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**Effect of application times of urease inhibitor (Agrotain®)
on NH₃ emissions from urine patches**

A thesis presented in partial fulfilment of the requirements for the degree
of

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Abstract

In grazed pastures about 80% of urine nitrogen (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 emissions from urine has been measured by applying urine mixed with the urease inhibitor 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 onto the soil.

This current research aimed to identify the best time to spray the Agrotain[®] above soil pasture to reduce NH_3 losses from urine patches. A field experiment was carried out on dairy farm # 4 at Massey University, Palmerston North, New Zealand. 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 and 3 days prior to urine deposition, on the same day and 1, 3 and 5 after urine deposition in autumn (April 2013). NH_3 losses were measured using the dynamic chamber method. After the application of the treatments, $\text{NH}_{3(\text{g})}$ volatilization was determined in the acid traps, and soil mineral N (NH_4^+ -N and NO_3^- -N) and pH were measured from soil plots at different times over a period of 30 days.

The application of the inhibitor prior to urine deposition reduced NH_3 losses with reductions of 27.6% and 17.5% achieved for UAgr-5 and UAgr-3, respectively; and there was also a reduction in both soil NH_4^+ -N concentration and soil pH in comparison with urine alone or with the treatments where Agrotain[®] was applied after urine deposition. Application of Agrotain[®] on the same day as urine reduced NH_3 losses by 9.6% but this was not statistically significant from treatments when Agrotain[®] was applied after urine. The application of Agrotain[®] after urine deposition had no effect on NH_3 losses from urine.

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Chapter 1

1.1 Introduction

In intensively managed grazed pasture, urine patches are one of the most important source of nitrogen (N) (Bolan et al., 2004; Saggar et al., 2009) which is well above N requirements of pasture and is therefore not utilized efficiently. This surplus of N is subject to loss through ammonia (NH₃) volatilization, nitrous oxide (N₂O) emission and nitrate (NO₃⁻-N) leaching. Due to the high urea N content in urine, NH₃ volatilization is the principal form of N loss from urine patches causing eutrophication and acidification of water and soils where it is deposited (Misselbrook et al., 2013; Sanz-Cobena et al., 2012; Zaman & Blennerhassett, 2010) with the second source being a greenhouse gas N₂O. These NH₃ losses have both environmental and economic implications.

Urease inhibitors (UI) have been widely studied to determine the reduction in NH₃ volatilization from animal urine deposition (Abalos et al., 2012; Menneer et al., 2008; Pereira et al., 2013; Sanz-Cobena et al., 2008; 2012; Singh et al., 2013; Zaman & Blennerhassett, 2010; Zaman & Nguyen, 2012; Zaman et al., 2009; 2013). In theory, UI slow down the conversion of urea ((NH₂)₂CO) into NH₄⁺-N so that less NH₄⁺-N is available for conversion into NH₃ which is susceptible to be volatilized (Bolan et al., 2004). In a number of studies reviewed by Saggar et al. (2013), UI (nBTPT [N-(n-butyl) thiophosphoric triamide]) sold under the trade name Agrotain® and applied at 0.025% w/w to urine or fertilizer has been shown to reduce NH₃ emissions from New Zealand pasture soils.

However, more information is required to investigate the mode of application of the UI to urine patch, and also the optimum time of application of the inhibitor. In all the trials reported previously, urine is mixed with the UI before the application in the soil, increasing the chance to better inhibit the urease enzyme, which is not a realistic scenario. Therefore, the main objective of this trial was to study the inhibitor effect of Agrotain® on NH₃ losses from urine deposition when it is sprayed onto a pasture soil before or after the deposition of animal urine.

1.2 Research objectives

The aim of this research was to investigate the effect of field-application of Agrotain® on NH₃ losses from urine patches.

The specific objectives were:

- 1) To determine the time for Agrotain® application before and after urine deposition to obtain optimum reduction in NH₃ emission
- 2) To understand the effect of Agrotain® on the transformations of mineral N
- 3) To assess the effect in soil pH with the addition of Agrotain®

1.3 Thesis structure

This thesis is divided into three chapters. Chapter 1 provides a brief outline of this research and the main objectives. Chapter 2 includes a review of the most relevant literature for this study and it is mainly focused in NH₃ volatilization and the use of UI as a mitigation option. Chapter 3 describes the field-plot experiment which presents quantitative data of NH₃ losses from urine patches when Agrotain was sprayed at different times. This chapter also includes the conclusion and also identifies the need of future research.

Chapter 2

Literature review

2.1 Introduction

In a grazed pasture system, the sources of nitrogen (N) are biological fixation of atmospheric N (BNF), animal dung and urine deposition during grazing, N fertilizers and manures, mineralization of soil organic N and atmospheric N deposition (Bolan et al., 2004). These N inputs often exceed the plant requirement, and therefore N is susceptible to gaseous and leaching loss which has a negative impact on aquatic, atmospheric and terrestrial environments. The N added to the soil from the above sources undergoes a series of transformations resulting in the production of different forms and species of N (Fig. 2.1). The N transformations include: mineralization, immobilization, nitrification, denitrification, ammonium (NH_4^+ -N) fixation, nitrate (NO_3^- -N) leaching and ammonia (NH_3) volatilization. All these processes have been widely studied and described in previous and recent reviews (Bolan et al., 2004; Saggar et al., 2004; 2009; 2013; Singh et al., 2008). Urine deposited by grazing animals and fertilizer urea used most widely in New Zealand, are susceptible to NH_3 volatilization. The loss of N mainly through NH_3 volatilization has both economic and environmental implications. NH_3 affects atmospheric visibility, aerosol chemistry, health and climate, and leads to soil acidification and eutrophication when deposited in soils and waters. Re-deposited NH_3 acts as an indirect source of a potent greenhouse gas, nitrous oxide (N_2O) which contributes to stratospheric ozone depletion (Saggar et al., 2005). This literature review describes the processes and soil, plant and climatic factors which influence NH_3 volatilization. It then discusses the different methods used to measure NH_3 emissions. It also provides data on NH_3 emission from urine and fertilizer urea collated from previous published and unpublished studies and describes the technologies available to mitigate NH_3 losses.

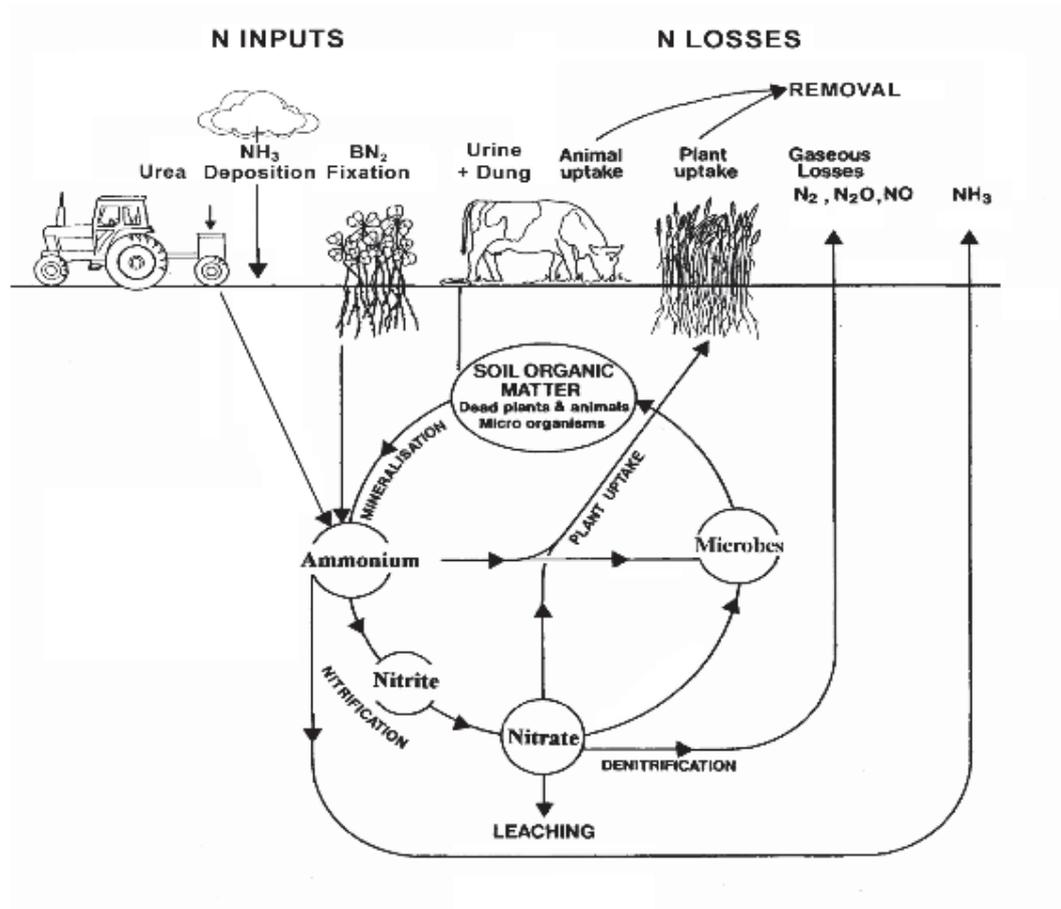
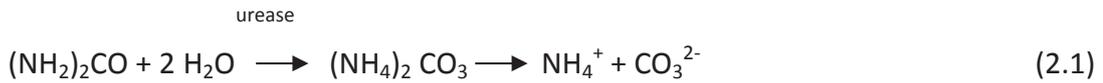


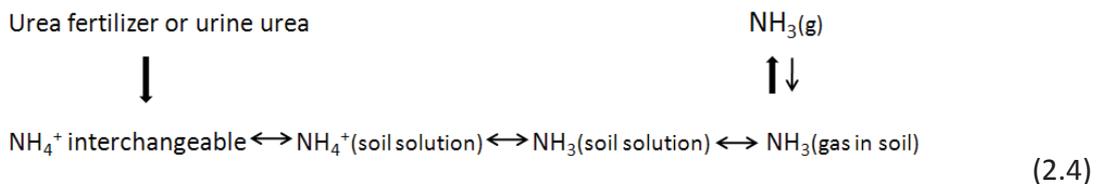
Figure 2.1. Nitrogen cycle. Source: Dawar (2010).

2.2 Ammonia volatilization

A major source of NH₃ in the atmosphere is the volatilization of NH₃ from animal excreta (mainly urine) and fertilizer N application (Bolan et al., 2004; Saggar et al., 2004; 2013). In a grazed system, NH₃ is produced at the soil surface wherever ruminants' urine is deposited or fertilizer urea is applied. Ammonia is formed when urea (NH₂)₂CO, the principal N compound contained within urine or in fertilizer urea, is hydrolysed by the urease enzyme to ammonium carbonate [(NH₄)₂CO₃], which is unstable and is dissociated into NH₄⁺-N and carbonate (CO₃²⁻) (Eq. 2.1) (Bolan et al., 2004). Then, CO₃²⁻ ions react with water to form bicarbonate (HCO₃⁻) and hydroxyl ions (OH⁻) which increase the soil pH (Eq. 2.2) to values greater than 7.2 (Saggar et al., 2004; Sherlock et al., 2008). Ammonium ions dissociate into NH₃ gas which is subject to volatilization losses (Eq. 2.3) (Bolan et al., 2004).



The rate of volatilization is controlled by the conversion of $\text{NH}_4^+\text{-N}$ to NH_3 gas which is a physio-chemical process that occurs only under alkaline conditions ($\text{pH} > 7.2$) (Bolan et al., 2004; Sherlock & Goh, 1984). The difference in partial pressure of NH_3 between soil and the atmosphere is one of the factors which drive the NH_3 volatilization process (Bolan et al., 2004; Saggar et al., 2004; Whitehead, 1995). This process depends on a chemical equilibrium which takes place in the soil solution (Eq. 2.4):



Urease enzyme is responsible for the hydrolysis of urea into $\text{NH}_4^+\text{-N}$ which occurs within one to two days of application (Saggar et al., 2004; Zaman et al., 2008; 2009). Urease is widely present in soils, plants and aquatic environments (Klose & Tabatabai, 1999; Saggar et al., 2004; Tabatabai & Bremner, 1972). The active site of the enzyme has two nickel ions (Ni) linked by a carbamate bridge and the Ni ions are involved in the catalytic process of the enzyme (Benini et al., 2001).

Urease activity can be altered by the presence of organic compounds which promote microbial activity (Zantua & Bremner, 1976). As the organic matter content of the soil decreases with depth, so does urease activity (Bremner & Mulvaney, 1978; Gould et al., 1973; Mulvaney & Bremner, 1981; Zantua & Bremner, 1976). Higher urease activity has been reported under grassland than cultivated soils due to differences in organic matter content and the microbial activity (O'Toole et al., 1985; Whitehead & Raistrick, 1993; Zaman et al., 2002). Urease activity is also affected by soil temperature. Moyo et

al. (1989) observed that a rise of soil temperature from 5 to 45^oC, increased urease activity in two different soils. According to Saggar et al. (2004), the volatilization process should be stopped if the temperature is below -4^oC because microbial activity ceases. Other factors that affect urease activity are soil pH, water content and urea concentration (Cabrera et al., 1991; Singh & Nye, 1984). The optimum pH for the activity of the urease is between 6 and 7.5 (Singh & Nye, 1984)

2.2.1 Factors that affect NH₃ volatilization

The rate of urea hydrolysis is affected by several environmental and soil factors at the time of the application/deposition of the N source. The environmental factors which most affect NH₃ volatilization are wind speed, rainfall and temperature. Soil temperature, pH, cation exchange capacity (CEC), buffering capacity and water content are the soil factors which affect the rate of NH₃ emission (Bolan et al., 2004; Saggar et al., 2004; 2013; Whitehead, 1995).

2.2.1.1 Climatic drivers

- **Temperature**

Temperature is one of the main factors affecting NH₃ volatilization process. The rate of urea hydrolysis and the transfer of NH₃ from soil solution to the atmosphere are accelerated by high temperatures (Cortus et al., 2008; He et al., 1999; McGarry et al., 1987; Moyo et al., 1989; Pereira et al., 2012; Salazar et al., 2012; Sommer et al., 2004; 2006). Hot and dry conditions favour NH₃ losses, while moist and winter conditions minimize the volatilization process (Pereira et al., 2012; Salazar et al., 2012). Sherlock and Goh (1984) found that NH₃ losses in sheep urine patches were 22.2%, 24.6% and 12.2% of the N applied in summer, autumn and winter, respectively. It was also found that on average, half of the concentration of urea in urine was hydrolysed in 3.0 and 4.7 hours in summer and autumn, respectively. This was due to the lower soil temperatures and the high moisture content. Lower temperature in autumn increased the formation of NH₄⁺-N in soil solution which reduces the concentration of NH₃

subject to be volatilized (Sherlock & Goh, 1984). Moreover, the high soil water content found in autumn (33.9%) diluted the NH_3 in soil solution, reducing the amount of 'volatilizable NH_3 ' in the surface. In a laboratory experiment, Pereira et al. (2012) studied the effect of four temperatures (5, 15, 25 and 35°C) on the rate of NH_3 emissions from a mix of urine (8 mL) and dung (8g) simulating a dairy house concrete floor. They reported an increase in NH_3 volatilization with an increase in soil temperature. They also observed that at 5°C, NH_3 volatilization peaked between 3 and 6 hours. However, at higher temperatures the peak occurred between 1 and 3 hours, indicating that urea hydrolysis occurs immediately after the excreta deposition and it is stimulated by high temperature and dry conditions. When temperature increased from 5 to 25°C and from 15 to 35°C, the cumulative NH_3 volatilization was 75% and 95% of the N applied as a mix of urine and dung, respectively. According to Sommer et al. (2006) urease activity is affected when soil temperature is lower than 10°C and increased in a range of 10°C and 40°C. Similarly, Cortus et al. (2008) observed that when temperature increased from 10°C to 20°C, NH_3 emissions increased 2 fold. Zaman et al. (2009) found that 8.2% of the N applied in urine was lost as NH_3 in summer and only 3.6% in spring due to the higher temperature and dry conditions in summer. However, in a field study under cold weather conditions where 100 kg N ha⁻¹ of urea was applied to winter wheat, Engel et al. (2011) found NH_3 losses of 30-44% of the N applied. These large losses were because the urea was applied to a soil with high-water content followed by a dry period without precipitation which promoted NH_3 loss.

Although there is a positive relationship between temperature and NH_3 volatilization, temperature affects other processes which compete with NH_3 volatilization such as nitrification, immobilization of N by microorganisms and also NH_4^+ -N uptakes by plants (Whitehead & Raistrick, 1991).

In summary, an increase in soil temperature generally accelerates NH_3 losses because of a stimulation effect on the urease activity, increasing NH_4^+ -N and OH^- in soil solution and accelerating the conversion of NH_4^+ -N to NH_3 . High NH_3 loss has been reported in summer due to dry conditions and high temperature which increase the urease

activity. On the other hand, when urea/urine are applied to a wet soil followed by a dry period, NH_3 loss increases.

- **Rainfall and irrigation**

NH_3 losses from fertilizer urea and urine are also affected by rainfall or the amount and timing of irrigation. Bouwmeester et al. (1985) reported that NH_3 losses were negligible when 25 mm of irrigation was added immediately after urea application due to the movement of unhydrolysed urea down into the soil profile. The same effect was found by Kissel et al. (2004) when simulated rainfall was added after urea application and NH_3 losses were <1% of the urea applied. Zaman et al. (2008) also reported low NH_3 losses after the application of urea and they attributed it to the moist soil conditions and a rainfall event of 17 mm which occurred one day after the fertilizer application. However, Craig and Wollum (1982) found that light rainfall (< 15mm) after urea application to dry soil stimulated urea hydrolysis because it was not enough to move urea down into soil profile, increasing NH_3 volatilization.

Recently, Sanz- Cobena et al. (2011) observed that the addition of 7 mm of water or more after fertilizer urea application reduced NH_3 losses due to the movement of urea into the soil. NH_4^+ -N ions produced during the hydrolysis can be uptaken by plants, immobilized on cation exchanges sites, transformed into NO_3^- -N, which reduced the risk of being volatilized. However, the addition of lower amount of water increased urea hydrolysis, enhancing NH_3 losses.

When urine is deposited into the soil, the soil water content has little influenced in the rate of NH_3 emission due to the water content in urine. However, if the soil is too dry, urease is inhibited and therefore NH_3 fluxes are very low or almost negligible (Bolan et al., 2004; Saggar et al., 2004). On the other hand, if the soil is moist at urination time but it is followed by a dry period, NH_3 volatilization would increase. Dry conditions favour evaporation of water soil content and therefore promote enhance of NH_3 fluxes (Saggar et al., 2004). Thus irrigation/rainfall immediately after a grazing event or fertilizer urea application can significantly reduce NH_3 volatilization (Zaman et al., 2013).

To summarize, rainfall/irrigation after fertilizer urea application can reduce NH_3 losses, and the effect is greater if the rainfall event occurs the first 24 hours after the application. Addition of water immediately after urea application moves the unhydrolysed urea down into the soil profile where is protected against the urease, reducing NH_3 emissions. However, as it was described, soil water content has a greater effect on NH_3 loss from fertilizer urea than urine due to the water content in urine.

- **Wind speed**

Volatilization of NH_3 from soil surface is mainly driven by the NH_3 concentration gradient between the soil and the atmosphere (Saggar et al., 2004). Increasing wind speed might enhance NH_3 volatilization due to the rapid movement of NH_3 from the soil-air interface (Balsari et al., 2007; Misselbrook et al., 2005; Whitehead & Raistrick, 1991).

Wind speed increases NH_3 losses due to an increase of the atmospheric diffusion and surface exchange of $\text{NH}_{3(g)}$ between the soil surface and the atmosphere (Asman et al., 1998). Thompson et al. (1990) found that wind speed had a positive effect on NH_3 volatilization from cattle slurry applied to the soil surface. They also observed that the effect of wind speed was more pronounced only in the first 24 hours when NH_3 fluxes were highest. The occurrence of strong wind also favoured the rapid reduction of $\text{NH}_{3(g)}$ which is commonly observed from urine patches (Sherlock & Goh, 1985). Misselbrook et al. (2005) also identified wind speed as one of the variable that most influenced NH_3 losses from slurry application. They found a linear relationship for wind speed between 0.5 and 4.5 m s^{-1} . Similarly, Huijsmans et al. (2003) found that NH_3 losses were affected by wind speed. They explained that increasing wind speed, NH_3 losses rise due to an increase of NH_3 diffusion from the N source. In contrast to these results, Bouwmeester et al. (1985) observed a decrease in NH_3 losses from 19% to 7.5% of the N applied when wind speed increased from 1.7 to 3.4 m s^{-1} due to a drying effect on the soil. Similar effect was observed by Whitehead and Raistrick (1991) who conducted a laboratory experiment where they studied the influence of five air flow rates on NH_3 emissions.

To summarize, it is expected that increasing the wind speed will increase NH_3 losses from the N source because of the movement of the NH_3 from the soil to the air interface. This positive effect is relevant immediately after excreta or fertilizer urea application when NH_3 fluxes are greatest. However, in some cases when the wind speed increase, there is a drying effect which reduce NH_3 losses from the N source.

2.2.1.2 Soil drivers

- **Soil water content**

Soil water content is an important factor affecting the rate of NH_3 emission (Bolan et al., 2004; Engel et al., 2011; Kissel et al., 2004; Prasertsak et al., 2001). Water content not only affects the dissolution of urea and its movement into the soil, but also influences the movement of NH_4^+ -N in the soils (Ferguson & Kissel, 1986). Vlek and Carter (1983) observed that low initial soil moisture restricted urea hydrolysis. This may be due to a poor urea diffusion affecting the interaction between urea and urease enzyme. The same effect was reported by Whitehead and Raistrick (1991), who observed that there was a difference on the rate of NH_3 emission between urine and fertilizer urea at low soil water content due to the addition of water in urine. Similarly, Prasertsak et al. (2001) found that applying urea to a dry soil, only 3.2% of the N applied was lost as NH_3 (114 kg N ha^{-1}) and 17.2% when urea was applied to wet soils. Contrary, Sherlock and Goh (1984) reported low NH_3 loss when urine was deposited in a soil with high moisture content (33.9%) due to a dissolution effect of NH_3 into the soil solution. According to Whitehead (1995), NH_3 volatilization is also affected for those conditions which increase the water evaporation rate because water moves up in the soil profile and so does NH_3 in soil solution.

These studies suggest that soil moisture has a greater influence on the rate of NH_3 losses from fertilizer urea than urine. If urine is applied to dry soil, NH_3 losses could be reduced because urea in urine is moved down in the soil profile. However, if the soil is moist when urea is applied or urine deposited, and is followed by a dry period, NH_3 losses could be increased. Therefore, soil moisture affects NH_3 losses due to the effect

of water content on the urea hydrolysis and also on the movement of $\text{NH}_4^+\text{-N}$ from the soil surface.

- **Soil pH**

Soil pH strongly influences NH_3 emission. It has been shown that NH_3 emission enhances when soil pH increases (Bolan et al., 2004; Freney et al., 1983; Pereira et al., 2013; Singh et al., 2013; Watson et al., 1994a; Zaman & Nguyen, 2012). During the hydrolysis reaction, H^+ ions are consumed and therefore soil pH increases. According to Freney et al. (1983), NH_3 concentration increases from 0.1, 1, 10 and 50% when soil pH increases from 6 to 7, 8 and 9. Similarly, Zaman and Nguyen (2012) and Singh et al. (2013) found that soil pH increased over 1.5 units immediately after urine deposition because of the production of OH^- ions during urea hydrolysis. Pereira et al. (2013) observed that applying urine at 200 kg N ha^{-1} , the soil pH increased in the first six days and then decreased until the end of the experiment. The initial rise in soil pH generally occurs at the same time that the highest peak of NH_3 loss, and when soil pH declines so does NH_3 fluxes (Singh et al., 2013). Watson et al. (1994a) in a laboratory study with 16 different soils, studied the effect of the soil properties on the effectiveness of the urease inhibitor nBTPT (N-9n butyl) thiophosphoric triamide)) to reduce NH_3 losses from fertilizer urea. They found that four soil properties, titratable acidity, pH, urease activity and CEC, explain the high variation in NH_3 loss between soils. They also observed that NH_3 losses from fertilizer urea were greater from soil with high pH. NH_3 losses ranged from 5.8% to 38.9% and soil pH varied between 5.7 and 7.6. Soil pH alters the equilibrium between $\text{NH}_4^+\text{-N}$ and NH_3 (Eq.2.3). Therefore, when fertilizer urea is applied, soil pH increases and the equilibrium in that equation moves to the right, increasing NH_3 losses (He et al., 1999).

To conclude, when urea/urine are applied to the soil, soil pH sharply increases because H^+ ions are consumed in the hydrolysis process. This rise in soil pH coincides with an increase in NH_3 fluxes. After this initial rise, soil pH declines so does NH_3 volatilization.

- **Cation exchange capacity and buffering capacity**

Along with soil pH, cation exchange capacity (CEC) affects NH_3 volatilization (Saggar et al., 2004). CEC influences NH_3 losses because of the reaction of NH_4^+ -N with the negatively charged cation exchange sites in soils (Bolan et al., 2004; Saggar et al., 2004). Soils with high CEC emit less NH_3 than soil with low CEC (Whitehead, 1995). Moreover, soil with high CEC can reduce NH_3 emission by restricting changes in soil pH or increasing the buffering capacity. Whitehead and Raistrick (1990) found that CEC presents a larger effect on NH_3 volatilization from urine than fertilizer urea due to the greater contact between soil and urine. A laboratory experiment was developed to compare the effect on NH_3 volatilization when urea fertilizer was mixed with different materials which increase soil CEC (humic acid and zeolite) and reduce soil pH in the microsites (triple superphosphate- TSP) (Ahmed et al., 2006). They found that applying these materials, NH_3 losses were reduced by between 32 and 61% in comparison with the urea treatment. Therefore, the increase in CEC and the reduction in soil pH, favoured the formation of NH_4^+ -N over NH_3 , promotes a greater buffering capacity and more NH_4^+ -N ions are retained by the cation exchange sites in soils (Ahmed et al., 2006). Although Watson et al. (2008) found that there was not a good relationship between CEC and total NH_3 losses, soil with lower CEC emitted more NH_3 compared to two soils with high CEC. In a previous work, they also showed that the total NH_3 loss was negatively correlated with CEC and this soil property contributed significantly to explain the variation in NH_3 volatilization between different soils (Watson et al., 1994a).

Buffering capacity is the ability of the soil to resist changes in soil pH (Ferguson et al., 1984) and hence it affects the rate of NH_3 losses. In soils with high buffering capacity once the fertilizer or urine is deposited, the increase in soil pH is small and less NH_3 is emitted (Ferguson et al., 1984; Whitehead, 1995). Ferguson et al. (1984) conducted an experiment to study the effect of H^+ buffering capacity on NH_3 losses in two soils. They found that soil amended with a resin enhanced the buffering capacity, hence NH_3 losses were reduced.

To conclude, CEC and buffering capacity are other factors which influence NH_3 loss from soil. Soils with high CEC emit less NH_3 due to the retention of NH_4^+ -N ions by the negative charged cation exchange sites. The buffering capacity of a soil influences the rise in soil pH which means that in soil with high buffering capacity, the increase in soil pH is small. Therefore, both CEC and buffering capacity are negatively correlated with NH_3 loss, when one of these properties increases, NH_3 loss is reduced.

- **Role of Plants**

The presence of plants also affects NH_3 volatilization process (Bolan et al., 2004; Saggar et al., 2004). Plants decrease NH_4^+ -N concentrations in the soil through plant uptake and also the pH of the rhizosphere is altered (Whitehead, 1995). Therefore, less NH_4^+ -N is converted into NH_3 which is susceptible to be volatilized. According to Saggar et al. (2004) plants also modify wind speed, temperature and moisture conditions at the soil surface and these factors affect the rate of NH_3 losses. However, Whitehead and Raistrick (1992) observed that the presence of dead leaf litter increases NH_3 volatilization due to an increase in surface area for urease activity.

Although the presence of plant reduces NH_3 losses through plant uptake of NH_4^+ -N, an important factor that affects the rate of volatilization could be the time when the fertilizer or urine is applied. In the case of urea fertilizer, it should be applied when the plant growth is active and is not affected by low temperature, drought or wetness (Sustainable Nutrient Management in NZ Agriculture/ FLRC, 2012). Therefore, plants uptake the NH_4^+ -N and less NH_4^+ -N will be converted into NH_3 .

In a temperate climate and grazed pastoral system which present seasonal variations during the year, the temperature and soil water content are the climatic and soil factors that contributed significantly to explain the variation in NH_3 losses. As mentioned, seasonal variations influence the rate of NH_3 volatilization throughout the year - high during summer and spring and almost negligible in winter (Pereira et al., 2012; Salazar et al., 2012). High temperature and dry conditions in summer, increase

NH₃ loss because these conditions stimulate urease activity, increasing the hydrolysis process (Moyo et al., 1989; Pereira et al., 2011; Sommer et al., 2004). As mentioned, soil pH is another soil factor which significantly influences NH₃ loss, because NH₃ volatilization is a process which occurs only under alkaline conditions (Bolan et al., 2004; Sherlock & Goh, 1984)

2.2.2 Environmental implications of NH₃ volatilization

Ammonia volatilization has gained attention due to its effect on environmental pollution, loss of biodiversity and climate change (Misselbrook et al., 2013; Sommer & Hutchings, 2001). The negative impacts of NH₃ can occur in different ways, with NH₃ deposition causing harmful effects in a variety of ecosystems. Over-enrichment of N with NH₃ in aquatic ecosystems can lead to eutrophication of surface water bodies and subsequent decline in aquatic species and a decrease in biological diversity (Misselbrook et al., 2013; Sanz-Cobena et al., 2012; Stevens et al., 2004; Zaman & Blennerhassett, 2010). Acidification of soils may also occur as a result of NH₃ deposition followed by oxidation of nitrate (Erisman et al., 2007; Grennfelt & Hultberg, 1986). Soil acidification can have negative effects if the soil is not able to buffer against the decrease in soil pH (Bolan et al., 2004).

NH₃ is an important contributor to the formation of atmospheric secondary aerosols and also contributes to the acid rain (Aneja et al., 2001; Asman et al., 1998; Menz & Seip, 2004). NH₃ reacts with nitrogen and sulphur oxides in the atmosphere and forms small particles, aerosols, which are a threat to human and animal health and also reduce visibility. These fine particles will be transported with the wind and may be re-deposited in other regions. Once it is deposited in the soil, it can be transformed into NO₃⁻-N with an accompanying release of H⁺ due to the nitrification (Aneja et al., 2001; Erisman et al., 2007).

The lifetime of NH₃ in the atmosphere is between 1 and 5 days, therefore it will likely be deposited near the source (Aneja et al., 2001; Asman et al., 1998). However, the aerosols have a longer lifetime (1-15 days) and they will be transported longer distances by the wind (Asman et al., 1998). Although NH₃ has a short lifetime in the

atmosphere, it acts as a secondary source of nitrous oxide (N_2O) which is a greenhouse gas.

To summarize, one of the main consequence of NH_3 loss to the atmosphere is the pollution of the environment. For example, NH_3 emissions cause soil acidification, eutrophication of water bodies, affects visibility and also health and climate. Moreover, it is a source of N_2O which is involved in the global warming (Martikainen, 1985). Therefore, several techniques have been developed to reduce NH_3 volatilization from excreta deposition and fertilizer urea application which are discussed in the following sections.

2.2.3 Methodology to measure NH_3 emission

NH_3 emissions from urine, nitrogen fertilizers, and animal waste management systems have been measured worldwide using different methodologies. The commonly used methods are micrometeorological, wind tunnels and dynamic chambers (Table 2.1).

Few studies compare the different field methods to measure NH_3 emissions. Smith et al. (2007) compared NH_3 losses using three methods: closed chamber, wind tunnel and a micrometeorological technique. Liquid swine manure was applied at $100 \text{ kg NH}_4^+\text{-N ha}^{-1}$ and NH_3 volatilization was measured during five days. The results from wind tunnels and the micrometeorological approach measured similar emission rates of NH_3 which ranged from 36% to 55% and from 38% to 42%, respectively. However, using static chambers, NH_3 volatilization ranged between 0.2% and 2%. Static chambers underestimated NH_3 emissions due to the lack of air exchange inside the chamber. Another reason of the low recovery of NH_3 losses by the static chambers could be that during the night the outside temperature is cooler than inside, causing condensation and therefore NH_3 could be diluted due to the high affinity of NH_3 for water. Given that the recovery percentage for static chambers is very low, dynamic chambers, wind tunnel and micrometeorological methods are widely used to measure NH_3 volatilization from treated areas.

These methods are discussed below.

Table 2. 1. *International and National References using the different methodology to determine NH₃ volatilization*

Reference	Region or country	Methodology	Flow rate or wind speed	N source	Rate of volatilization
Bussink (1992)	The Netherlands	MM mass balance		Urea	550 kg N 250 Kg N
Cai et al.(2002)	China	MM mass balance ^A	--	Urea	75 kg N 200 kg N 120 kg N
Engel et al. (2011)	USA	MM-Integrated horizontal flux (HIF)		Urea	100 kg N
Laubach et al. (2012)	New Zealand	MM-mass budget		Urine	29.1 kg N
Petersen et al. (1998)	Denmark	Wind tunnel	3-3.5 m s ⁻¹	Urine	2.55 kg N
Prasertsak et al. (2001)	Australia	MM mass balance ^A		Urea	115 kg N
Ryden et al. (1987)	UK	Wind tunnels	1.5 m s ⁻¹	Urine Urea	420 kg N 70 kg N
Salazar et al. (2012)	Chile	Mass balance -HIF		Urea	100 kg N
Sanz-Cobena et al. (2011)	Spain	Small wind tunnels		Urea	100 kg N

Sanz-Cobena et al.(2008)	Spain	MM -HIF		Urea	170 kg N
Sherlock and Goh (1984)	New Zealand	Dynamic chambers	21 L min ⁻¹	Urine	500 kg N
Sherlock and Goh (1984)	New Zealand	Dynamic chambers	21 L min ⁻¹	Urea	500 kg N
Singh et al. (2013)	New Zealand	Dynamic chamber Lab.study	1 L min ⁻¹	Urine Urea	476 kg N 100 kg N
Turner et al. (2012)	Australia	MM- ZINST		Urea	46 Kg N 92 kg N
Vallis et al. (1982)	Australia	Dynamic chamber	0.07 - 1.9 m s ⁻¹	Urine	370-480
Wang et al. (2004)		Closed chamber		Urea	60 -180

Wang et al.(2004)	China	Vented chamber		Urea – winter wheat	120 kg N 240 kg N 360 kg N
				Urea- summer maize	120 kg N 240 kg N 360 kg N
Watson and Kilpatrick (1991)	Ireland	Ventilated enclosure	2 L min ⁻¹	Urea	50-100- kg N ha ⁻¹
Whitehead and Raistrick (1990)	UK	Dynamic chamber	3 L min ⁻¹	Urea	100 kg N
Whitehead and Raistrick (1993)	UK	Dynamic chamber		Urine	265 Kg N
Zaman and Nguyen (2012)	New Zealand	Dynamic chambers Field study	6-7 L min ⁻¹	Urine	600 Kg N
Zaman et al. (2008)	New Zealand	Dynamic chambers	6-7 L min ⁻¹	Urea	150 kg N
Zaman et al. (2009)	New Zealand	Dynamic chambers	6 L min ⁻¹	Urine	600 kg N
Zaman et al. (2013)	New Zealand	Dynamic chambers	7-8 L min ⁻¹	Urine	600 kg N

Micrometeorological method

Micrometeorological methods (MM) are based on the measurement of gas concentration gradients, wind speed, and other micrometeorological variables (wet and dry bulb air temperatures, net radiation) used to estimate the NH_3 volatilization from large areas. The three micrometeorological methods most used are: ZINST (height, z , independent of stability), the backward-Lagrangian stochastic (bLS) method and mass-balance (MB) method.

The Integrated horizontal flux (IHF) is a MM commonly used to measure NH_3 losses in small areas. This method assumes that all the NH_3 gas emitted from control sources within a defined ground area leaves via the downwind side, and carried by the wind (Denmead, 1995). Circular shape is given to the source area so this method does depend on wind direction, and the horizontal flux of NH_3 is measured in the centre of the plot. A mast is located in the centre of the treated area and devices are located at different heights on the mast to measure NH_3 and wind speed. Salazar et al. (2012) measured NH_3 losses from urea application at 100 kg N ha^{-1} using this method. They found that cumulative NH_3 emissions were 1.4 and 7.7 kg N ha^{-1} during winter, and 12.2 and 26.7 kg N ha^{-1} for the spring application.

The ZINST method assumes that at a field-plot scale of an irregular surface where gas is emitted at a constant rate, there is a height at which the horizontal flux is almost independent of the environmental stability conditions (Wilson et al. 1982 cited by Laubach et al. 2012). This method is a simplified micrometeorological approach and it requires only a measurement of the NH_3 loss and the wind speed at one height (z), although large plots are needed. Both NH_3 and wind speed can be measured independently or together using 'Leuning samplers'. Turner et al. (2012) carried out an experiment in two different locations (with different average annual rainfall and annual temperature) where N fertilizers were applied (urea, urea ammonium nitrate, and ammonium sulfate) at different rates in circular plots of 25 m radius. They used the ZINST method to measure NH_3 losses from the different treatments. In terms of urea fertilizer, two different rates were used: 46 and 92 kg N ha^{-1} . The NH_3 losses from urea fertilizer ranged from 5.4% (at a rate of 46 kg N -

urea ha^{-1} in the drier location) to 23% of the N applied (at a rate of 46 kg N ha^{-1} in second location with an annual rainfall of 413mm). This variation of NH_3 volatilization can be a combination of the different factors that influence the rate of emissions such as: moisture of the soil, texture, wind speed, the fertilizer and the application techniques and also the rainfall or irrigation after the fertilization.

Finally, the bLS model estimates the concentration of a gas measured downwind which is emitted from a source, and models the flight paths of thousands of gas particles backward from the location of NH_3 samplers (Flesch & Wilson, 1995; Flesch et al., 2004). This method was used by Denmead et al. (2004). Urea fertilizer was applied after grazing at a rate of 50 kg N ha^{-1} , and the pastures were irrigated immediately afterward. They found high rate of NH_3 loss during the day due to high temperatures, moisture contents, moderately strong winds and high evaporation rates. The cumulative NH_3 volatilized after grazing period was equal to 14.8 kg N in summer and only 2.0 kg N in autumn. Assuming a value of $150 \text{ kg N/cow per year}$ for N inputs from grazing dairy cows, the emissions factor would be 12% in summer and 1% in autumn. After urea application, 6% of the urea N was lost when the irrigation was delayed for 3 days, although when the irrigation followed the application of urea immediately, only 1% of urea-N was lost as NH_3 . Soil with high moisture content accelerates the process of urea hydrolysis and NH_3 volatilization occurs immediately.

A comparison between the bLS and ZINST methods was carried out by Sommer et al. (2005). The cumulative NH_3 volatilized was $23.3 \text{ kg N ha}^{-1}$ which corresponded to 29.4% of the N applied as urea ($79.25 \text{ kg N ha}^{-1}$). They also found that the bLS method underestimated NH_3 emissions by 16-24% when it was applied to a measurement height between 0.5 and 1.55 m. They suggested that ZINST is a reliable and easy to use technique, although it can only be carried out in circular plots. In non-circular plots, bLS technique is recommended. They recommended also that for long sampling intervals neutral atmospheric stability conditions have to be assumed. Recently, Laubach et al. (2012) measured NH_3 emissions from urine patches in a field experiments using the three micrometeorological methods

described. They observed that using the MB method, 25.7% of the N applied was emitted as NH_3 the first 6 days. Although the rate of NH_3 emitted calculated by the ZINST method was similar to the rate estimated using the MB, it showed a 50% larger random error. On the other hand, they found that bLS method underestimated the emission rate.

Micrometeorological methods are advantageous because they do not alter the environmental conditions, and they do not disturb the process of emissions (Bolan et al., 2004; Laubach et al., 2012; 2013). They are continuous methods because MM run from excreta deposition, when NH_3 losses are very high, until it is almost negligible (Sherlock et al., 2008). Also they estimate an integrated flux over the area; therefore these methods reduce the variability between samples. However, micrometeorological methods also have some limitations. They are site-specific, depend on weather conditions, and they are expensive in terms of instrumentation and difficult to use in practice.

Wind tunnels

Wind tunnels were developed to study NH_3 volatilization to minimize the disturbance of natural conditions between chambers and field conditions (Sherlock et al., 2008). The tunnel consists of a transparent and flexible polycarbonate canopy covering a source area, and a steel circular duct with an anemometer and an electrical fan inside which moves air through the canopy section at a speed that is controlled and recorded over time (Fig. 2.2) (Lockyer & Whitehead, 1990). The concentration of NH_3 in the airflow which enters and leaves the tunnel is measured using gas washing bottles containing an acid solution (acid trap). It is calculated as the product of the difference between the concentration of NH_3 that enters and leaves the tunnel multiplied by the volume of air which passes through the tunnel (Sanz-Cobena, 2010).

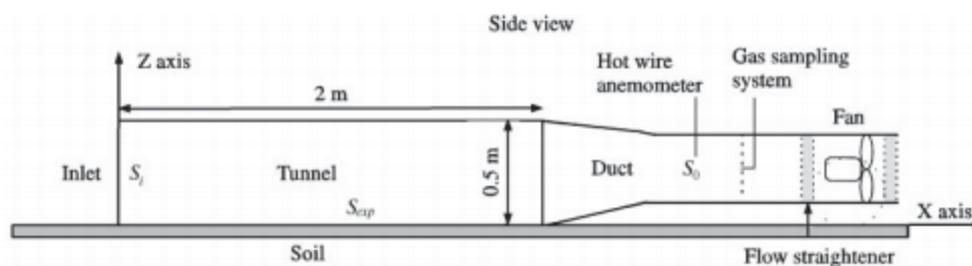


Figure 2.2. Diagram of a wind tunnel. Source: Loubet et al. (1999).



Figure 2.3. Wind tunnels used in a field work. Source: Sanz-Cobena (2010)

This method was used by Sanz-Cobena et al. (2011) to assess the mitigation effect on NH_3 losses, using nBTPT as urease inhibitor with or without irrigation (Fig. 2.3). Similarly, Ryden et al. (1987) used also a system of small wind tunnels to measure NH_3 losses from application of livestock excreta and fertilizer. They found that in the first 5 days, 80% of the total emissions occurred; NH_3 losses from urea ranged between 5.7% and 20.7% at a rate of application of 70 kg N ha^{-1} . For urine, NH_3 volatilization reached 8.8- 24.7% of the N applied, but from dung the losses were small.

Wind tunnel method creates a better approximation of the real microclimate with the use of fans, which minimize the effect of chambers (described below) (Sanz-Cobena, 2010). The other advantage is that any problem with rainfall is addressed by moving the tunnel to a slightly different place under the same conditions. However, this method also has some limitations. It is expensive because lots of instrumentation is needed and also large area is required in comparison with chambers, and electricity is required. Given that, in the study developed in this thesis, 48 wind tunnels for each trial would be needed, because each treatment has to be replicated 6 times, it would prove too costly.

Enclosure method

Enclosure methods are the most commonly used to determine NH_3 losses under field-plot conditions (Blennerhassett et al., 2006; Bussink, 1992; Dawar et al., 2012; Singh et al., 2013; Soares et al., 2012; Vallis et al., 1982; Whitehead et al., 1989; Whitehead & Raistrick, 1993; Zaman & Blennerhassett, 2010; Zaman & Nguyen, 2012; Zaman et al., 2008). It involves the use of chambers located above the soil surface with low vegetation cover. However, not all of them are accurate because the determination of NH_3 requires a flow-through system which allows NH_3 to be removed from the source and passed to the acid trap (Fig. 2.4). Therefore, the dynamic chamber has been developed. It is a type of enclosure method which includes the flow-through system which allows an accurate determination of NH_3 volatilization directly from the source. Kissel et al. (1977) designed a dynamic chamber to measure NH_3 volatilization under field conditions, and they found that using flow rates above 15 volume exchanges per minute there was no difference in NH_3 emissions. This result was also consistent with those obtained by Sommer and Ersbøll (1996), where flow rates higher than 3.9 L min^{-1} did not alter NH_3 losses. However, different results were obtained by Singh (2007) in laboratory work where using a chamber of approximately 7.4 litres of head space, she achieved 100% NH_3 recovery using a flow rate equal to 1.0 L min^{-1} .

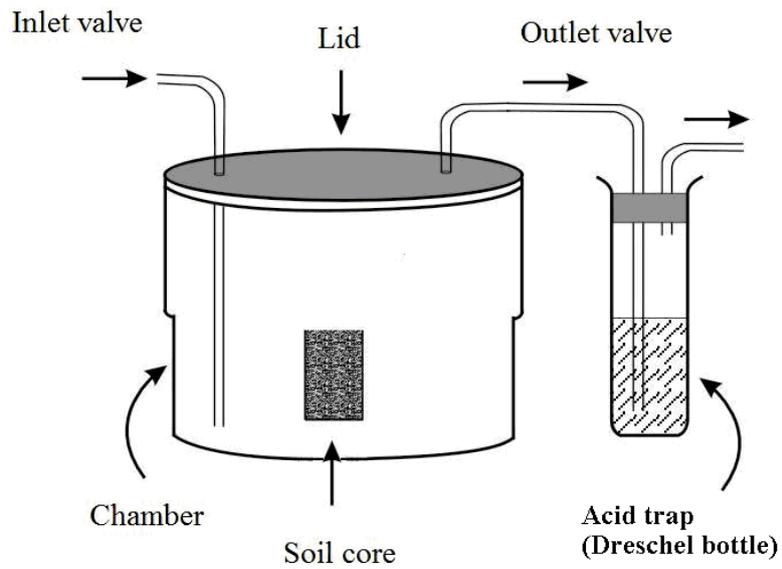


Figure 2.4. Schematic diagram of the chamber used to measure NH_3 volatilization.

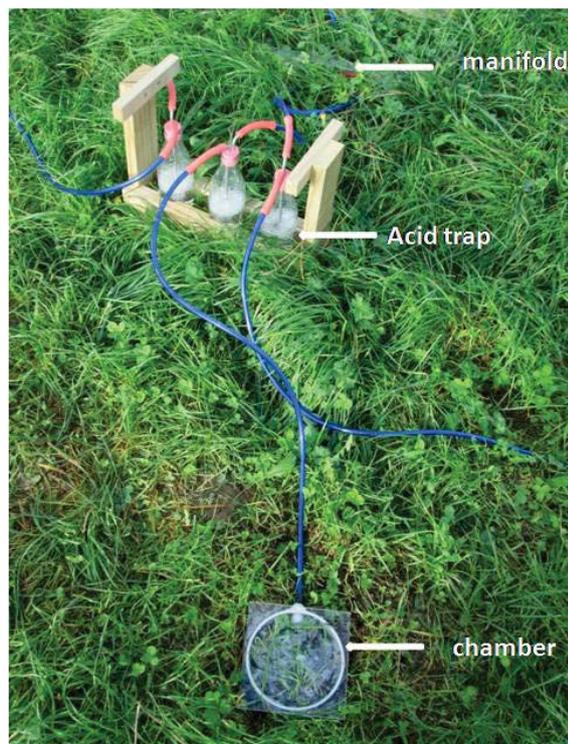


Figure 2.5. Gear used in the present study to measure NH_3 losses from urine applied to the chambers.

Figure 2.5 shows the dynamic chamber system used in the current study to measure NH_3 losses from urine patches. This method was also used previously by Soares et al. (2012) in a laboratory study. They measured NH_3 losses from soils after applying urea fertilizer along with urease and nitrification inhibitors. The volatilization chambers had a volume of 1.5 L, therefore the airflow rate used was 1.5 L min^{-1} . NH_3 losses from unamended urea ranged between 28% and 37% of the N applied. Recently, the dynamic chamber technique has been widely used in New Zealand mainly in field works (Dawar et al., 2011b; Saggar et al., 2013; Zaman & Blennerhassett, 2010; Zaman & Nguyen, 2012; Zaman et al., 2008; 2009; 2013). For example, Zaman and Nguyen (2012) studied the effect of applying a double inhibitor (urease and nitrification inhibitor) prior to urine deposition on NH_3 emission. They measured changes in NH_3 emissions by inserting a PVC chamber into the soil with a volume above the soil surface of 1.47 L and the air from each chamber was sucked at a flow rate of 6 L min^{-1} , giving an air exchange rate of 4.8 times/min. They found that NH_3 loss from urine patches and the reduction on NH_3 losses after applying the double inhibitor was similar to the results obtained in previous studies. The flow-through system inside the chamber allows NH_3 to be removed from the source and passed to the acid trap where NH_3 concentration is determined.

All the methods described have advantages and disadvantages which have been reported previously. Wind tunnels as well as dynamic chambers have a system to draw outside air through the chamber headspace, moving the NH_3 to the acid trap. However, due to the characteristics of the field-plot experiment developed in this thesis, dynamic chamber was used to measure NH_3 loss from urine patches. The choice of this method was because dynamic chamber can be used in small areas, allows the many replications of the treatments and it is an accurate method due to the air flow -through system inside the chamber.

The next section of the literature review focus on fertilizer urea and urine patches as the major sources of NH_3 emissions.

2.2.4 NH₃ volatilization from N fertilizer

Urea is the most common source of inorganic N used in agriculture worldwide, accounting for more than 50% of the total world fertilizer consumption (FAO, 2011). Its consumption is increasing due to its high N content (46%) and relatively low production cost (Saggar et al., 2013; Soares et al., 2012). However, its efficiency is affected by losses of N as NH₃. According to previous studies, these losses can account for up to 30% of the N applied. In a field experiment conducted by Sanz-Cobena et al. (2011), 31% of the N applied as urea at a rate of 100 kg N ha⁻¹ was emitted as NH₃. Similar results were reported by Soares et al. (2012) in a laboratory experiment. After applying urea at 300 kg N ha⁻¹, NH₃ losses ranged between 28% and 37% of the N applied. van der Weerden and Jarvis (1997) compared NH₃ losses between fertilizer urea, ammonium nitrate (NH₄NO₃) and calcium nitrate (Ca(NO₃)₂). They found that NH₃ volatilization from urea was much higher than volatilization from the other fertilizers (less than 1%). The reason of this difference is the increment in soil pH during urea hydrolysis process which favoured NH₃ volatilization.

Losses of NH₃ from urea fertilizer are affected not only by climatic and soil conditions, but also by agronomic practices and management of the fertilizer application. According to Black et al. (1985) when the rate of urea fertilizer increases so does NH₃ loss. They applied urea at 0, 15, 30, 60, 100 and 200 kg N ha⁻¹ and they found that NH₃ volatilization increased from 13% to 33% of the N applied when the application rates increased from 30 to 200 kg N ha⁻¹ due to the increase in soil surface pH. Similar results were obtained by Engel et al. (2011) who found NH₃ losses of 20.5% when 100 kg ha⁻¹ of urea was applied, they also observed losses as high as 44.1% of the N applied in a soil with a pH of 8.4. Similarly, Ryden et al. (1987) applied 70 and 100 kg N ha⁻¹ as urea and NH₃ volatilization ranged from 5.7% to 20.7% and 19.9% to 35.6%, respectively. However, when ammonium nitrate fertilizer was applied as the N source, the percentage of NH₃ losses sharply decreased and it was almost negligible (0- 2.5%). Smaller losses (4.74%) were

reported by Singh et al. (2013) from urea fertilizer at a rate of 100 kg N ha⁻¹. Table 2.2 summarizes the measured NH₃ losses from urea fertilizer.

Table 2. 2. *NH₃ volatilization from urea fertilizer*

Reference	Rate of urea (kg N ha ⁻¹)	NH ₃ -N losses (kg ha ⁻¹)	Season	% N lost as NH ₃
Singh et al. (2013)	100	4.9	Summer	4.74
Abalos et al. (2012)	120	8.1	Winter	6.7
Salazar et al. (2012)	100	7.7	Winter	8
		1.4	Winter	1
		12.2	Spring	12
		26.7	Spring	27
Sanz-Cobena et al. (2011)	100	31.4	Spring	31
Soares et al. (2012)	300	97.5	Summer	32.5
Zaman et al. (2008)	150	7.7	Spring	4.2
Sanz-Cobena et al. (2008)	170	17.3	Spring	10.1

In summary, NH₃ losses from fertilizer urea can be as high as 30% of the N applied during summer. NH₃ volatilization from urea is affected by different climatic and soil factors such as rainfall and temperature and it increases with an increase in the rate of urea applied.

2.2.5 NH₃ volatilization from urine patches

NH₃ volatilization from urine patches ranges between 7 and 14% of the N applied in urine (Menneer et al., 2008; Singh et al., 2013; Zaman & Blennerhassett, 2010; Zaman & Nguyen, 2012; Zaman et al., 2009; 2013) (Table 2.3). Sherlock et al. (2008) collated previous studies which used the aspirated chamber method and found that on average 15.9% of urine-N was lost as NH₃. However, using a mass budget method, Laubach et al. (2012) found NH₃ losses as high as 25.7% of N applied in urine, although overall emission rates were compatible with an annually-averaged

emission value of 10%. Similarly, Zaman et al. (2013) measured NH_3 emissions after applying urine at 600 kg N ha^{-1} during autumn and spring in two years. They observed that the average NH_3 losses in autumn and spring were 8.4% and 17% of the N applied, respectively. Smaller percentages were found by Zaman et al. (2009) and Pereira et al. (2013). Zaman et al. (2009) reported NH_3 losses of 8.2% and 3.6% of the N applied in urine during summer and spring, respectively. Pereira et al. (2013) conducted an incubation study where they measured NH_3 emissions from soils after applied 200 kg N ha^{-1} as urine. They reported NH_3 losses of 3.7% of the N applied which corresponded to $7.4 \text{ kg NH}_3\text{-N ha}^{-1}$ over 72 days. The difference in the percentage emitted as NH_3 in the experiments described reflects the difference in soil temperature, soil water content and rainfall. Another factor which accelerates NH_3 losses in urine is the hippuric acid which has a stimulatory effect on urea hydrolysis (Whitehead et al., 1989).

However, higher NH_3 losses from urine were reported by Lockyer and Whitehead (1990) and Petersen et al. (1998). For example, Petersen et al. (1998), using the wind tunnel method, found that NH_3 losses ranged from 3 to 52% of the total N content in urine, and the peak of maximum loss was reached 1-2 days after urine deposition. After 15 days of urine deposition, losses of NH_3 are almost negligible (Whitehead & Raistrick, 1991; Zaman & Blennerhassett, 2010).

Table 2. 3. *NH₃ volatilization from urine applied to pasture: New Zealand data*

Reference	Rate of urine (kg N ha ⁻¹)	% N lost as NH ₃	Season
Singh et al. (2013)	476	8.88	Summer
Zaman et al. (2013)	600	12	Autumn
		23	Spring
		4.9	Autumn
		10.9	Spring
Zaman et al. (2009)	600	3.6	Spring
		8.2	Summer
Zaman and Nguyen (2012)	600	7.0	Spring
		1.5	Autumn
Zaman and Blennerhassett (2010)	600	5	Autumn
		7	Spring
Menneer et al. (2008)	775	14.1	

To conclude, NH₃ losses from urine patches can account for up to 15% but higher percentages also have been reported by Laubach et al. (2012) and Petersen et al. (1998). As mentioned, NH₃ loss from urine patches is affected mainly by the temperature. However, water content has less influence on NH₃ loss from urine than fertilizer urea due to the water content in urine. The presence of hippuric acid in urine is another factor that influences NH₃ volatilization due to a stimulatory effect, increasing NH₃ loss in comparison with losses from fertilizer urea.

2.2.6 Technologies to reduce NH₃ emissions

Rapid hydrolysis of the urea component after the fertilizer application or urine deposition can lead to NH₃ volatilization. Some strategies have been recommended as mitigation tools to reduce NH₃ losses from these sources. One approach is the use of inhibitory compounds which delay the urea hydrolysis and hence these compounds limit the accumulation of NH₄⁺-N and the increase in soil pH which promote NH₃ volatilization (Dawar et al., 2010; 2011b; Henning et al., 2013; Sanz-Cobena et al., 2008; Singh et al., 2013; Zaman & Blennerhassett, 2010; Zaman & Nguyen, 2012; Zaman et al., 2009). Sanz-Cobena et al. (2011) recommended also as a mitigation technique the application of water immediately after urea application. This technique incorporates the unhydrolysed urea into the soil and reduces the amount of NH₄⁺-N ions susceptible to be transformed into NH₃. Recently, the addition of biochar into the soil has been identified as another mitigation tool (Mendes de Paiva et al., 2012; Taghizadeh-Toosi, 2011). Biochar improves soil cation exchange capacity and therefore increase the retention of NH₄⁺-N in soil. Mendes de Paiva et al. (2012) studied the effect of applying coated urea granules with *Eucalyptus grandis* charcoal on NH₃ losses. They observed that charcoal application reduced NH₃ emissions by 43% in comparison to uncoated urea. Urea coated with acidic compounds that are obtained by the oxidation of the charcoal decrease the rate of volatilization due to the acidic property, the high buffering capacity and CEC (Mendes de Paiva et al., 2012)

Due to the focus of this study on the impact of rainfall/irrigation and urease inhibitors in reducing NH₃ losses these mitigation strategies are discussed below.

Rainfall or irrigation

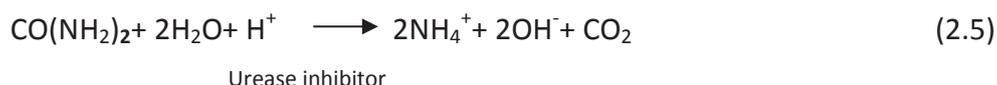
NH₃ losses from N fertilizers especially urea, can be reduced when the fertilizer is incorporated into the soil (Saggar et al., 2013; Sanz-Cobena et al., 2008; 2011). Two mechanisms have been proposed to incorporate the fertilizer: deep placement of the fertilizer or applying irrigation (Sanz-Cobena et al., 2011). In pasture systems,

the best option is the addition of water which moves the urea into soil, increasing the contact between $\text{NH}_4^+\text{-N}$ and the soil matrix and reducing the probability of NH_3 losses from the soil surface (Bouwmeester et al., 1985; Sanz-Cobena et al., 2011). For example, Sanz-Cobena et al. (2011) achieved a significant reduction on NH_3 losses of 77% and 89% after urea application when 7 and 14mm of irrigation were added respectively. Similarly, Saggar et al. (2013a) observed that NH_3 emissions were reduced more than 50% when both 5 and 10 mm were applied at eight hours after fertilizer urea application compared to irrigation applied after 48 hours. However, low amount of water may not be enough to move urea into the soil profile and may promote urea hydrolysis in the soil surface increasing the rate of NH_3 volatilization (Sanz-Cobena et al., 2011). To reduce NH_3 losses from dry soils, they suggested adding a greater volume of water, at least 10mm of water.

Therefore, the addition of water immediately after fertilizer urea application is a mitigation tool and reduction of NH_3 loss ranged from 50% to 89% depending on the amount of water added. However, an increment on NH_3 volatilization could be reported if the amount of water is small and does not move the unhydrolysed urea into the soil profile.

Urease inhibitors (UI)

Urease inhibitors are chemical compounds which slow the conversion of urea to $\text{NH}_4^+\text{-N}$ by inhibiting the activity of the urease enzyme (Eq. 2.5) (Watson & Miller, 1996; Watson et al., 1994a; 1994b; Zaman & Nguyen, 2012). This reduces the concentration of $\text{NH}_4^+\text{-N}$ into the soil and prevents the formation of zones with high pH, hence less $\text{NH}_4^+\text{-N}$ is available to be transformed into NH_3 which is susceptible to be volatilized (Saggar et al., 2013; Singh et al., 2013; Watson, 2005; Watson et al., 1994a; 1994b). Delaying urea hydrolysis will also increase the probability of rainfall or irrigation events occurring, so urea will be dispersed from the surface soil layer to deeper layers where it is taken up by plant roots (Dawar et al., 2011a; Zaman et al., 2009).



Several compounds have been tested as inhibitors of urease activity (Christianson et al., 1990; Creason et al., 1990; Watson, 1990). Some of these inhibitors alter the conformation of the active site and other groups react with the active site of the urease or key functional group in the molecule. These compounds have been classified as organic and inorganic compounds and also metal ions which complex the Ni ion in the enzyme, and other groups react with the sulfhydryl groups or the carboxylic acid group in the enzyme. According to Singh et al. (2008) the principal groups of urease inhibitors are hydroxamic acids and phosphorodiamidates.

Hydroxamic acids are metal chelates which bind to Ni ions, the active site of the urease enzyme (Manunza et al., 1999). Acetohydroxamic acid (AHA) is the best studied inhibitor in this group. The mechanism of binding of AHA has been proposed as a monodentate ligand with its carbonyl oxygen oriented towards one Ni atom (Manunza et al., 1999). However, Benini (2001) also proposed that there is a more stable complex structure, a bidentate structure where both Ni atoms are binding.

Phosphorodiamidates have been shown to be stronger inhibitors than AHA (Singh et al., 2008). nBTPT [N-(n-butyl) thiophosphorictriamide] known as Agrotain[®], a strong urease inhibitor, is classified as a phosphorodiamidate inhibitor which forms a bridge between the two Ni atoms in the enzyme. nBTPT is more stable than AHA inhibitors because nBTPT is linked to three points in the urease enzyme (two Ni atoms and one oxygen atom in the bridging carbamate group) (Manunza et al., 1999). Once nBTPT is applied to the soil, it is quickly converted into the oxygen analogue, nBTPTO, which is the actual inhibitor of the urease enzyme (Christianson et al., 1990; Creason et al., 1990).

According to Saggar et al. (2005), the ideal inhibitor for use in agricultural soils should:

- be specific to block an enzymatic reaction; for example an UI should block the urease enzyme
- remain close to the N compounds. In the case of UIs have to move with the urea which is not absorbed by soil
- not negatively affect other beneficial soil microorganisms and plants
- remain effective in the soil for weeks after urea/urine application into the soil.
- not be toxic to animals and humans at the level used to inhibit the urease
- be cost-effective to use.

Several data have been published describing the effectiveness of the Agrotain® reducing NH₃ losses from fertilizer urea. However, the effect of the UI on NH₃ losses from urine patches has not been widely studied and the majority of the data set published is from New Zealand.

The next section will describe the effect of the UI on NH₃ volatilization from urea fertilizer application and urine deposition.

Effect of nBTPT on NH₃ volatilization from urea and urine

It has been shown that N-(n-butyl) thiophosphoric triamid is the most effective and promising urease inhibitor, and can reduce NH₃ losses by more than 50% at low concentrations (Carmona et al., 1990; Saggar et al., 2013; Sherlock et al., 2008; Watson et al., 1994a; 1994b; 2008). According to Saggar et al. (2013b), UI can reduce NH₃ loss from urea and urine on average 44.7% and 53% respectively. The higher reduction of NH₃ from urine is due to the better interaction between urine and the UI. Agrotain® is formulated as a green clear liquid which contains 25% of the active ingredient nBTPT. The nBTPT is in a mixed solvent consisting of 10% by

weight of the N methyl-pyrrolidone with the balance consisting of non-hazardous solvent and inert ingredients (IMC-Agrico, 1997). In New Zealand, Sustain Green, Sustain Rapid S and Sustain FPA are the three products which contain the inhibitor. Sustain Green is granular urea coated with Agrotain® at 1L per tonne of urea and Sustain Rapid S is similar but also contains ammonium sulphate. Sustain FPA is manufactured by grinding Sustain into fine particles, then adding water to create a slurry. This product can then be sprayed on to pasture. According to the manufacturer, there are more than 1000 FPA particles where there would normally be just one of Sustain Green granule (<http://www.altum.co.nz>). Singh et al. (2013) studied the effect of applying different amended forms of urea at 100 kg N ha⁻¹ (Sustain Green and Sustain Rapid S) on NH₃ volatilization. They observed that 2.9 kg NH₃-N ha⁻¹ was emitted which accounted for a 42-48% reduction in NH₃ emissions compared to urea alone treatment. In a field experiment, Zaman et al. (2008), found a 45% and 48% of reduction of NH₃ losses when they applied Sustain Green and Agrotain® coated with sulphur (S) at 150 kg N ha⁻¹, respectively.

Many overseas published data have studied the effect of nBTPT on NH₃ losses from fertilizer urea. For example, Watson et al. (1990; 1994a; 1994b; 1998; 2008) have conducted several field and laboratory experiment. They observed that increasing the rate of nBTPT, the reduction of NH₃ emissions also increased. Watson et al. (1994b) observed that the percentage of inhibition was 50.4%, 82.8%, 89.0%, 96.5% and 97.0% when nBTPT was applied at 0.01, 0.05, 0.1, 0.25 and 0.5% w/w respectively. They also found that nBTPT also delayed the time at which the maximum NH₃ losses occurred, increasing the chance that a rainfall event occurs which moves the urea deeper into the soil. Watson et al. (2008) studied the effect of different rates of nBTPT on NH₃ losses (0, 0.01, 0.025, 0.05, 0.075 and 0.01% w/w) at three temperatures (5, 15 and 25°C) under four different soils. They showed that NH₃ losses were reduced by 61.2%, 69.9%, 74.2%, 79.2% and 79.8% for 0, 0.01, 0.025, 0.05, 0.075 and 0.01% nBTPT w/w for all soils and temperatures. They also concluded that commercially the benefit on NH₃ reductions using concentrations above 0.025% is not significantly important.

Although nBTPT is the most effective urease inhibitor, it is negatively affected by different soil factors such as clay, soil organic matter contents and temperature (Carmona et al., 1990; Gioacchini et al., 2002; Watson et al., 1994a; 2008). Carmona et al. (1990) observed that high concentrations of nBTPT were required when the inhibitor was applied to high temperature conditions and soils under plant residues due to a reduction on the effectiveness of the inhibitor. They showed that NH_3 losses were reduced by 81% when nBTPT was added at 0.01% at 18°C. However, applying the same nBTPT concentration at 32°C, NH_3 emission was reduced by only 42%. The reason for these differences could be that the increase in soil temperature causes a higher rate of urea hydrolysis than the conversion of nBTPT in its oxygen analogue. Therefore, under high temperature, nBTPT should be applied at greater concentration. Similarly, a field trial was conducted by Rawluk et al. (2001) to study the effect of applying nBTPT at four different rates 0, 0.05, 0.1 and 0.15% to a single urea rate of 100 kg N ha⁻¹ under two different soil (clay vs sandy soils). In the clay loam soil, NH_3 losses were reduced by 85% under cool temperature and 75% under warm temperature. In the sandy soil, 81% of reduction was achieved under cool temperature but only 37% in warm temperature. Therefore, it is clear that the activity of the urease enzyme is influenced by temperature due to an increase in urease activity under warm conditions. However, Gioacchini et al. (2002) observed that the UI was much more efficient under sandy loam soil than in a clay loam soil. The diffusion of urea in light textured soil is greater than in heavy soil which protects the unhydrolysed urea from the volatilization process in the soil surface. As urease activity is also influenced by soil organic matter, and this is higher under the clay soil, urea is therefore rapidly hydrolyzed. On the other hand, clay soil presents high CEC and therefore less NH_4^+ -N is available to be transformed into NH_3 .

Several studies have been conducted on NH_3 losses using Agrotain® along with urea; however, only few studies have been conducted applying Agrotain® with urine and they have been undertaken mainly in New Zealand (Table 2.4 and 2.5). There is only one international research developed by Pereira et al. (2013) who

studied the effect of applying Agrotain® to urine. They conducted a laboratory experiment adding nBTPT at two concentrations (0.1 and 1.0%) which were mixed with urine at 200 kg N ha⁻¹ and then applied to the soil. Total NH₃ emissions were reduced by 48% compared to urine alone, and there was no effect of the nBTPT concentration.

In New Zealand, there are a few laboratory and field-plot experiments which studied the effect of adding nBTPT to urine patches and are summarized in Table 2.5. However, in all these experiments UI was mixed with urine prior to the application to the soil which is not a situation that could be carried out by farmers. For example, Menneer et al. (2008) carried out a field lysimeter experiment where they observed a reduction of NH₃ loss of 64% when Agrotain® was applied to urine patches (775 kg N ha⁻¹). Similarly, in another field experiment, Zaman et al. (2009) showed that the application of Agrotain® with urine to pasture soil reduced NH₃ volatilization by 29%, 93% and 31% in autumn, spring and summer respectively. A reduction of 22.4% on NH₃ losses from urine patches after adding the UI was achieved by Singh et al. (2013). However, in some previous work Agrotain® was applied along with a nitrification inhibitor to also reduce nitrous oxide emissions. For example, Zaman et al. (2009) applied a double inhibitor (UI + DCD); (DI), and NH₃ losses decreased to 14%, 78% and 9% in autumn, spring and summer, respectively. Zaman and Blennerhassett (2010) found that the application of the DI at a ratio of 1:7 reduced NH₃ losses by 48% in autumn and 51% in spring. More recently, Zaman and Nguyen (2012) showed a reduction of 38% and 28% in NH₃ losses from urine when the DI was added 5 days before urine application compared to urine alone (600 kg N ha⁻¹) in autumn and spring, respectively.

Table 2. 4. NH_3 reductions with application of nBTPT from urea fertilizer in New Zealand and overseas

Inhibitors	Rate of UI	Rate of N in urea ($kg\ N\ ha^{-1}$)	% of reduction	Ref
Sustain Green/	1L Agrotain® t^{-1} urea	100	42-48 (summer)	Sing
Sustain Rapid S	1L Agrotain® t^{-1} urea	100		
Sustain	0.025% w/w	60	45 (summer)	Sag
	0.025% w/w	100	65-69 (spring)	Dav
Agrotain®	0.025% w/w	150	45 (spring)	Zan
Sustain Rapid S	0.025% w/w		48 (spring)	
Agrotain®	0.01%	100	61.2	Wa
	0.025%		69.9	
	0.05%		74.2	
	0.075%		79.2	
	0.10%		79.8	
Agrotain®	0.10%	100	83	Wa
Agrotain®	0.01%	100	28.4	Wa
	0.058%		67.9	
	0.28%		87.1	
Agrotain®	0.01%	100	52	Wa
	0.05%		83	
	0.1%		88	
	0.25%		96	
	0.5%		97	

Table 2. 5. *NH₃ reductions with application of nBTPT from animal urine in New Zealand*

Inhibitors	Rate of UI	Rate of N in urine (kg N ha ⁻¹)	% of reduction	Season
Agrotain®	1L 460kg N ha ⁻¹	476	22.4	Glasshouse
Agrotain® & DCD	1:7 w/w	600	44	Autumn
			55	
Agrotain® & DCD	1:7 L kg ha ⁻¹	600	38	Spring
			28	Spring
Agrotain®	3L ha ⁻¹	600	29	Autumn
			93	Spring
			31	Summer
Agrotain® & DCD	1:7::L ha ⁻¹ :kg ha ⁻¹	600	48	Autumn
			51	Spring

In a recent New Zealand publication, Saggar et al. (2013a) concluded that the efficiency of fertilizer urea can be considerably improved either by treating it with Agrotain® or by applying 5-10 mm of irrigation/rainfall to suppress NH₃ emission. In a situation where irrigation is not possible and 5-10 mm of rainfall is unlikely to occur within 8 to 24 hours of urea application, urea treated with Agrotain® can be recommended to reduce NH₃ emission.

To summarize, several experiments have been conducted to study the effectiveness of UI on NH₃ losses from fertilizer urea. On average, when nBTPT is applied to urea, NH₃ volatilization is reduced by 45%. However, only a few studies describe the effectiveness of UI on NH₃ volatilization from urine. The majority of these experiments have been conducted in New Zealand and all of them mixed the nBTPT with urine before the application, therefore the reduction percentage obtained from these researches are only an approximation of the real situation. In these studies, after applying the UI, NH₃ loss is reduced by 53%. Therefore, an aspect that needs to be addressed is the method of application of the UI to urine patches and the timing of application.

2.3 Conclusions

1. Fertilizer urea and urine are the major contributors to NH₃ losses in grazed pasture soils. It has been estimated that NH₃ losses from urea and urine can account for up to 30%, and from 7% to 14% of applied N, respectively. Ammonia volatilization has both economic and environmental implications. The negative impacts of NH₃ in the environment are environmental pollution, soil acidification and eutrophication of water bodies.
2. NH₃ losses are influenced by climatic and soil factors at the time of the deposition of urine or application of fertilizer urea. Wind speed, rainfall, temperature, pH, CEC, and soil water content are the climatic and soil factors which influence NH₃ volatilization.

3. Different technologies have been developed to reduce NH_3 losses from fertilizer urea and urine. It has been showed that urease inhibitor effectively reduced NH_3 volatilization from those N sources. UI can reduce NH_3 losses on average by 44.7% and 53% when is applied to fertilizer urea and urine, respectively.
4. These high reduction percentages have been obtained in laboratory and field-plot experiments in which the UI is mixed with urine before the application, increasing the effectiveness of the inhibitor on the urease activity, and this is not a likely situation under field conditions. The inhibitor should be applied before or after the grazing day. Therefore, there is no research which has considered the effect on NH_3 losses when Agrotain® is sprayed singularly before, on the same day or after the grazing day.

Chapter 3

Effect of application times of urease inhibitor (Agrotain®) on NH₃ emissions from urine patches

3.1 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 from 50 to 200 g m⁻² in urine patches (Bolan et al., 2004; Saggar et al., 2009), which is well above the N requirements of pastures. Approximately 80% of urine N is in the form of urea (Bolan et al., 2004; Zaman et al., 2007). The urea N in urine is rapidly hydrolysed by the urease enzyme in soil to ammonium (NH₄⁺-N). Under alkaline conditions, ammonium is converted to NH₃, which is volatilized. Ammonia emission can cause eutrophication and acidification of water and soils where it is deposited and is also a second source of N₂O, which is a greenhouse gas. Loss of N as NH₃ gas 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). Higher figures have also been reported in New Zealand: Laubach et al. (2012) found NH₃ losses as high as 25.7% of N applied in urine. 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.

Many approaches have been suggested to mitigate the environmental impacts of N losses from urine patches. Among these, urease inhibitors (UI) have been used as a mitigation tool to decrease the rate of NH₃ volatilization from fertilizer urea and animal urine deposition (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). UI are chemical compounds that control the conversion of urea ((NH₂)₂CO) into NH₄⁺-N making less NH₄⁺-N available to be converted into NH₃, which is susceptible to volatilization (Bolan et al., 2004). Urease inhibitor (UI) nBTPT [N-(n-butyl) thiophosphoric triamide], sold under the trade name Agrotain® and applied at 0.025% w/w to urine or fertilizer urea, has been shown to reduce NH₃ emissions from New Zealand pasture soils (Singh et al., 2013; Zaman & Nguyen, 2012; Zaman et al., 2008; 2009).

The value of UI for mitigating NH₃ 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 NH₃ is emitted from urea-N (Manunza et al., 1999).

Only a limited number of published data sets are available that describe the effectiveness of UI in reducing NH₃ losses from urine in a grazed pasture system. The method of UI application in these studies is flawed as all experiments were conducted by mixing UI with urine before its application to soil. As yet no research has studied the effect on NH₃ emissions when Agrotain® is sprayed over the grazed pasture. Therefore, based on the existing data, it is not possible to estimate accurately the effect of UI on reductions in the NH₃ lost from animal urine-N deposited during grazing.

It is impractical for the farmers to apply UI to each paddock on the grazing day, but Agrotain® can be applied within a week of grazing. Thus, the emissions reduction and the value of Fra_{C_GASM} (fraction of total N excretion emitted as NO_x and NH₃) will vary considerably, depending on the time of UI application following excretal deposition by grazing animals. No study has investigated the effect of varying the time of UI application on reduction in NH₃ emission from urine deposition in the field. There is only one study in New Zealand that has investigated the application of nitrogen inhibitors (urease inhibitor+ nitrification inhibitor) before and after urine deposition (Zaman & Nguyen, 2012).

The main objective of this experiment therefore was to study the inhibitor effect of Agrotain® on NH₃ losses from urine deposition when it is sprayed onto a pasture soil before or after the deposition of animal urine.

The specific objectives of this experiment were to study:

- the effect of time of Agrotain® application before (5 and 3 days), on the same day and after (1, 3, 5 days) urine deposition on reduction in ammonia emission
- the effect of Agrotain® on the transformations of mineral N
- changes in soil pH with the addition of Agrotain®

3.2 Materials and methods

3.2.1 Site description

The experiment was set up on dairy farm # 4 at Massey University, Palmerston North. The site pasture was a mix of ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). The soil is Tokomaru silt loam classified as Pallic soil. These soils have compact clay loam with compact subsoil, and poor natural drainage with a tendency to dry out in summer (Hewitt, 1998). A-horizon consists of a weak to moderately developed brown silt loam. B-horizon combines a weakly developed, grey, strongly mottled, clay loam and a highly compacted, weakly developed pale gray, silt loam fragipan C-Horizon. Physical and chemical characteristics of the soil are shown in Table 3.1.

Table 3. 1. *Physical and Chemical Characteristics of the Tokomaru Silt Loam*

	pH	Bulk Density (g cm ⁻³)	Total C (%)	Total N (%)	NH ₄ ⁺ -N (mg/kg soil)	NO ₃ ⁻ -N (mg/kg soil)
Tokomaru silt loam	5.8	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, to minimize the effect of previous dung and urine patches, and to reduce the inherent variability.

3.2.2 Experimental design

The experiment was laid out in a completely randomized block design with eight treatments, replicated 6 times, resulting in 48 sampling plots for soil and NH₃ volatilization measurements. Treatments comprised 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) (Table 3.2).

Agrotain® was applied to the soil surface as a water-based solution and the chosen application rate of 0.025%w/w has been shown to be the most effective in reducing NH₃ emissions (Watson et al., 2008). The application rate of Agrotain® to the field was 800 L ha⁻¹ which is the DCD application rate commonly used. This means that 20 mL and 1.41 mL of the dilute solution were sprayed to the plot and chamber area, respectively. It was sprayed above the pasture in treatments T2, T3, T4, T5, T6 and T7 (Table 3.2). The experiment also had an untreated control.

The experiment comprised two areas: the soil plots and the area of the volatilization chambers (Fig. 3. 1) where the same treatments were applied at the same time. In each soil plot (0.5 m x 0.5 m separated by a 0.5 m buffer), soil samples were taken and ammonia was sampled from the acid trap. The sampled soil was analysed for soil mineral-N (NH₄⁺-N and NO₃⁻-N) and soil pH described below.

On day 1 after treatment application, NH₃ emissions from U, UAgr1, UAgr3 and UAgr5 were essentially similar and were averaged.

The pasture was mown in all treatment plots before the application of urine on day 0 to mimic the grazing effect and stimulate pasture growth. In UAgr0, the application of urine was followed by the inhibitor.

As chambers and soil plots were covered during the first week to avoid any rainfall events, rainfall did not influence NH_3 losses during the first week.



Figure 3.1. Experimental set up including chambers and soil plots

3.2.3 Urine collection and analyses

Urine was collected from Friesian cows while they were milking. After collection, the urine was transferred to 20-L containers, and stored below 4°C to avoid urea hydrolysis until the application in the field. Four urine samples of 100 mL were used for the analyses of total N and C, $\text{NH}_4^+\text{-N}$, urea-N and pH. The urine collected in different containers was transferred to a 200-L container and thoroughly mixed before application. Urine was applied to the chambers and soil plots with a watering can.

Urea-N analysis was done by AgResearch laboratory. The method used was ‘Diacetyl Monoxime’. Briefly, it has been shown that urea can be extracted from soils by 2M KCl. Urea is quantitatively determined by a colorimetric method in which red color is formed when the extraction is heated along with diacetyl monoxime (DMA),

thiosemicarbazide (TSC), sulphuric and orthophosphoric acid and ferric chloride hexahydrate (Mulvaney & Bremner, 1979; Wybenga et al., 1971).

Table 3. 2. *Description of the Treatments*

	Treatments	Replicates	Chamber/Plot numbers
Control	T0	6	1-9-17-25-33-41
Urine only	T1	6	2-10-18-26-34-42
Urine+ Agrotain® day -5	T2	6	3-11-19-27-35-43
Urine+ Agrotain® day -3	T3	6	4-12-20-28-36-44
Urine+ Agrotain® day 0	T4	6	5-13-21-29-37-45
Urine+ Agrotain® day +1	T5	6	6-14-22-30-38-46
Urine+ Agrotain® day +3	T6	6	7-15-23-31-39-47
Urine+ Agrotain® day +5	T7	6	8-16-24-32-40-48
TOTAL		48	

3.2.4 Ammonia emission measurement

Ammonia volatilization in this experiment was measured using the dynamic chamber method (Kissel et al., 1977) that comprised 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 transparent tops (to allow photosynthesis) were inserted into the soil to a depth of 0.01 m, giving a headspace volume of 0.5 m³. The chamber had a vent on its vertical surface connected to an acid trap (250mL, 0.025 M H₂SO₄) using a tube 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 (Fig. 2.5). Sub-samples of the H₂SO₄ solution in the acid traps were analysed for NH₄⁺-N concentrations, as above. Samples were taken every day for the first 12 days and then on days 15, 18, 21, 24, 27 and 30.

3.2.5 Analysis

3.2.5.1 Soil sampling

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

3.2.5.2 Soil analyses

Mineral N and pH

Before soil analysis, soil samples were sieved (2mm) to remove plant roots. A sub-sample of 5 g of field moist soil was extracted with 50 mL of 2M potassium chloride (KCl) solution by shaking for 1hour. The extract was analysed for nitrate (NO_3^- -N) and ammonium (NH_4^+ -N) concentrations colorimetrically using Technicon AutoAnalyser (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 wet soil sample per plot was weighed and then dried at 105°C for 24 hours. After drying, these samples were again weighed and the gravimetric water content was calculated.

3.2.6 Statistical analysis

Gaseous emissions and soil parameters (mineral N and soil pH) were analysed 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, 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 heterogeneous 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 NH₃ volatilization, and days 1, 3, 5, 9, 12, 15, 18, 21, 24, and 30 in soil parameters analyses. Differences between treatment means were assessed using a one way analysis of variance (ANOVA). Fisher's test was used to determine if those differences were significant or not with 95% confidence limits ($P < 0.05$).

3.3 Results

3.3.1 Urine composition

The chemical composition of urine applied to the treatments is given in Table 3.3. It had a pH of 7.6 ± 0.40 and a total C concentration of $11.50 \pm 0.16 \text{ g L}^{-1}$. The total N concentration was $4.95 \text{ g L}^{-1} \pm 0.22$, of which $3.65 \pm 0.22 \text{ g L}^{-1}$ was urea component (73.7%) (Table 3.3).

Table 3. 3. *Chemical Composition of Urine*

Urine chemical composition	Values
$\text{NH}_4^+\text{-N}$ (mg/L)	$267 \text{ mg L}^{-1} \pm 55.08$
Urea-N (g/L)	$3.65 \text{ g L}^{-1} \pm 0.36$
Total N	$4.95 \text{ g L}^{-1} \pm 0.22$
Total C	$11.5 \text{ g L}^{-1} \pm 0.16$
pH	7.6 ± 0.40

Data are mean \pm sd (n = 4).

3.3.2 Meteorological data

The mean daily temperature during the experiment and over the last 20 years is reported in Figure 3.2a. The average daily temperature during the experiment ranged between 8.4°C and 18.9°C , and for the first 3 weeks of the experimental period it was higher than the 20 year average temperatures.

During the experimental period a total of 58 mm rainfall was recorded (Fig. 3.2b), 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 3.2b). Almost no rain occurred after day 11.

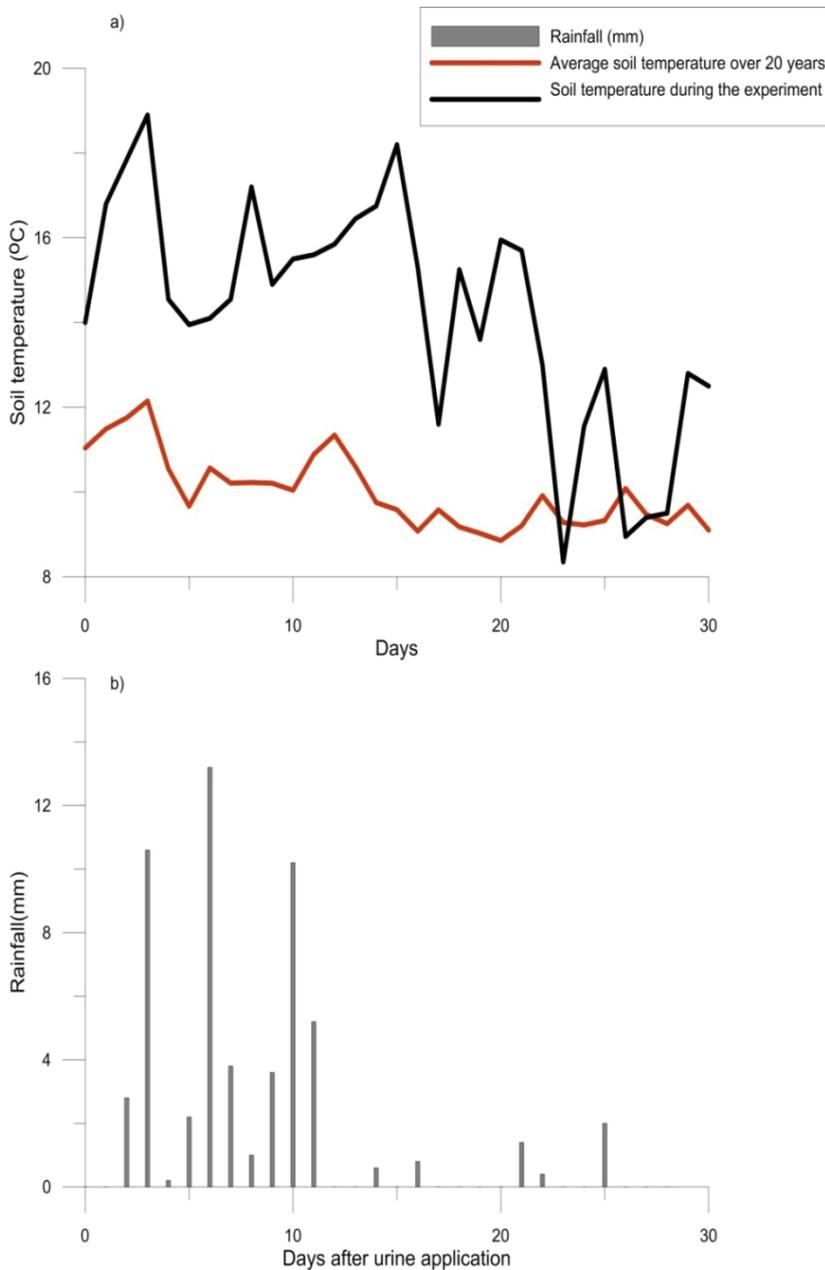


Figure 3. 2. Meteorological data obtained from a meteorological station near the site of the experiment over the experimental period.

During the measurement period a comparison was made between soil temperature inside and outside of the chambers to avoid the effect of temperature inside and outside 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. There was significant linear relationship between temperature inside and outside the chambers ($r^2 = 0.971$) (Fig. 3.3). Similar temperature inside and 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.

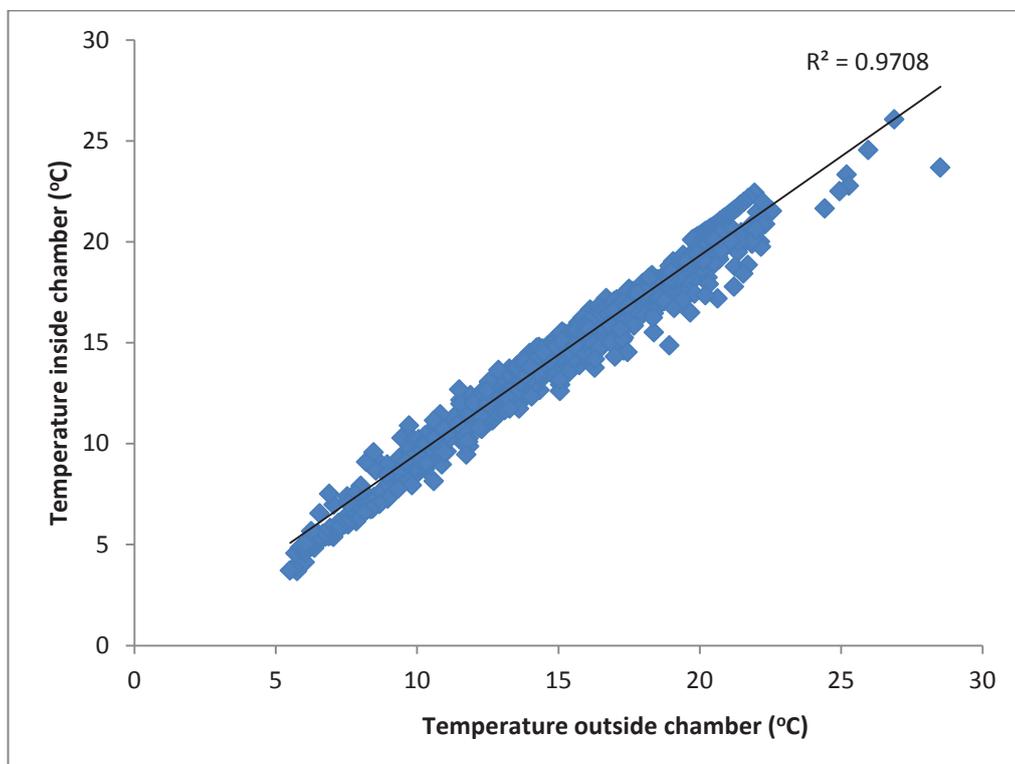


Figure 3. 3. Relationship between temperature inside and outside the chamber during part of the experiment.

3.3.3 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 during the remaining measurements (Figs 3.4 and 3.5). The emissions reached the background levels within 15 days.

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 $36.31 \pm 2.18 \text{ kg NH}_3\text{-N ha}^{-1}$ (mean \pm standard deviation) was measured within 24 hours from urine-only treatments (urine, UAgr1, UAgr3, and UAgr5), which did not receive Agrotain® at the time of urine application (Fig. 3.5). In the treatments that did not receive Agrotain® before urine application, 46.5% of the urine-N was lost as NH_3 during the first 24 hours, and 72% in 3 days. In the treatments (UAgr0, UAgr-3, and UAgr-5) where Agrotain® was applied the same day or 3 and 5 days before urine application (Fig. 3.4), NH_3 losses were significantly reduced ($P < 0.0001$) within 24 hours, compared with the urine-only, UAgr1, UAgr3, and UAgr5 treatments. The amount of NH_3 emitted during the first 24 hours was 28.82 ± 2.91 , 27.77 ± 3.12 , and $23.05 \pm 2.32 \text{ kg NH}_3\text{-N ha}^{-1} \text{ day}^{-1}$, for UAgr0, UAgr-3, and UAgr-5, respectively. This resulted in a reduction of emissions during first 24 hours by $20.7 \pm 8.0\%$, $23.5 \pm 8.6\%$, and $36.5 \pm 6.4\%$, respectively. Those treatments that received Agrotain® before urine application were significantly different till day 2. On day 1, NH_3 losses from UAgr-5 were significantly lower than losses from UAgr-3 and UAgr0 ($P < 0.0001$); however, NH_3 volatilization between UAgr-3 and UAgr0 was not significantly different ($P < 0.37$). On day 2, only UAgr-5 and UAgr0 were significantly different ($P < 0.0001$). Subsequent NH_3 losses from all urine treatments with or without Agrotain® were nearly the same and remained significantly higher than the Control treatment until day 10 (Figs 3.4 and 3.5). Losses of NH_3 were negligible from all treatments after 15 days of urine application.

NH_3 emissions in the Control treatment were very low and ranged from 0.01 to $0.28 \text{ kg NH}_3\text{-N ha}^{-1}$ during the 30 days of the experiment. Table 3.4 shows the total NH_3 losses and the reduction percentages for each treatment. Cumulative NH_3 loss

for each treatment is shown in Figure 3.6. Total NH₃ losses in the U, UAgr1, UAgr3, and UAgr5 treatments did not differ and over 30 days averaged 77.12 kg N ha⁻¹, giving an average emission factor (EF) of 14.4% in these treatments. The cumulative NH₃ emissions for the UAgr-5, UAgr-3, and UAgr0 treatments recorded average reductions of 27.3 ± 5.5%, 17.5 ± 11.1%, and 9.6 ± 7.4%, corresponding to EF values of 10.2, 11.8, and 13.1%, respectively. Cumulative NH₃ loss from UAgr-5 was significantly different ($P < 0.0001$) from urine, UAgr0, UAgr1, UAgr3 and UAgr5. Similar, at the end of the experiment NH₃ emission from UAgr-3 was significantly lower ($P < 0.013$) than urine, UAgr1, UAgr3 and UAgr5. Figure 3.7 summarizes the cumulative NH₃ losses for all treatments.

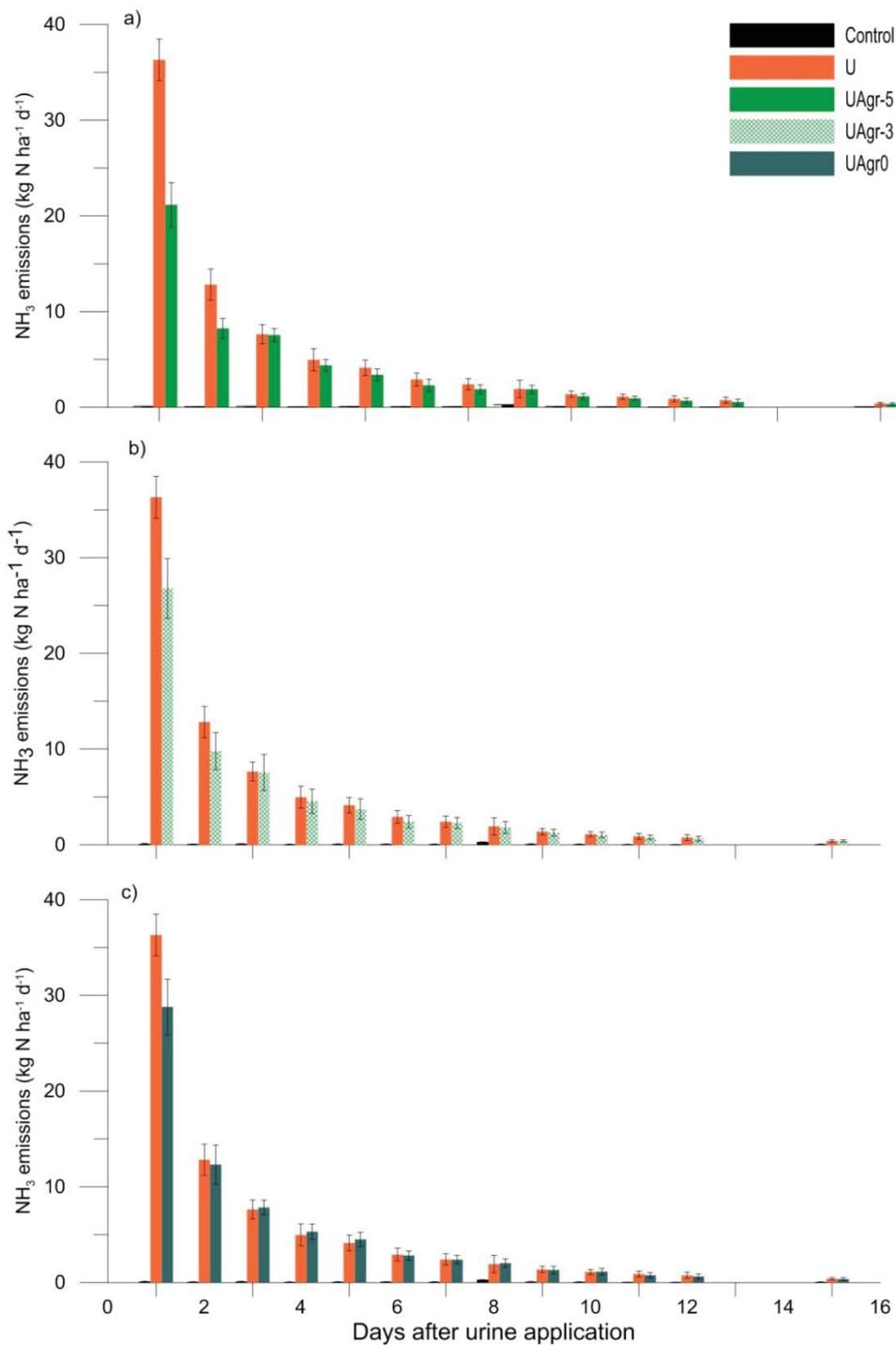


Figure 3.4. Daily ammonia emissions from the treatments where Agrotain[®] was applied before urine deposition in autumn, a) Volatilization from UAgr-5, b) Volatilization from UAgr-3, and c) Volatilization from UAgr0. Data are mean \pm sd ($n = 6$).

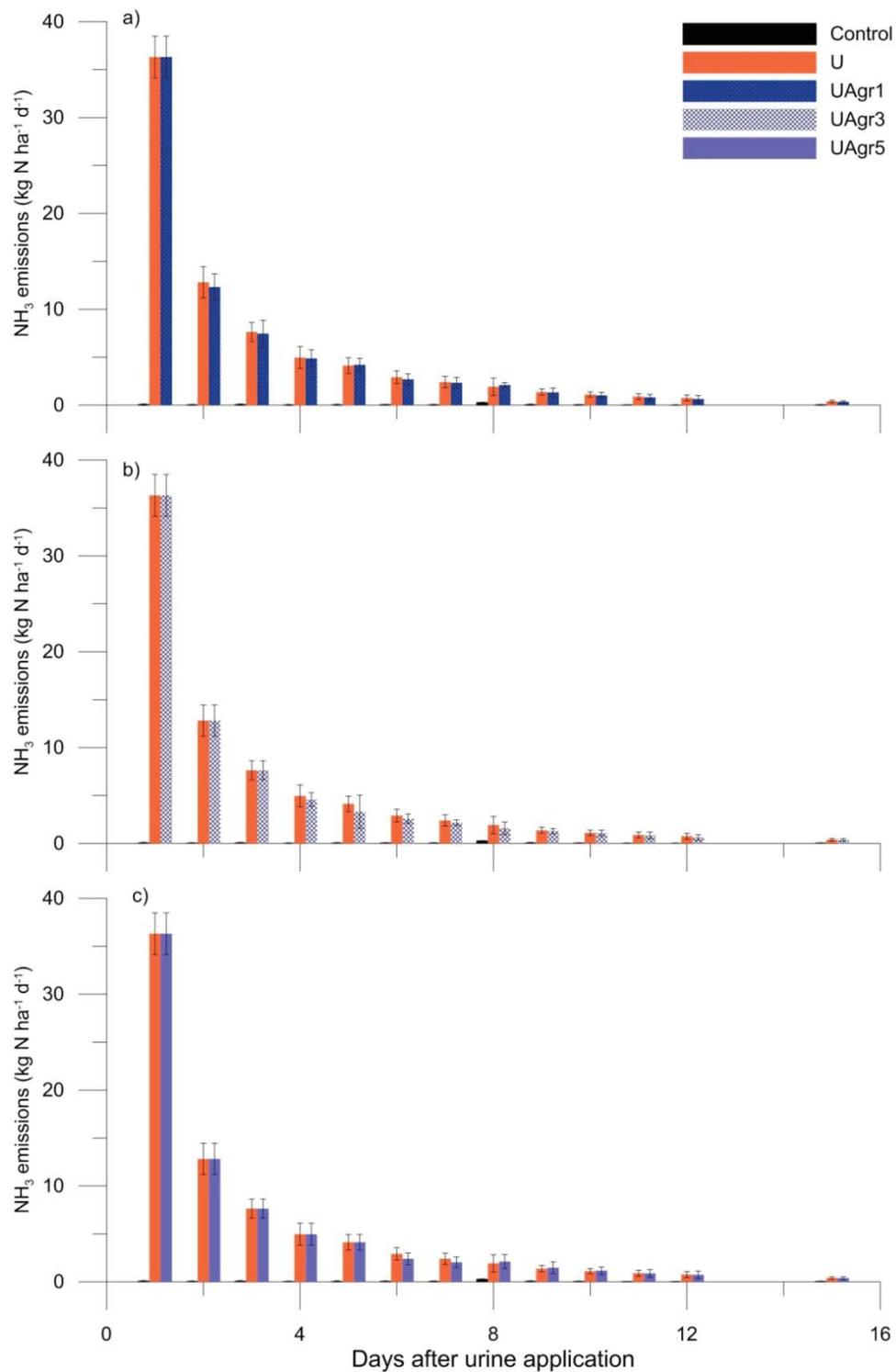


Figure 3. 5. Daily ammonia emissions from the treatments where Agrotain® was applied after urine deposition in autumn, a) Volatilization from UAgr1, b) Volatilization from UAgr3, and c) Volatilization from UAgr5. Data are mean \pm sd ($n = 6$).

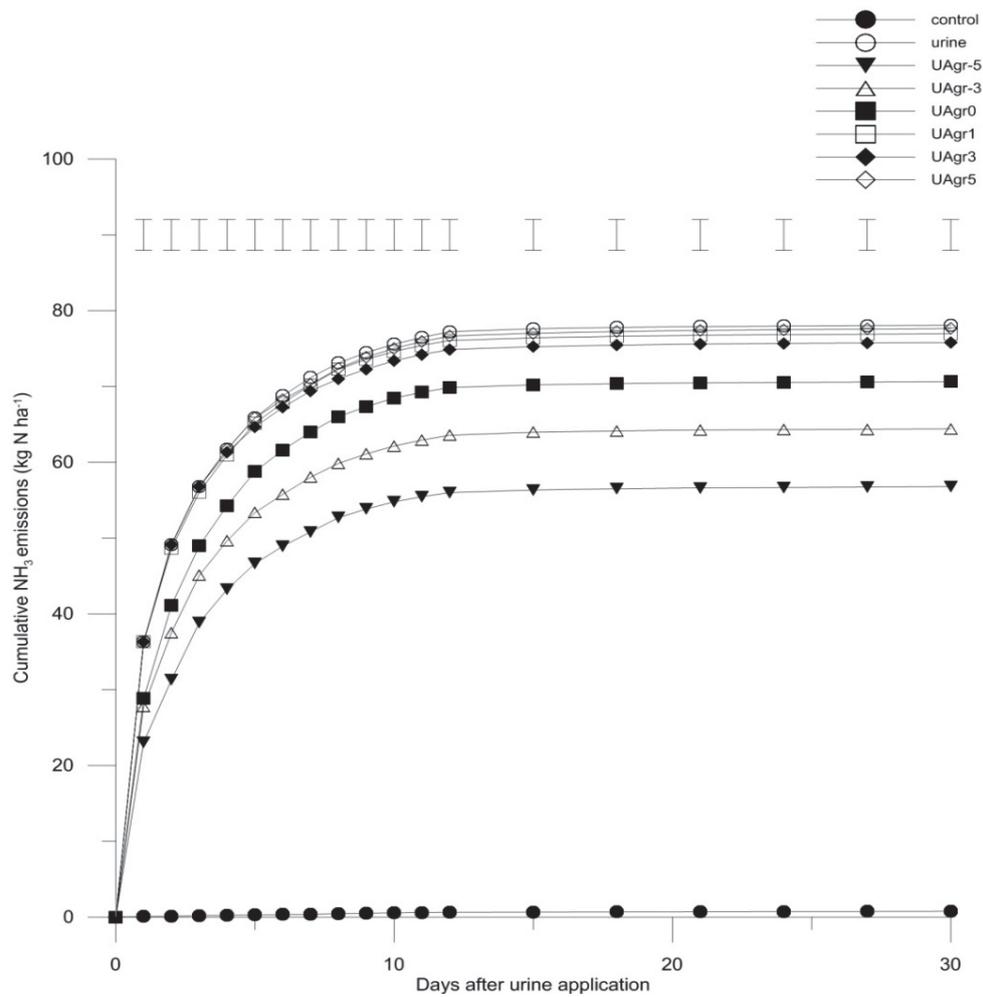


Figure 3.6. Cumulative NH₃ emissions following urine deposition before, on the same day, and after Agrotain® application in autumn. Data are mean ± sd (*n* = 6).

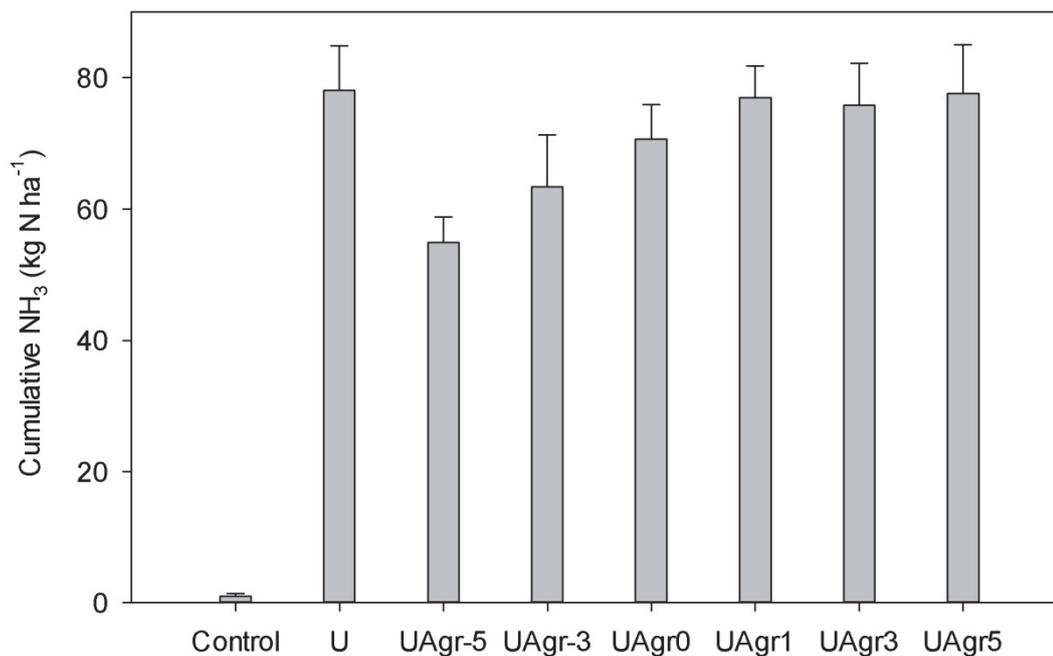


Figure 3.7. Bar graph of cumulative NH₃ emissions following urine deposition before, on the same day and after Agrotain® application in autumn. Data are mean+sd ($n = 6$).

Table 3. 4. Total NH₃ Losses by Treatment with urine applied at 530 kg N ha⁻¹

Treatment	Total NH ₃ emissions ± sd (kg N ha ⁻¹)	Reduction % *
Control	1.02	-
Urine	78.08 ± 7.44	-
UAgr-5	56.79 ± 4.30	27.3
UAgr-3	64.40 ± 8.64	17.5
UAgr0	70.62 ± 5.7	9.6
UAgr1	76.96 ± 5.31	1.4
UAgr3	75.79 ± 6.60	2.9
UAgr5	77.65 ± 8.15	0.6

*Reduction % calculated from urine only

3.3.4 Soil results

3.3.4.1 Soil pH

Application of urine resulted in a significant increase ($P < 0.0001$) in surface (0–10 cm depth) soil pH in all treatments in comparison with the Control treatment (Fig. 3.8). 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. UAgr1 exhibited a second peak on day 5. After this initial rise, soil pH dropped gradually in these treatments until the end of the experiment with values of 5.80, 5.47, 5.68, and 5.60, for the urine, UAgr1, UAgr3, and UAgr5 treatments respectively. 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. At the end of the experiment the values reached were 5.58, 5.54, and 5.59 for UAgr-5, UAgr-3, and UAgr0, respectively.

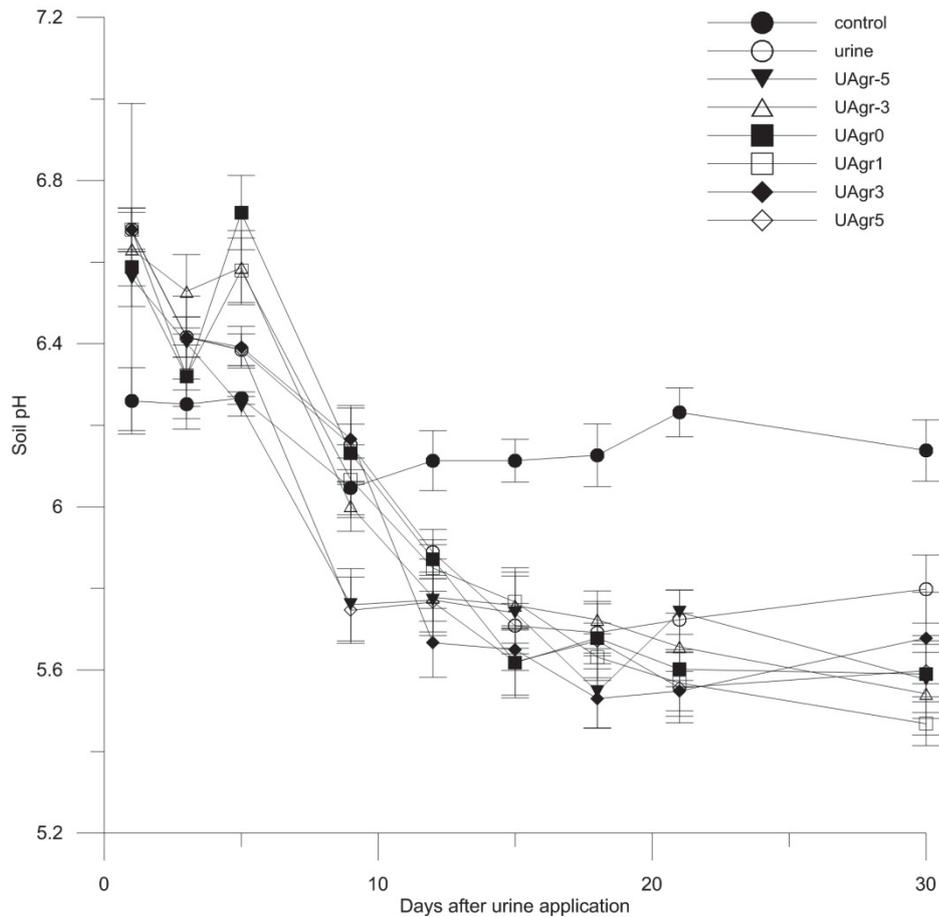


Figure 3. 8. Soil pH at 0–10 cm depth following ruminant urine deposition before, on the same day, and after Agrotain® application in autumn. Data are mean \pm sd ($n = 6$).

3.3.4.2 Mineral N

Inorganic N concentration at soil surface increased significantly ($P < 0.0001$) with urine deposition compared with the Control treatment. Soil NH_4^+ -N concentration sharply increased soon after urine addition compared with the Control treatment, followed by a subsequent decline during the remaining measurements. Soil NH_4^+ -N concentration in all treatments was close to background level after 21 days of the experiment (Fig. 3.9a).

In urine, UAgr1, UAgr3, and UAgr5 treatments, exchangeable NH_4^+ -N in the top soil layer reached a maximum value of $305.32 \pm 9.35 \text{ mg NH}_4^+\text{-N kg}^{-1}$ of soil after 24 hours.

UAgr1 and UAgr3 treatments exhibited a second peak of 241.11 ± 11.79 and 229.51 ± 10.22 mg NH_4^+ -N kg^{-1} of soil on day 5 and 9, respectively.

Application of Agrotain® before urine deposition (UAgr-3 and UAgr-5) was effective in significantly ($P < 0.0001$) reducing the concentration of NH_4^+ -N compared with 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 hours, NH_4^+ -N concentration was 207.19 ± 12.89 , 213.10 ± 18.03 , and 226.34 ± 109.29 mg NH_4^+ -N kg^{-1} of soil in UAgr-5, UAgr-3, and UAgr0, respectively. UAgr-5 treatment exhibited a second peak on day 5 of 203.01 ± 11.37 mg NH_4^+ -N kg^{-1} of soil. In UAgr0, the highest soil NH_4^+ -N concentration of 236.01 ± 9.95 mg NH_4^+ -N kg^{-1} of soil was on day 3, and it remained high until day 9. After that, it sharply decreased.

After day 9, soil NH_4^+ -N concentrations in all treatments gradually decreased until day 21, when all treatments almost reached background levels (14.14 ± 0.88 mg NH_4^+ -N kg^{-1} of soil). Soil NH_4^+ -N concentration in control plots ranged between 13.08 ± 0.95 and 62.62 ± 7.28 mg NH_4^+ -N kg^{-1} of soil during the experiment.

Soil NO_3^- -N concentration rose with a concomitant decreased in soil NH_4^+ -N. Figure 3.9b shows the changes in soil NO_3^- -N concentration in all treatments. No significant differences in soil NO_3^- -N concentration were observed between treatments within the experiment. Treatments reached the maximum values at the end of the experiment, except for Control plots and UAgr-5. In the Control treatment soil NO_3^- -N concentration varied from 3.33 ± 1.27 to 7.46 ± 1.02 mg NO_3^- -N kg^{-1} of soil. At the end of the experiment NO_3^- -N concentration ranged from 114.36 ± 7.78 mg NO_3^- -N kg^{-1} of soil in UAgr-5 to 213.81 ± 10.39 mg NO_3^- -N kg^{-1} of soil in the urine treatment.

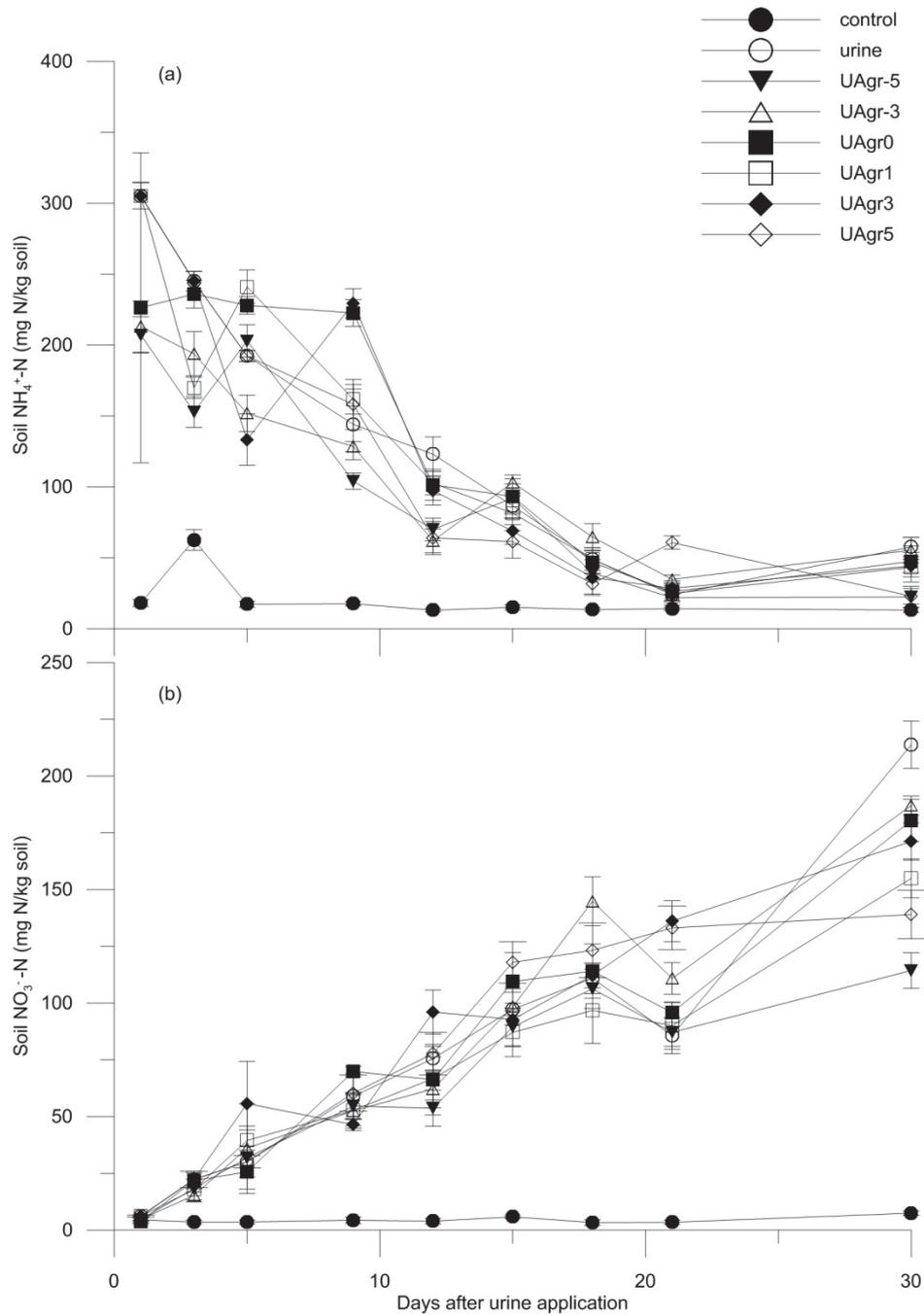


Figure 3. 9. Soil mineral N concentrations at 0–10 cm depth for (a) NH_4^+ -N and (b) NO_3^- -N, following ruminant urine deposition before, on the same day, and after Agrotain[®] application in autumn. Data are mean \pm sd ($n = 6$).

3.4 Discussion

This study investigated the effect of spraying the UI (Agrotain®) up to 5 days before or after urine application on NH₃ emission and N transformations. This study differs from other New Zealand studies on NH₃ losses from urine patches where previous studies mixed UI with urine before application (Menneer et al., 2008; Singh et al., 2013; Zaman & Blennerhassett, 2010; Zaman & Nguyen, 2012; Zaman et al., 2009; 2013).

The results indicate that: i) spraying the Agrotain® in a solution at the rate of 800 L ha⁻¹ before or on the same day of urine application reduced NH₃ losses, ii) the application of the Agrotain® after urine application did not reduce NH₃ losses, iii) application of Agrotain® reduced the accumulation of NH₄⁺-N in soil and iv) the application of Agrotain® also slightly reduced the increase in soil pH; however, the differences were not statistically significant.

These results are discussed below and compared with previous studies, to explain these observations.

3.4.1 Ammonia emission from applied urine

Total NH₃-N emitted from applied cattle urine (530 kg N ha⁻¹) in the present experiment was 78.08 kg NH₃-N ha⁻¹ (14.7% of the urine-N) which is within the range reported in previous New Zealand studies (Menneer et al., 2008; Sherlock et al., 2008; Zaman et al., 2013). Menneer et al. (2008) reported a 14% of urine-N (775 kg N ha⁻¹) loss as NH₃ where a little rainfall occurred during the first days. Similarly, Sherlock et al. (2008) collated previous studies which used the aspirated chamber method and found that on average 15.9% of urine-N was lost as NH₃. Recently, Zaman et al. (2013) reported 12% of urine-N loss as NH₃ (applied at 600 kg N ha⁻¹) with significant rainfall during the initial days, and high soil water content and low temperature. Pereira et al. (2012) suggested that moist and winter conditions can minimize the NH₃ volatilization loss. Lower NH₃ emissions have also been reported in other studies (Singh et al., 2013; Zaman & Nguyen, 2012; Zaman et al., 2009; 2013). These losses varied between 3.6%

and 8.9% of urine-N, depending on the soil, experimental and climatic conditions (Cortus et al., 2008; He et al., 1999; Pereira et al., 2012; Salazar et al., 2012; Sommer et al., 2004; 2006).

In this study, the majority of NH_3 (46.5%) was emitted within 24 hours of urine application in urine only, UAgr1, UAgr3 and UAgr5 treatments, which is supported by high soil NH_4^+ -N concentration and a sharp rise in soil pH. Soil NH_4^+ -N concentration following urine application increased from 13.08 mg NH_4^+ -N kg^{-1} of soil in control treatments to 305.32 mg NH_4^+ -N kg^{-1} of soil in urine treatments. Similar or higher increases in NH_4^+ -N have been reported in earlier New Zealand studies (220 to 900 mg NH_4^+ -N kg^{-1} of soil) during the first 24 hours of urine deposition (Menneer et al., 2008; Zaman & Nguyen, 2012; Zaman et al., 2009). The increase in soil pH of 0.5 units following urine application observed in the present study was lower than 1 pH unit increase reported in other studies (Singh et al., 2013; Zaman & Nguyen, 2012). This lower increase in soil pH observed in this study may reflect the lower amount of urine-N used. 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).

Other factors that could stimulate NH_3 losses in this study were the warm soil temperature and appropriate moisture conditions. High soil temperatures induce both conversion of urea to NH_4^+ -N and an increase in soil pH which promote a rapid increase in NH_3 losses due to a stimulation effect in the urease activity (Cortus et al., 2008; Sommer et al., 2006). However, moist and cold winter conditions can minimize NH_3 losses (Pereira et al., 2012; Salazar et al., 2012). In the current study, soil temperature inside the chamber averaged 19°C the first two days and the moisture content at urine application was approximately 24%. Thereby, warm conditions and the water content probably accelerated NH_3 losses the first day. Zaman and Nguyen (2012) observed that approximately 23.3% and 42% of urine-N (600 kg N ha^{-1}) were lost the first day during autumn (May) and spring (September), respectively, where average temperatures were either below 10°C (autumn) or between 10°C and 15°C (spring).

It is also important to highlight that other urine factors which contribute to high NH_3 losses are urea-N content, urine pH and hippuric acid. The presence of hippuric acid in urine (not measured) accelerates NH_3 losses due to a stimulatory effect on urea hydrolysis (Whitehead et al., 1989). 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 (4.95g L^{-1}) is below the data reported by others authors, 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. The difference in total N can vary with the diet and the water consumption of the cows.

Ammonia emissions from urea and urine is also affected by other soil factors such as cation exchange capacity (CEC), pH, and buffering capacity, and by climatic factors like wind speed, temperature and rainfall/irrigation (Bolan et al., 2004; Saggar et al., 2004; 2013; Whitehead, 1995). The experimental plots were covered during the first week to avoid the differences in the movement of UI sprayed at different times before urine application; thereby the rainfall did not affect NH_3 losses.

3.4.2 Effect of Agrotain® spray on reducing ammonia emission from urine

3.4.2.1 Effect of Agrotain® spray before urine application on reducing ammonia emission

In treatments where Agrotain® was applied before urine application, NH_3 losses were lower than urine only treatments. The application of Agrotain® before urine or on the same day (UAgr-5, UAgr-3 and UAgr0) probably decreased urea hydrolysis because a reduction in soil NH_4^+ -N was observed in those treatments, thereby decreasing the potential for NH_3 losses. Ammonia losses were reduced by $27.6 \pm 5.5\%$, $17.5 \pm 11.1\%$ and $9.6 \pm 7.4\%$ for UAgr-5, UAgr-3 and UAgr0, respectively. The significant reductions of NH_3 losses achieved in UAgr-5 and UAgr-3 indicated that the inhibitor effectively inhibited the activity of the urease before urine application in comparison with urine alone. Other studies have reported immediate reductions in NH_3 losses after urine

deposition, where the nBTPT (in Agrotain®) is quickly transformed into its active form NBPTO (Manunza et al., 1999); however its effectiveness is quickly reduced as NBPTO is degraded quickly (Rawluk et al., 2001). Similar result was reported by Singh et al. (2013) who observed that Agrotain® also reduced NH₃ losses from 999 mg NH₃-N m⁻² to 375 mg NH₃-N m⁻². Slowing the rate of urea hydrolysis allows more time for urea to diffuse away from the soil surface decreasing the likelihood of being volatilized (Dawar et al., 2011a; Zaman & Nguyen, 2012).

However, the application of the inhibitor on the same day of urine application was expected to be more effective in reducing NH₃ losses. This was not observed in the present study as the grass was mown, and then Agrotain® was applied after urine deposition. This may have resulted in less Agrotain® reaching the urine which moved quickly into the soil. Another possible reason for the difference between applied Agrotain® before and on the same day may be that Agrotain® applied 5 and 3 days prior to grazing inhibited the urease activity in the plant and soil before urine application. For instance, Watson and Miller (1996) observed that shoot urease activity was reduced dramatically in the first few days after nBTPT application. The time taken by urease to recover completely ranged between 4 and 10 days following nBTPT application. Alternatively/concurrently Agrotain® may have been moved from leaf surfaces to the soil surface by dew action, inhibiting soil urease activity. The reasons behind the differences seen need further consideration as they were not investigated in the current study.

Previous studies conducted in New Zealand in which Agrotain® and urine were applied on the same day, have reported reductions in NH₃ losses which range from 11% to 93% (Menneer et al., 2008; Singh et al., 2013; Zaman & Blennerhassett, 2010; Zaman & Nguyen, 2012; Zaman et al., 2009). The reductions obtained in UAgro treatment in this study is in the lowest range. This could be attributed to differences in the method of Agrotain® application. In previous studies, Agrotain® was mixed with urine before application which allowed a maximum opportunity to inhibit the urease by the inhibitor. In the present study, urine was applied either after the inhibitor was sprayed

or inhibitor was sprayed after urine deposition to simulate the natural field grazing conditions. This method is likely to decrease the interaction between the inhibitor and the urease, resulting in lower reduction in NH_3 losses.

After urine application, soil NH_4^+ -N concentration and pH increased in comparison to the control treatment. Agrotain® effectively reduced soil NH_4^+ -N concentration when it was sprayed 3 and 5 days prior to urine in comparison with urine, UAgr1, UAgr3 and UAgr5. These results are in agreement with previously published results of Zaman and Nguyen (2012) where Agrotain® was applied 5 days prior to urine. They reported that soil NH_4^+ -N concentration was reduced by approximately $400 \text{ mg NH}_4^+\text{-N kg}^{-1}$ of soil, and soil pH was reduced by more than 1 unit. They also found that applying the inhibitor 5 days prior to urine, NH_3 losses were reduced by 38% and 28% in autumn and spring, respectively. After the initial increase, both soil NH_4^+ -N and pH decreased over the present 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.

3.4.2.2 Effect of Agrotain® spray after urine application on reducing ammonia emission

Agrotain® application after urine deposition (UAgr1, UAgr3 and UAgr5) did not show any reduction on NH_3 volatilization compared to the urine only treatment. According to the results observed in the present study, the urea hydrolysis took place during the first 24 hours because there was no reduction in NH_3 losses in the UAgr1 treatment. These results are in agreement with the results reported by Zaman and Nguyen (2012)

who found no reduction in NH_3 emissions from Agrotain® applied 5 days after urine deposition.

The application of Agrotain® after urine application showed no reduction on NH_3 losses, and this is supported by the high soil pH and soil NH_4^+ -N concentration on day 1 and 2 in these treatments. These are two main parameters regulating NH_3 emissions from soils which have been discussed above.

3.5 Conclusions and future research

- 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.
- Total $\text{NH}_3\text{-N}$ emitted in urine-only treatment during the experiment was $78.08 \text{ kg NH}_3\text{-N ha}^{-1}$ and equaled to 14.7% of the urine-N applied (530 kg N ha^{-1}).
- When Agrotain® was applied 5 and 3 days prior to urine NH_3 losses were effectively reduced. The reduction percentages observed were 27.6% and 17.5% for UAgr-5 and UAgr-3, respectively.
- Agrotain application on the same day that urine was applied reduced NH_3 losses. However, it was not statistically different from treatments where Agrotain was applied after urine deposition.
- When Agrotain® was applied after urine deposition (UAgr1, UAgr3 and UAgr5), no reduction on NH_3 losses were observed as majority of the NH_3 was already lost by the time Agrotain® was applied.
- Application of Agrotain® before urine deposition (UAgr-5 and UAgr-3) reduced the accumulation of $\text{NH}_4^+\text{-N}$ in the soil surface which is subjected to NH_3 volatilization. Although the sharp increase in soil pH was reduced in those treatments, it was not statistically different from urine, UAgr1, UAgr3 and UAgr5 treatments.
- Urease inhibitor increased soil $\text{NO}_3^-\text{-N}$ concentration in all treatment that received urine which may be susceptible to leaching or denitrification, but the differences were not significant.

The results found in this thesis highlight some areas that require further investigation.

Future studies should include the next areas:

- According to the results in the current study, Agrotain® application 3 to 5 days before simulated grazing event was more effective in reducing NH_3 losses than the application on the same day. Further quantitative data is required about the effect of Agrotain® application before or on the same day as the grazing event. Further work is also required to explore this difference and the biophysical factors contributing to this difference.
- Pasture samples were collected but could not be analysed for the proportion of Agrotain® retained on the pasture canopy due to limited resources. Information on the retention of the inhibitor by the canopy can provide more understanding of the effectiveness of the nBTPT applied to pasture soil and to study the fate of retained Agrotain® in the pasture grazed by the animals.
- The use of polymers to coat urease inhibitor and slow the release to enhance the effectiveness of the Agrotain® could be studied to test the longevity of urease inhibitor.

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