Williams, 1992; Jones et al., 2007; Zaman et al., 2008). Soil NH_4^+-N was also reduced due to the processes described previously. The nitrification process discussed in previous studies can explain the rise in soil NO_3^--N in urine treatments (Bolan et al., 2004). After 15 days of urine application, NO_3^--N was the dominant ion due to the nitrification process in which NH_4^+-N is transformed into NO_3^--N and H^+ ions were released into the soil (Fig. 6b).

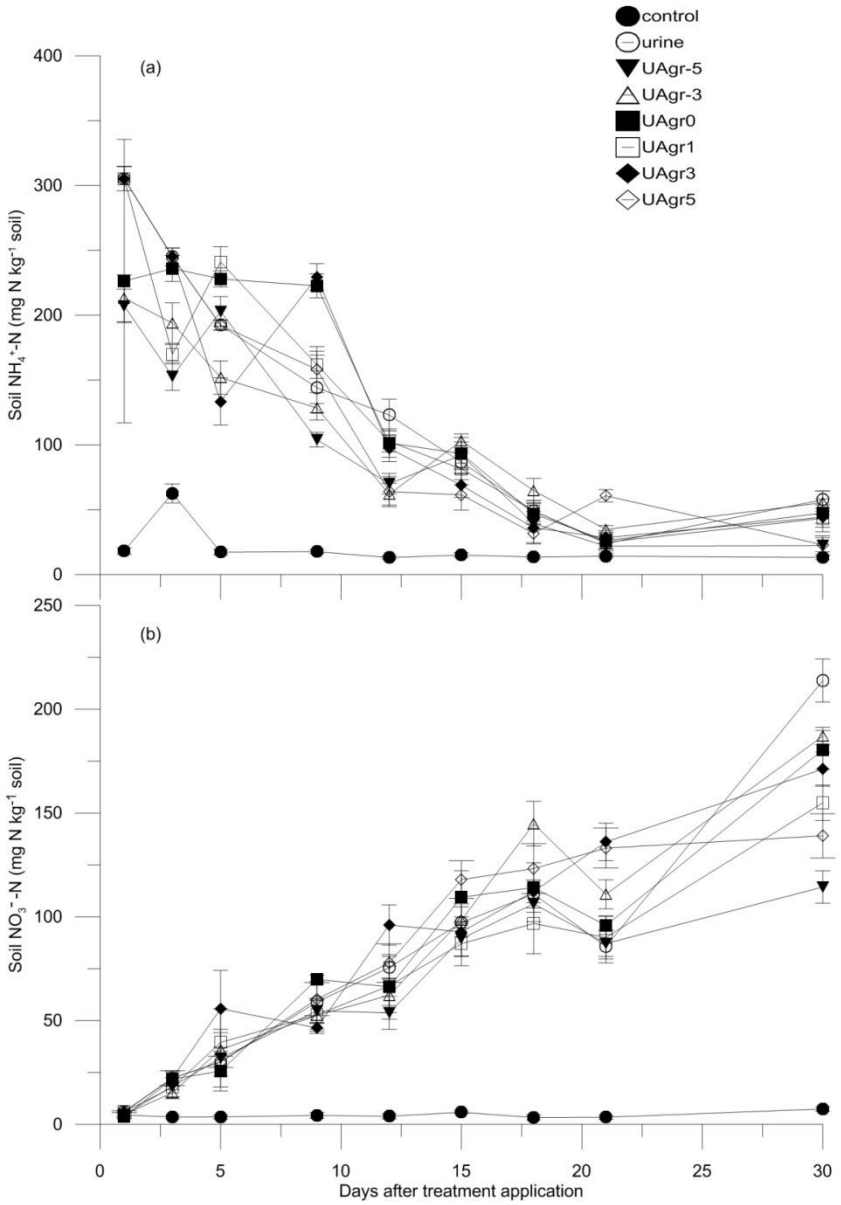

Figure 6. Soil mineral N concentrations at 0-10 cm depth for (a) NH_4^+-N and (b) NO_3^--N, following ruminant urine deposition before, on the same day, and after Agrotain® application in autumn. Data are mean ± sd (n = 6).

Conclusion

This study is the first attempt to simulate a real grazing scenario and assess the effect of Agrotain® by spraying it before or after urine deposition and not mixing UI in urine and then applying to the soil. Here when Agrotain® and urine were applied on the same day, the grass was mown to mimic the grazing event, urine was applied and then the inhibitor was sprayed. It may be desired to apply the inhibitor before urine and then measure the NH_3 losses. Agrotain® application 5 and 3 days before urine application and on the same day reduced NH_3 losses by 27.3, 17.5, and 9.6%, respectively. However, the application of Agrotain® after urine deposition had no effect on NH_3 losses. The lower reduction percentage observed in the present study in comparison with previous studies could be due to the method of application. Although the method used here has practical limitations, it is more realistic than that employed in other studies where Agrotain® was mixed with urine before application.

The spraying of Agrotain® onto pastures prior to grazing is not a label or recommended use of the product. Agrotain® use in this way could result in residues remaining on the pasture canopy being subsequently grazed by the animals. Any further research would need to consider the uptake of Agrotain® by pasture plants, and its fate in the body of grazing animals.

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