EFECT OF APPLICATION TIMES OF AN UREASE INHIBITOR ON NH_3 EMISSIONS FROM URINE PATCHES

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INTRODUCTION

In grazed pastures about 80% of urine N in the form of urea is rapidly hydrolysed and is subjected to ammonia (NH_3) losses (Bolan et al., 2004). 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 & Nguyen, 2012; Zaman et al., 2013). Ammonia itself is not a greenhouse gas but when re-deposited on land acts as an indirect source of nitrous oxide (N_2O) (Misselbrook et al., 2013). Therefore, many approaches to mitigating NH_3 loss have been investigated in New Zealand.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 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., 2013). 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 only urine is deposited on to the soil. 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.

MATERIALS AND METHODS

The experiment was set up in dairy farm # 4 at Massey University, New Zealand. The pasture site was a mix of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). 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.

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% w/w) 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.
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.

Ammonia emission measurement
Ammonia volatilization in this experiment was measured using the dynamic chamber method (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 was 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.

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

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 .

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. 1) 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 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). 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) 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 where Agrotain® was applied the same day or 3 and 5 days before urine application, NH_3 losses were significantly reduced. 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.

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.

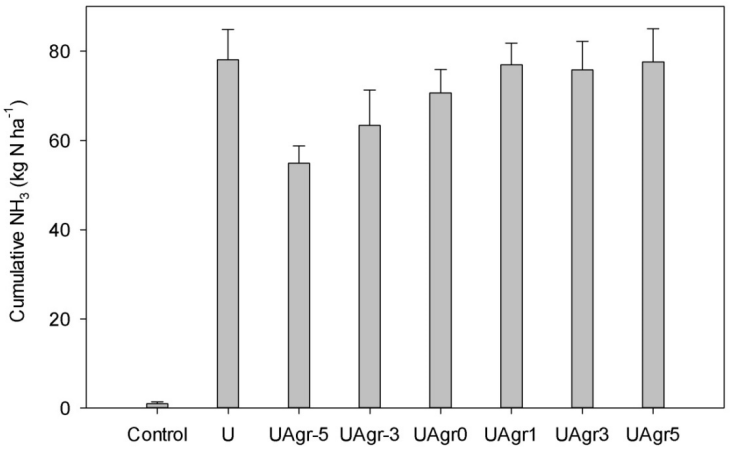

Fig. 1. Cumulative NH_3 emissions following urine deposition before, on the same day, and after Agrotain® application in autumn. Data are mean ± sd (*n* = 6).

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. 2). 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).

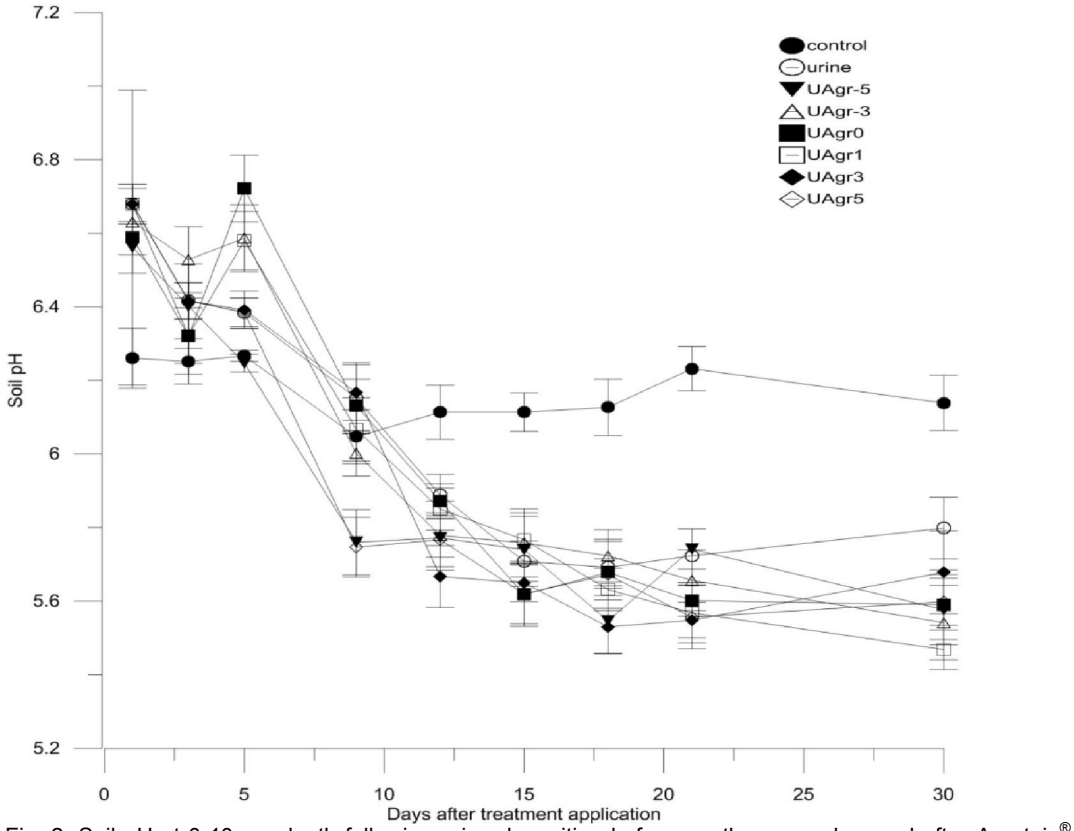

Fig. 2. Soil pH at 0-10 cm depth following urine deposition before, on the same day, and after Agrotain® application in autumn. Data are mean ± sd (n = 6).

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. 3a). 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. 3a).
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.

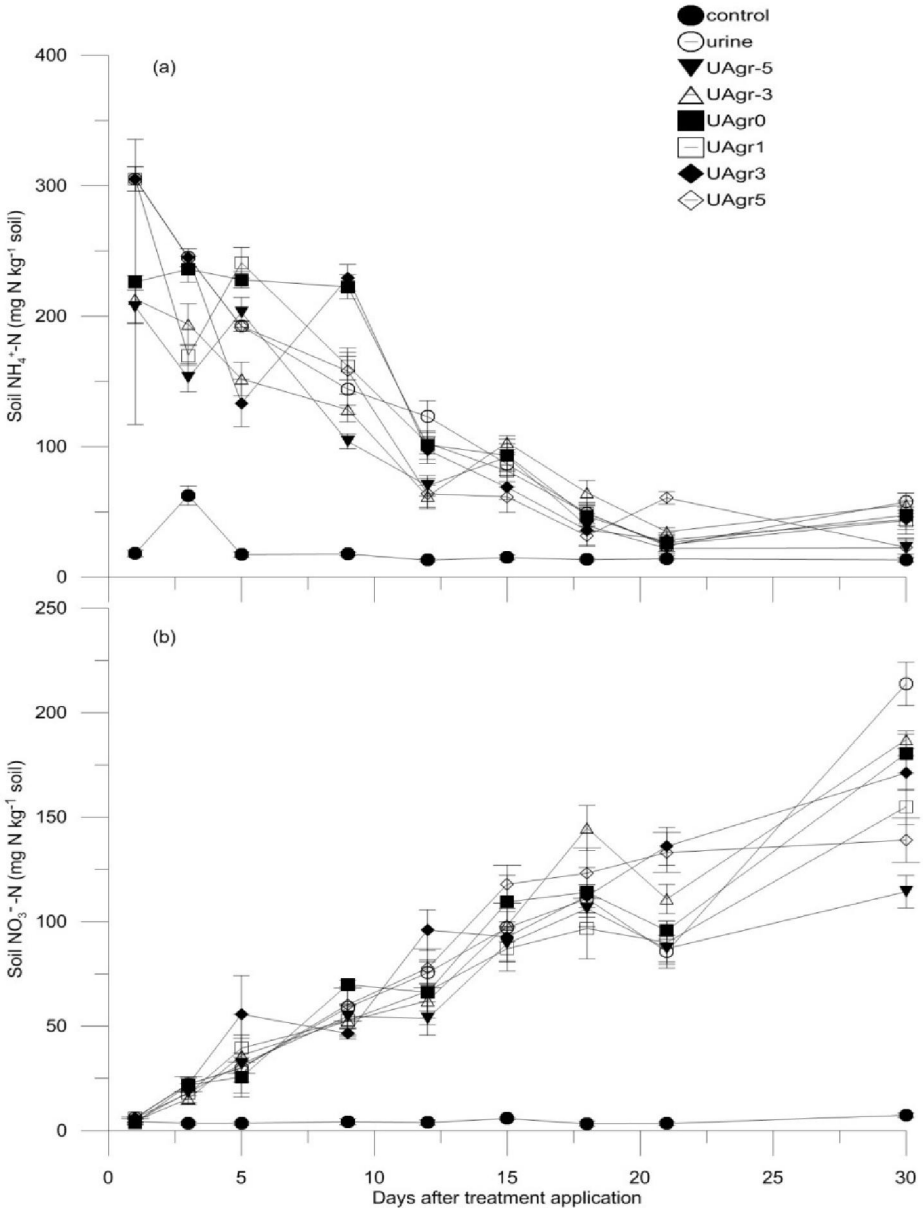

Fig. 3. Soil mineral N concentrations at 0-10 cm depth for (a) NH_4^+-N and (b) NO_3^--N, following ruminant urine deposition before, on the same day, and after Agrotain® application in autumn. Data are mean ± sd (n = 6).

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; 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. 3b).

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.

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