MITIGATION POTENTIAL OF UREASE INHIBITORY COMPOUNDS IN REDUCING AMMONIA EMISSIONS FROM CATTLE URINE IN DAIRY-GRAZED PASTURE SOILS

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Kamal Prasad Adhikari

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Abstract

The excretal deposition by grazing animals, especially urine containing about 80% urea, and urea fertiliser use on pastoral farms are major sources of ammonia (NH_3) emissions in New Zealand (NZ) agriculture. Recent intensification of dairy farming in NZ has resulted in a substantial increase in the use of nitrogen (N) fertiliser, especially urea, and the quantity of urine deposited by grazing dairy cows onto pasture soils, and as a consequence, higher NH_3 emissions. These emissions represent economic and environmental losses.

The urease inhibitor (UI) N-(n-butyl) thiophosphoric triamide (nBTPT) has shown effectiveness in reducing emissions when applied with fertiliser urea or cattle urine in NZ dairy-grazed pasture soils. However, the inhibitory effect of nBTPT on reducing NH_3 emissions is effective for a relatively short period (7 - 14 days), during which emissions from urea fertiliser is inhibited. But, in the context of grazed pasture, to reduce the NH_3 emitted from deposited urine following each grazing event, regular applications of nBTPT are required, which would be prohibitively expensive. To mitigate NH_3 emissions from cattle urine deposited during more than a single grazing, in dairy-grazed pasture soils, it is necessary to identify alternative longer lasting inhibition approaches to using nBTPT. Therefore, the overall objective of this thesis was to assess the effectiveness and longevity of potentially longer-lasting non-specific inhibitors copper (Cu) and zinc (Zn), and the specific inhibitor N-(2-Nitrophenyl) phosphoric triamide (2-NPT) in reducing NH_3 emissions following cattle urine applied to pasture soils.

The study presented in this thesis initially examined the influence of inherent and added Cu and Zn in inhibiting soil urease activity (UA), and the role of soil organic carbon (C) and soil textural and mineralogical properties on influencing the ability of these metals at inhibiting soil UA of dairy pasture soils under laboratory incubations. The study then evaluated the effect of the recently introduced UI 2-NPT on NH_3 emissions, soil microbial biomass C, pasture dry matter yield and N uptake, which was compared with the more commonly used UI nBTPT. This study involved both laboratory and field experiments.

The first laboratory experiment assessed the effect of inherent and added Cu and Zn in inhibiting soil UA of dairy-grazed pasture soils. The results showed significant positive correlations between soil total C and N with soil UA for 23 soils from the Waikato region of NZ. However, there were no significant negative correlations between soil UA with inherent Cu and Zn levels. Similarly, the addition of Cu up to 20 mg kg^{-1} soil and the combination of 5 mg Cu and 5 mg Zn kg^{-1} soil did not significantly reduce soil UA of 4 dairy-grazed pasture soils, with contrasting organic C levels.

In the second laboratory experiment, the influence of the soil C factor (soil organic C, and other related soil properties, such as clay content and cation exchange capacity (CEC)) on the effectiveness of Cu and Zn to inhibit urea hydrolysis in soil supernatants were studied. When Cu was added to 2 different soil supernatants, at rates of 5, 10, and 20 mg Cu kg^{-1} soil, there was a significant reduction in hydrolysis of urea applied at either 120 or 600 mg urea-N kg^{-1} soil. Additions of Zn, at a rate of 20 mg kg^{-1} soil achieved negligible or small reductions in urea hydrolysis after either 120 or 600 mg urea-N kg^{-1} soil applications to soil supernatants. These results suggest that Cu

has a urease inhibitory effect, but its ineffectiveness in C rich pasture soils is caused by reduced bioavailability as a result of high Cu complexation. However, Zn had a negligible inhibitory effect on soil UA at the rate used in this experiment. Overall these results support the conclusion that neither metal is likely to be a practical UI for reducing NH_3 emissions from NZ dairy-grazed pasture soils.

The effectiveness and longevity of 2-NPT and nBTPT in reducing NH_3 emissions from cattle urine applied to 2 dairy-grazed pasture soils were evaluated under laboratory conditions. The inhibitors were applied at the start of the experiment and urine was applied at 4 stages; (A) immediately before, (B) 29 days after, (C) 56 days after, and (D) 29 days after and again 60 days after inhibitor application, and NH_3 emissions were measured following each urine application. There were 3 application rates of 2-NPT; 0.025, 0.050, and 0.075% of total urine-N applied at Stage-A, and one rate of nBTPT; 0.025% of total urine-N applied at Stage-A. The application depth of urine applied was 10 mm for Stages A, B and C and 7.2 mm for Stage-D. The % applied urine-N that was emitted as NH_3 at the different stages ranged from 14.2 to 50.5% for the soils studied. Both UIs equally reduced total NH_3 emissions (20.6 - 27.3%), from both of the soil types, when urine was applied immediately before inhibitor application. The inhibitor 2-NPT continued to reduce emissions (5.6 - 7.4%) from urine applied up to 56 days after the inhibitor application, but only for the soil with lower microbial biomass C and UA, suggesting that 2-NPT has slightly greater longevity of efficacy than nBTPT. When urine was applied immediately before inhibitor application, inhibitors had no effect on soil microbial biomass C, measured 31 days after inhibitor application, which suggest specificity of UIs on inhibiting UA.

Two field experiments were conducted during summer and autumn to assess whether the differences observed between the inhibitors 2-NPT and nBTPT in the laboratory experiment are also achieved under field conditions. In the summer experiment, the inhibitors were applied at the start of the experiment and urine was applied at 3 stages; (A) 3 hrs before, (B) 28 days after, and (C) 68 days after inhibitor application, and NH_3 emissions were measured following each urine application. The application rates of inhibitors to the urine treatments for all 3 stages were based on the percentage (0.025%) of total urine-N applied at Stage-A. In the autumn experiment, urine was only applied either immediately before or 3 hrs before inhibitor application, also at a rate of 0.025% of total urine-N. The application depth of urine applied in both of the summer and autumn experiments was 10 mm. The NH_3-N emitted in the summer experiment was between 15.3 - 23.6% of the applied urine-N (equivalent to 111 - 142 kg N ha^{-1}), however, in autumn the emissions were only 4.5% (equivalent to 27 kg N ha^{-1}) of the total N applied. In the summer experiment, only 2-NPT significantly reduced total NH_3 emissions (19.5% reduction), which was only when urine was applied 28 days after the inhibitor application (Stage B). Both inhibitors significantly reduced emissions in autumn when urine was applied either 3 hrs before or immediately before inhibitor application. However, the effectiveness was greater when urine was applied immediately before inhibitor application (52.3 - 72.7% reduction) compared to urine applied 3 hrs before inhibitor application (35.0 - 41.2% reduction). The reduction was greater with 2-NPT (72.7% reduction) compared to nBTPT (52.3% reduction) when urine was applied immediately before inhibitor application. The field study confirmed the findings of laboratory study that the effectiveness and longevity of 2-NPT, in reducing NH_3 emissions from cattle urine applied to pasture soils, is greater

compared to nBTPT. There was no effect of inhibitors on pasture dry matter yield and N uptake in either of the field experiments.

The 2-NPT applied up to about a month prior to a grazing event in summer reduced NH_3 emissions from urine patch areas at a rate equivalent to 26 kg N ha^{-1} (based on 19.5 % reduction in summer) at the subsequent grazing. If 3 applications of 2-NPT are applied in summer (period when emissions are typically the highest) following 3 grazings that would reduce losses by 78 kg N ha^{-1} from 3 subsequent grazings. When these reductions are extrapolated to determine the overall benefit on whole paddock basis, the total reduction in N loss (26 kg N ha^{-1} x 3% x 3 grazings) is 2.3 kg N ha^{-1} yr^{-1}, assuming the urine patches cover 3% of the grazed area per grazing. Thus, overall benefit from using 2-NPT is greater than nBTPT in reducing NH_3 emissions from cattle urine deposited in dairy-grazed pastures. However, the size of the reduction from using 2-NPT on whole paddock was low, compared to the amount of N cycling in grazed pastures annually.

To further improve the effectiveness of inhibitors, applied after urine application in summer, future research could focus on enhancing the contact between the inhibitor and urine urea by increasing the volume of inhibitor used (>800 L ha^{-1}) and/or by implementing shorter durations (<3 hrs) between urine and inhibitor application. Further changes in volume of inhibitor applied and timing of inhibitor application should consider cost involved and feasibility in dairy farms with the current technology.

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Chapter 1

Introduction

1.1 The issue

Globally, the agriculture sector (cropping and livestock farming) remains responsible for most ammonia (NH_3) emissions. In New Zealand (NZ) agriculture, excreta deposited by grazing animals (particularly urine containing about 80% urea) and the use of urea fertiliser are the key sources of NH_3 emissions. Intensification of dairy farming in NZ over the last three decades has resulted in substantial increases in the use of nitrogen (N) fertiliser (most commonly urea representing more than 80%) and in the amounts of excreta-N returned to pastures, causing higher NH_3 emissions. Using a 10% emission factor, adopted in the NZ National Greenhouse Gas Inventory (Ministry for the Environment 2018), for NH_3 from applied N fertiliser and for livestock excreta-N, it is estimated that NZ grazed pastures lost 150.04 Gg of N as NH_3 in 1990, which increased to 198.8 Gg in 2016 (~32 % increase).

The NH_3 emissions have negative impacts on human health and the wider environment and represent agronomic and economic losses. The emitted NH_3 causes detrimental effects through the generation of secondary aerosols and a greenhouse gas nitrous oxide (N_2O), eutrophication of aquatic bodies and acidification of soil (Saggar et al. 2004; Pozzer et al. 2017). Using an excreta-N deposition rate of 119.5 kg N $head^{-1}$ yr^{-1} for dairy cattle (Ministry for the Environment 2018), the annual losses of NH_3 from deposited excreta in NZ dairy pastures are estimated to have a value of about $94 million, of which the losses from cattle urine account for about 60 - 70%. In addition,

Ballance Agri-Nutrients (2015) reported that an estimated value of $30 million of urea fertiliser is lost annually as NH_3 in NZ, most of which is from dairy farms. Reducing NH_3 emissions can potentially improve N use efficiency on farms and reduce the environmental impacts and economic losses (Pozzer et al. 2017).

Irrigation (5 - 10 mm) following ≤ 8 hrs of urea fertilisation has shown to reduce NH_3 emissions by up to 50% in dairy-grazed pasture soils (Zaman et al. 2013a). While irrigation following urine application has been shown to mitigate NH_3 emissions (Hoogendoorn et al. 2017), the majority of NZ dairy-grazed farms are not irrigated except dairy farms from Canterbury region. Furthermore, in some cases, irrigation can move urine-N below the root zone, making it unavailable to plant uptake and, thus, susceptible to nitrate (NO_3^-) leaching (Thomas 2015).

A number of NZ and international studies have found that application of urease inhibitors (UIs) with urea fertiliser or animal urine can slow down the hydrolysis of urea by inhibiting soil urease activity (UA), which reduces the concentration of ammonium (NH_4^+) in the soil solution and, thereby, reduces NH_3 emissions (Saggar et al. 2013; Abalos et al. 2014; Khariri et al. 2016; Pan et al. 2016; Silva et al. 2017; Modolo et al. 2018). Among the various available UIs, N-(n-butyl) thiophosphoric triamide (nBTPT) has shown effectiveness in reducing emissions from NZ dairy-grazed pasture soils when applied with fertiliser urea or cattle urine (Saggar et al. 2013; Hoogendoorn et al. 2017). About 28 % of total urea fertiliser used in NZ agricultural farms are coated with nBTPT (Agrotain®) constituting SustaiN and N-Protect® traded by Balance Agri-Nutrients Limited and Ravensdown Limited, respectively (Ministry for the Environment 2019). However, nBTPT has a short-lived inhibitory effect of approximately 7 - 14 days

(Saggar et al. 2009). The degradation nBTPT in soil results in its loss of effectiveness. Most NH_3 emissions from urea fertiliser application occurs usually within the first few days after application. Therefore, when nBTPT is added with urea fertiliser it can be effective at reducing emissions during this critical period. However, reducing NH_3 emissions from cattle urine following grazings, would require nBTPT application after each grazing event. This is because grazing events typically occur at intervals ranging from 3 - 8 weeks, depending on region, season and weather conditions (Brougham 1970; Parker 2002). Therefore, to reduce the NH_3 emitted from deposition of urine in dairy-grazed pastures, regular applications of nBTPT are required, which would be prohibitively expensive.

In order to mitigate NH_3 emissions from deposited urine during grazing events in dairy-grazed pasture soils, it is necessary to consider alternative longer-lasting inhibition approaches to using nBTPT. These inhibition approaches should not have harmful effects on beneficial soil microorganisms, plants, humans, grazing animals and environment at the recommended levels. Metal compounds copper (Cu) and zinc (Zn) have been shown to inhibit soil UA for 8 - 12 weeks and are also plant micronutrients (Hemida et al. 1997; Wyszkowska et al. 2006; Chien et al. 2009). The efficiency of Cu and Zn at inhibiting soil UA and reducing NH_3 emissions is affected by the physical and chemical properties of the soil (Tabatabai 1977), which regulate their bioavailability, plant and microbial uptake and leaching losses (Kumpiene et al. 2008; Nwachukwu and Pulford 2009). However, the application rates of these elements need to be carefully managed to avoid the long-term risk of excessive Cu and Zn accumulation levels in soil, as this is detrimental to soil microorganisms, plants and grazing animals (Bolan et al. 2003; Broadley et al. 2007).

A recently introduced UI, N-(2-Nitrophenyl) phosphoric triamide (2-NPT) has been reported to be stable, more efficient and longer lasting than nBTPT in reducing soil UA (Domínguez et al. 2008). The mechanism of soil UA inhibition by 2-NPT is similar to that of nBTPT, as both inhibitors are members of a group of chemicals called phosphorodiamidates (Domínguez et al. 2008). There is also little risk of accumulation of 2-NPT in soil from its continued application as this organic compound degrades by chemical hydrolysis and microbial activity over time (Kappaun et al. 2018).

Although there is evidence of Cu, Zn, and 2-NPT being longer-lasting UIs compared to nBTPT, there is minimal NZ and international research information available on the effectiveness and longevity of these alternative inhibitors at reducing NH_3 emissions from urine patches in dairy-grazed pasture soils.

1.2 Research objectives

The current study was carried out with the following objectives in mind:

- To determine the influence of inherent and added Cu and Zn on inhibition of soil UA in dairy-grazed pasture soils.
- To investigate the processes regulating Cu and Zn inhibition of UA in soil and how these processes affect their effectiveness in reducing soil UA and NH_3 emissions following urine addition to dairy-grazed pasture soils.
- To evaluate the effectiveness and longevity of 2-NPT and nBTPT in reducing NH_3 emissions from cattle urine applied to pasture soils under laboratory and field conditions.

The specific objectives of the laboratory and field experiments are presented in the respective chapters.

1.3 Thesis structure

The overview of chapters included in this thesis are briefly summarised in this sub-chapter. Including this chapter, the thesis is structured into 7 chapters. Chapter 1 gives an overview of the study including issues of NH_3 emissions associated with intensification of dairy farming in NZ, economic and environmental implications of these losses and indicate the need for alternatives to existing mitigation approaches focusing on the longevity of NH_3 emission reductions from urine deposited by grazing cattle in dairy-grazed pastures. The overall objectives of the several laboratory and field experiments presented in this thesis are also highlighted in this chapter. Chapter 2 reviews the literature describing the NH_3 emissions from cattle urine patches in pasture soils, factors affecting these emissions, the limitations of currently available mitigation options, and the research gaps relating to information on potentially more effective mitigations.

Chapter 3 covers the series of laboratory experiments that were conducted to evaluate the influence of inherent and added Cu and Zn in inhibiting soil UA, for the use of these micronutrients in reducing NH_3 emissions from cattle urine patches.

Chapter 4 highlights the role of soil organic carbon (C) and soil textural and mineralogical properties on influencing the ability of Cu and Zn at inhibiting soil UA of dairy pasture soils. The results from Chapters 3 and 4 have been published as a full research paper in the journal Soil Research (Adhikari et al. 2018).

Chapter 5 evaluates the effectiveness and longevity of the UIs 2-NPT and nBTPT in reducing NH_3 emissions from cattle urine applied to dairy-grazed pasture

soils. Four laboratory incubation experiments with two pasture soils, with contrasting soil organic C levels, were used to assess the effect of the UIs on emissions following urine applied to soil at different stages before or after UIs application. The influence of soil type on the longevity of UIs in reducing emissions is also described in this chapter along with effect of UIs on soil microbial biomass. The results from chapter 5 have been accepted for publication in the journal Soil Research.

Chapter 6 assesses the effectiveness and longevity of the UIs 2-NPT and nBTPT in reducing NH_3 emissions from cattle urine in a series of field plot experiments. The effect of soil and environmental conditions and inhibitor application time on the effectiveness of UIs applied following urine application is examined along with describing the reasons for differences in longevity of effectiveness of 2-NPT in field conditions compared to laboratory incubations. This chapter also quantifies the effect of UIs on pasture yield and on N uptake from urine.

All the findings from this thesis are summarised in Chapter 7 along with the overall conclusions of the research, general discussion and recommendations for future work.

The scientific articles in peer reviewed journals, conference proceedings and conference abstracts, which are based on the results from this thesis are listed below. Experimental chapters in this thesis are written in a manuscript format, so some repetition on discussion of results may occur in the different chapters.

List of publications:

Refereed journal papers

Adhikari KP, Saggar S, Hanly JA, Guinto DF, Taylor MD (2018) Why copper and zinc are ineffective in reducing soil urease activity in New Zealand dairy-grazed pasture soils. *Soil Research* **56**, 491-502.

Adhikari KP, Saggar S, Hanly JA, Guinto DF (2019) Comparing the effectiveness and longevity of the urease inhibitor 2-NPT with nBTPT in reducing ammonia emissions from cattle urine applied to dairy-grazed pasture soils. *Soil Research* (accepted).

Conference proceedings

Adhikari KP, Saggar S, Hanly JA, Guinto DF (2018) Laboratory evaluation of urease inhibitors 2-NPT and nBTPT in reducing ammonia emissions from cattle urine applied in dairy-grazed pasture soils. 'In: Farm environmental planning science, policy and practice'. (Eds LD Currie, CL Christensen). Occasional Report No. 31. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand.

Adhikari KP, Saggar S, Hanly JA, Guinto DF (2017) Understanding the ineffectiveness of Cu and Zn in reducing urea hydrolysis in grazed dairy pasture soils. 'In: Science and policy: nutrient management challenges for the next generation'. (Eds LD Currie, MJ Hedley). Occasional Report No. 30. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand.

Adhikari KP, Taylor MD, Saggar S, Hanly JA, Guinto DF (2016) Assessing the relationship between common measures of soil Cu and Zn status and soil urease activity of dairy-grazed pasture soils. 'In: Integrated nutrient and water

management for sustainable farming'. (Eds LD Currie, R Singh). Occasional Report No. 29. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand.

Conference abstracts

Adhikari KP, Saggar S, Hanly JA, Guinto DF (2019) Effectiveness and longevity of the urease inhibitors 2-NPT and nBTPT in reducing NH_3 emissions from cattle urine applied to pasture soils – Laboratory and field evaluation. 'In: New Zealand Agriculture Greenhouse Gas Inventory Research Conference'. Palmerston North, New Zealand.

Adhikari KP, Saggar S, Hanly JA, Guinto DF (2018) Effectiveness and longevity of 2-NPT and nBTPT in reducing NH_3 emissions from cattle urine-patches. 'In: Diverse soils - Productive landscapes'. New Zealand Society of Soil Science Conference, Napier, New Zealand.

Adhikari KP, Saggar S, Hanly JA, Guinto DF (2018) Determining and comparing the effectiveness and longevity of 2-NPT with nBTPT in reducing NH_3 emissions from cattle urine applied to dairy-grazed pasture soils. 'In: New Zealand Agriculture Greenhouse Gas Inventory Research Conference'. Wellington, New Zealand.

Adhikari KP, Saggar S, Hanly JA, Guinto DF (2017) Cu and Zn do not inhibit urea hydrolysis in New Zealand dairy-grazed pasture soils. 'In: Annual NzONet, MethaNet and CarboNet meeting'. Wellington, New Zealand.

Adhikari KP, Saggar S, Hanly JA, Guinto DF (2017) Why Cu and Zn applications do not reduce urea hydrolysis in New Zealand dairy-grazed pasture soils? 'In:

Visions for the future'. Institute of Agriculture and Environment Symposium, Massey University, Palmerston North, New Zealand.

Adhikari KP, Saggar S, Hanly JA, Guinto DF Taylor MD (2016) Assessing the relationship between soil Cu and Zn levels and urease activity in dairy-grazed pasture. 'In: Soil, a balancing act downunder'. Joint Conference for the New Zealand Society of Soil Science and Soil Science Australia, Queenstown, New Zealand.

Adhikari KP, Taylor MD, Saggar S, Hanly JA, Guinto DF (2016) Assessing the relationship between common measures of soil Cu and Zn status and soil urease activity of dairy-grazed pasture soils. 'In: Annual NzONet, MethaNet and CarboNet meeting'. Wellington, New Zealand.

Chapter 2

Review of literature

2.1 Introduction

Nitrogen is an essential nutrient required for productive pasture growth. In NZ livestock farms, much of the N cycling in grazed pastures originates from atmospheric N_2 fixed by Rhizobium bacteria associated with the root nodules of white clover (*Trifolium repens*). However, a large proportion of this N is returned to pastures inefficiently in concentrated animal urine patches. Recent intensification of dairy farming in NZ has resulted in substantial increase in the national dairy herd size from 3.4 million cows in 1990 to 6.6 million in 2016 (an increase of 94%). This has resulted in a significant rise in the volumes of cattle urine deposited onto pasture soils, which are equivalent to the addition of approximately 232.8 Gg N in 1990 to 514.1 Gg N in 2016. The Ministry for the Environment (2018) indicated that N fertiliser (432.2 Gg N) applied in NZ in the year ending June 2016 was more than seven times greater, relative to that applied in the year ending June 1990 (59.3 Gg N). Among N fertilisers, urea is the most common representing approximately 83% of N fertiliser used in 2016 in NZ pasture, most of which is used in dairy farms (Ministry for the Environment 2018). The greater quantities of N inputs in grazed dairy pastures has also resulted in increases in gaseous and leaching losses of N, including NH_3 emissions. Quantitatively, NH_3 emissions can be a substantial loss of N, representing both an economic loss and also has environmental implications.

Various approaches, such as irrigation management and UIs, have been used to reduce NH_3 emissions from urea fertiliser or cattle urine patches. Despite the ubiquitous and the highly specific activity of soil urease, which generally makes urease inhibition difficult to achieve in soils, there is growing interest in the evaluation of UIs, particularly for use with fertiliser urea (Saggar et al. 2013; Ni et al. 2014; Khariri et al. 2016; Modolo et al. 2018). Urease inhibitors include both specific and non-specific compounds. The specific inhibitors tend to control enzymes involved in specific biochemical reactions during the ammonification process (i.e. urease), whereas the non-specific inhibitors tend to also have a broad biocidal effect on microbial community in soils. The most prominent UIs are grouped into 2 categories based on their structures and/or their binding modes with urease: (a) competitive inhibitors (e.g., hydroxyurea, phenyl phosphorodiamidate (PPDA), nBTPT, 2-NPT) resemble the substrate's structure closely and compete with the substrate for binding to the same active site on the urease enzyme, which block it from reacting with urea, (b) non-competitive inhibitors alter the structure of the urease enzyme and demolish its ability to react with urea. One type of non-competitive inhibitor is reactive organic or inorganic compounds (e.g., metal ions (Cu^{2+}, Zn^{2+}), alk(en)yl thiosulfinate, hydroquinone, p-Benzoquinone) that react with the sulfhydryl (SH) group that is required for the proper function of the enzyme. Another type of non-competitive inhibitors are metal chelating compounds (e.g., caprylohydroxamic acid, acetohydroxamic acid), which react with one of the nickel (Ni) atoms in the active site of urease and forms a complex that causes inhibition (Hasan 2000; Saggar et al. 2009). Several phosphorodiamidates, quinones and hydroxamic acids have been patented for inhibition of urea hydrolysis and their effectiveness have been evaluated under various soil conditions. However, only a few (e.g., nBTPT, hydroquinone (HQ), and acetohydroxamic acid (AHA)) are thought to be sufficiently

effective compounds for use in mitigating urea hydrolysis. As discussed in the Introduction (Chapter 1) the specific UI called nBTPT has been shown to be effective in reducing emissions when applied with urea fertiliser or cattle urine in NZ dairy-grazed pasture soils. Its duration of effectiveness at reducing NH_3 emissions is up to 7 - 14 days when applied with either urea fertiliser or cattle urine (Saggar et al. 2013). Thus, if nBTPT is applied to reduce emissions from urine deposited in grazed pastures it will potentially be effective for only a single grazing event. To mitigate NH_3 emissions from cattle urine deposited over more than a single grazing it is necessary to identify alternative longer lasting UIs. Therefore, the main aim of this chapter is to review the published NZ and international research information on the effectiveness and longevity of UIs in reducing NH_3 emissions from cattle urine deposited in dairy-grazed pasture soils.

Cattle urine contains various organic and inorganic forms of N that undergo different transformations in soils. An understanding of fundamental aspects of the processes and the factors regulating NH_3 emissions from urine can help to illustrate the important characteristics of effective UIs in pasture soils. There is a well-established body of published literature dealing with the NH_3 emissions from urine in pasture soil, factors affecting these emissions and the evaluation of nBTPT as a mitigation option to reduce emissions. Therefore, the chapter will only briefly summarise these topics and the reader is referred to appropriate reviews that provide more in-depth coverage of respective topics (Bolan et al. 2004; Singh 2007; Sherlock et al. 2008; Saggar et al. 2013). This chapter attempts to mainly focus on potentially longer lasting non-specific UIs, Cu and Zn, and the specific UI, 2-NPT, and in particular their mode of action and the factors affecting their effectiveness. The various areas relevant to mitigating NH_3

emissions from urine patches included here are: i) the mechanism of NH_3 emissions, ii) soil and environmental factors affecting emissions, iii) NH_3 emissions from urine in pasture soils, iv) the mechanisms of the soil urease enzyme, v) mitigation strategies to reduce emissions, including the evaluation of nBTPT and potentially longer lasting UIs Cu, Zn, and 2-NPT and factors affecting effectiveness of UIs, and vi) conclusions presenting gaps in existing knowledge and research needs, with an emphasis on potential longer lasting UIs to reduce emissions from dairy-grazed pastures.

2.2 Mechanism of NH_3 emissions

In the context of grazed pastures, NH_3 emissions start almost immediately after urine deposition, and exhibit a peak within 1 or 2 days as a result of rapid hydrolysis of the urea present in urine (Zaman et al. 2009). During urea hydrolysis, the enzyme urease forms ammonium carbonate ($(NH_4)_2CO_3$) (Eqn. 2.1) and then NH_4^+ and carbonate (CO_3^{2}) ions (Eqn. 2.2). Hydroxyl (OH^-) ions are released by the CO_3^{2-} ions, which results in an increase pH, often > pH 9 at the soil surface (Eqn. 2.3) (Sherlock et al. 2008), which facilitates the dissociation of NH_4^+ to gaseous NH_3 and ultimately emissions into the atmosphere (Eqn. 2.4) (Saggar et al. 2004).

$$(NH_2)_2CO + 2H_2O \xrightarrow{\text{Urease}} (NH_4)_2CO_3 \quad \text{(Eqn. 2.1)}$$

$$(NH_4)_2CO_3 \rightarrow 2NH_4^+ + CO_3^{2-} \quad \text{(Eqn. 2.2)}$$

$$CO_3^{2-} + H_2O \rightarrow HCO_3^- + OH^- \quad \text{(Eqn. 2.3)}$$

$$NH_4^+ + OH^- \rightarrow NH_3 \uparrow + H_2O \text{ (pKa 9.24)} \quad \text{(Eqn. 2.4)}$$

2.3 Factors affecting NH_3 emissions

The main factors that determine the level of NH_3 emissions from soil are those that affect the conversion rate of NH_4^+ to gaseous NH_3 and the movement of gaseous

NH_3 between soil solution and atmosphere (Saggar et al. 2004). Similarly, any factor which alters the concentration of NH_4^+ in soil solution by affecting urea hydrolysis or other mechanisms will have an influence on NH_3 emissions. Soil and environmental factors that have an important role in controlling these features of NH_3 emissions are initial soil pH, soil pH buffering capacity, temperature, soil moisture content, and cation exchange capacity (CEC). Soil pH changes the equilibrium between NH_4^+ and NH_3 in the soil solution; with increasing pH the concentration of NH_3 is increased and vice-versa (He et al. 1999). Freney et al. (1983) found in their study that the concentration of NH_3 in the soil solution increases from 0.1% of total ammoniacal-N (NH_4^+ and NH_3) at pH 6 to 1% at pH 7, 10% at pH 8, and 50% at pH 9. In NZ dairy pasture soils, the soil pH is typically maintained within the range of 5.8 - 6, therefore, differences in NH_3 emissions after urine application are likely to be influenced more by variations in pH buffering capacity between soils than by their initial pH. Soil moisture content affects the diffusion of NH_3 gas in soil. When water content is high then the diffusion will be slow and result in minimal NH_3 emissions. The high water content could also dilute the NH_3 concentration in soil solution and lowers the emissions (Sherlock and Goh 1984). The rate of urea hydrolysis is increased by high temperatures. The movement of NH_3 from the soil solution to the atmosphere is also increased by high temperatures, because increasing temperature reduces NH_3 solubility in water (Bouwman et al. 2002). Zaman et al. (2013b) reported the NH_3 emissions of 12% of urine N applied in NZ dairy grazed pasture soil during autumn (soil water filled pore space (WFPS); ~ 0.7 - 0.8, and soil temperature; ~ 5.5 - 8.5°C), which was significantly lower compared to emissions of 23% during spring (WFPS; ~ 0.55 - 0.66, and soil temperature; ~ 7 - 12°C), suggesting soil moisture content and temperature having an influence in total emissions. The NH_3 concentration in soil solution is also influenced by

the CEC of soil through the retention of NH_4^+ onto negatively charged cation exchange sites (Bolan et al. 2004; Saggar et al. 2004). Soils with higher CEC have lower NH_4^+ concentration in soil solution, which reduces NH_3 emissions from the soil surface. Whitehead and Raistrick (1993) found a strong negative correlation (R = 0.97) between NH_3 emissions and soil CEC.

2.4 Ammonia emissions from urine in pasture soil

Loss of N through NH_3 emissions is the major concern following urine deposition by grazing cattle in grazed pastures. A global review of previous field studies by Cai and Akiyama (2016) reported average emissions of 12.4% of N applied following cattle urine deposited to pasture soils. Similar emissions have been presented in a review of NZ studies by Sherlock et al. (2008), which reported average emissions of 15.9%, ranging from 1.5 to 66 %, of N applied following cattle urine deposition. They also reported that the average NH_3 emissions from livestock urine were 12.9%, ranging from 3.5 to 28.4% of N applied, from overseas studies, which included two studies conducted with sheep urine and one with synthetic urine. Recent information available on NH_3 emissions in NZ and overseas since the review by Sherlock et al. (2008), are presented in Table 2.1. The NH_3 emissions from NZ pastures range from 1.5 to 25.7% averaging 10% of urine-N applied. This is similar to the NH_3 emissions from recent overseas studies range from 3.7 to 25.2% of urine-N applied. The large variation in emissions in both NZ and overseas data is attributed to the different soil types and climatic conditions during the study period for reported studies. The average emissions from the more recent NZ studies are lower compared to studies reported by Sherlock et al. (2008), which is partly due to less of the data being collected in summer in the recent studies.

Table 2.1 Ammonia emissions from pasture soils following cattle urine deposition

Reference	Country	N applied (kg N ha^{-1})	Season	N lost as NH_3 (% of N applied)
Fischer et al. (2016)	Ireland	638	spring	14.9
		731	summer	9.8
		716	autumn	8.7
Lessa et al. (2014)	Brazil	421	spring	30.4
		267	summer	16.8
		396	winter	20.8
Burchill et al. (2017)	Ireland	825	spring	6.3
		569	summer	7.1
Misselbrook et al. (2014)	England	625	summer	25.2 (average)
		625	autumn	
		488	spring	
		470	autumn	
Laubach et al. (2012)	NZ	600	summer	25.7
Zaman et al. (2013b)	NZ	600	autumn	12.0
			spring	23.0
			autumn	4.9
			spring	10.9
Zaman et al. (2009)	NZ	600	spring	3.6
			summer	8.2
Zaman and Nguyen (2012)	NZ	600	spring	7.0
			autumn	1.5
Zaman and Blennerhassett (2010)	NZ	600	autumn	5.0
			spring	7.0
Rodriguez (2014)	NZ	530	autumn	14.7
Hoogendoorn et al. (2017)	NZ	660	autumn	7.0

2.5 Mechanisms of urease enzyme activity

The active site of the urease enzyme is comprised of two Ni atoms connected by a carbamylated lysine (Lys) through its two oxygen (O) atoms, and two histidines (His) are tied to each Ni atom through their N atoms. In addition, the Ni atoms are connected by an OH^- ion along with two terminal water molecules, one on each Ni atom situated towards the opening of the active site forming an H-bonded water tetrahedral cluster (Fig. 2.1). It is this cluster that urea replaces at the time of binding to the active site for the reaction (Benini et al. 1999; Saggar et al. 2009). The two Ni atoms that exist in the active site are mandatory to the enzyme's proper functioning, and the release of these atoms from the enzyme may occur under acidic conditions, leading to irreversible loss of UA (Mobley et al. 1995). Additionally, SH groups (cysteine) are commonly present in the mobile flap of the active site (Hasan 2000; Krajewska et al. 2012), which was evidenced by reacting the enzyme with SH-reactive agents, alkylating and disulfides (Riddles et al. 1983; Todd and Hausinger 1991) as shown in Eqn. 2.5. Among the SH groups present in the flap, the essential one reacts slowly with SH reagents and loses its activity, while the others that readily react without loss of catalytic activity are not essential (Takishima et al. 1988; Hasan 2000; Krajewska et al. 2012). The former SH group is very critical for mobility of that flap, which serves as a gate for the substrate molecule (urea) to enter into the active site (Martin and Hausinger 1992; Krajewska et al. 2012), and inactivation of the enzyme occurs when this SH group is covalently modified (Ambrose et al. 1951; Riddles et al. 1983; Todd and Hausinger 1991).

$$U-SH + HOCH_2CH_2SSCH_2OH \rightarrow HOCH_2CH_2SH + U-SSCH_2CH_2OH \quad \text{(Eqn. 2.5)}$$

where, U-SH = SH group in urease, $HOCH_2CH_2SSCH_2OH$ = dithiothreitol, a disulphide, $HOCH_2CH_2SH$ = mercaptoethanol, and U-$SSCH_2CH_2OH$ = enzyme product which is inactive and contains a mixed disulphide involving SH group and mercaptoethanol.

The urea hydrolysis mechanism at the active site of the urease enzyme is not completely understood. The urea molecule enters the active site cavity when the mobile flap is open, then the higher electrophilic Ni atom may bind to the carbonyl O of urea with the less electrophilic Ni atom binding to an amino N on urea, creating a carbonyl C, which is more electrophilic, hence more susceptible to nucleophilic attack by water (Saggar et al. 2009; Kappaun et al. 2018). The SH groups present in the mobile flap are involved in substrate binding through H-bonding and accelerate the catalytic reaction (Krajewska 2009) after the mobile flap is closed (Benini et al. 1999). This reaction results in the tetrahedral intermediate structure that decomposes to release NH_4^+ and carbamate facilitated by a SH group acting as a general acid catalyst (Dixon et al. 1980; Benini et al. 1999; Zimmer 2000). Another possible mechanism suggests that only the first Ni atom is bound to the urea and the second Ni atom supplies a nucleophilic water molecule to urea leading to the tetrahedral intermediate structure, which releases NH_4^+ and carbamate (Karplus et al. 1997; Benini et al. 2001; Krajewska 2009).

2.6 Mitigations to reduce NH_3 emissions from cattle urine in grazed pastures

2.6.1 Evaluation of UIs

Several NZ and overseas studies have found that UIs have potential to reduce NH_3 emissions following application of urea or after urine deposition. However, when selecting UIs to mitigate emissions from urine in grazed pastures, the consideration should also be given to its longevity, because there is continuous deposition of urine following each grazing event. The UIs with longer lasting inhibitory effect could mitigate the emissions following multiple grazing events from a single application, which would make the use of inhibitor more feasible and cost effective, by reducing the number of applications required. In addition to greater longevity, the ideal inhibitor for

use in agriculture should have the following characteristics as reported in previous studies (Saggar et al. 2009; Trenkel 2010; Modolo et al. 2018).

- be compatible with urea molecules of fertiliser urea or urine.
- be effective at inhibiting soil UA at relatively low concentrations.
- be very specific to block targeted enzymatic reactions (UIs should inhibit only soil UA).
- move closely with the target N compounds. Urease inhibitors should move with the urea molecules of fertiliser urea or urine, which are not quickly absorbed by soil.
- be longer lasting in soil. Urease inhibitors should remain effective for several weeks to inhibit soil UA following urea-N input through fertiliser application and urine deposition by grazing animals.
- not be greatly affected by soil and environmental factors in terms of efficacy. In particular, the effectiveness of UI should not vary much with changes in soil pH, soil temperature, soil organic C, soil moisture content and soil type.
- be stable during storage for long period (long shelf life).
- not possess phytotoxicity and negative effect on beneficial soil microorganisms, at the levels used to inhibit soil UA effectively.
- not possess toxicity to humans and grazing animals at the levels used for inhibiting soil UA effectively.
- be sustainable for use both environmentally and economically.

Among several tested UIs, only hydroxamic acids and phosphorodiamidates have gained importance for agricultural use, mainly due to their high efficiency at low concentrations, non-toxicity, and degradability in the soil (Saggar et al. 2009; Trenkel

2010). However, phosphorodiamidates are stronger inhibitors than hydroxamic acids (Martens and Bremner 1984; Liao and Raines 1985) and, as a result, are more widely used in agriculture (Trenkel 2010). The main phosphorodiamidates evaluated as UI are nBTPT, 2-NPT, N-(n-propyl) thiophosphoric triamide (NPPT), PPDA and thiophosphoryl triamide (TPT) (Schraml et al. 2016; Modolo et al. 2018). Among them, nBTPT is one of the most intensively studied and widely distributed UI (Modolo et al. 2018) and has shown effectiveness in reducing emissions from fertiliser urea and cattle urine in NZ pasture soils (Saggar et al. 2013; Hoogendoorn et al. 2017). In a few studies a recently introduced UI 2-NPT, has been reported to be effective and to last longer than nBTPT in reducing soil UA (Domínguez et al. 2008; Hucke et al. 2010; Saggar et al. 2013).

Micronutrients Cu and Zn have not been widely used as UIs in reducing NH_3 emissions either from urea fertiliser or cattle urine. However, these micronutrients have shown longer effectiveness in reducing soil UA compared to nBTPT in limited studies (Hemida et al. 1997; Wyszkowska et al. 2006; Saggar et al. 2013).

2.6.1.1 Effectiveness of nBTPT

A review of previous NZ studies reported an average reduction of 53% of NH_3 emissions after applying cattle urine with nBTPT (Saggar et al. 2013). In the majority of these experiments urine was mixed with nBTPT before application. A limited number of recent studies (Zaman and Nguyen 2012; Rodriguez 2014; Hoogendoorn et al. 2017) have reported emissions from cattle urine, under conditions similar to real grazing, by applying nBTPT before or after urine deposition. In these studies, the reductions in emissions using nBTPT ranged from 9.6 to 27.6%. However, the inhibitory effect of

nBTPT on soil UA and NH_3 emissions is effective for only a relatively short period of 7-14 days (Saggar et al. 2009). Because of this shorter effectiveness, an application of nBTPT will potentially only reduce emissions from a single grazing, when used to treat urine patches in grazed pastures. Therefore, other UIs with longer lasting inhibitory effects are required to mitigate emissions from deposited urine during more than a single grazing event, in order to reduce the number of applications required.

The short period of effectiveness of nBTPT in inhibiting soil UA and NH_3 emissions is attributed to its degradation in soil. When nBTPT is applied in soil, its oxidation occurs resulting in N-(n-butyl) phosphoric triamide (NBTPO), which is susceptible to degradation through chemical hydrolysis and microbial activity resulting in the decay product n-butylamine (NBA) (Engel et al. 2015).

2.6.1.2 Effectiveness of Cu and Zn

The micronutrients Cu and Zn have been reported to inhibit soil UA over a relatively long durations of 8 weeks when applied at 50 mg kg^{-1} soil (Wyszkowska et al. 2006) and 12 weeks when applied at 200 mg kg^{-1} soil (Hemida et al. 1997). Similarly, Tabatabai (1977) found that Cu at 32 mg kg^{-1} soil and Zn at 33 mg kg^{-1} soil resulted in inhibition of UA by 24 and 7%, respectively, while Cu at 320 mg kg^{-1} soil and Zn at 330 mg kg^{-1} soil achieved 94 and 34% reductions, respectively, when applied on a fine loamy soil. This study also reported that the inhibition of UA was lower in silty clay loam soil, being between 17 and 6% when Cu was applied at 32 mg kg^{-1} soil and Zn was applied at 33 mg kg^{-1} soil, respectively, and between 51 and 30% when Cu was applied at 320 mg kg^{-1} soil and Zn was applied at 330 mg kg^{-1} soil, respectively. The results from this study suggest that Cu rather than Zn can potentially have a high

inhibitory effect which was also observed by Kim et al. (2008). Several soil and environmental conditions that influence the effectiveness of Cu and Zn in inhibiting soil UA are described later in the sub-chapter on the factors affecting the efficacy of UIs (Section 2.6.2).

When Cu and Zn are used to coat urea at mass ratios of 1.8% (Cu:urea) and 3.6% (Zn:urea), NH_3 emissions were reduced by 28 and 19.4%, respectively, in a laboratory incubation study (Khariri et al. 2016). Subsequently, urea coated with Cu (5% (Cu:urea), 400 mg N kg^{-1} soil) or Cu + Zn (5% (Cu:urea) + 5% (Zn:urea), 400 mg N kg^{-1} soil) reduced emissions by 50% relative to urea alone (Junejo et al. 2013). The combined application of urea with 25% potassium chloride (KCl) + 2% copper sulphate ($CuSO_4$) reduced emissions by 24.6%, and enhanced N-use efficiency by 23.9%, respectively (Reddy and Sharma 2000). This study was conducted in India to evaluate the effect of Cu on NH_3 emissions and N-use efficiency in an incubation experiment (200 kg N ha^{-1}) and a pot experiment (120 kg N ha^{-1}), respectively. Similarly, another greenhouse study reported that the combination of urea and zinc sulphate ($ZnSO_4$) (N and Zn content; 33.4 and 10.4% respectively) reduced emissions losses by 22.6, 18.1 and 20.6% from a Vertisol, Inceptisol and Alfisol, respectively (Purakayastha and Katyal 1998).

Inherent soil Cu and Zn levels in NZ dairy-grazed pasture soils vary depending on soil type. Measurements taken from 530 topsoil samples from NZ dairy farms averaged 16 mg kg^{-1} (range 3 - 71 mg kg^{-1}) and 62 mg kg^{-1} (range 9 - 380 mg kg^{-1}) for Cu and Zn, respectively (Taylor 2016). Having large variations in Cu and Zn levels, some of these pasture soils are potentially deficit in Cu (some yellow brown earths, sand

and peat soils) and Zn (some calcareous soils and Waitohi silt loam soils) for plant and animal requirements (McLeod and Quin 1979; Johnson 1995). The wide variations in these elements on pasture soils could also influence differences in soil UA and NH_3 emissions. In addition, application of Cu and Zn to deficit soils may have co-benefits of meeting plant and animal requirements in NZ dairy-grazed pasture soils. Copper and Zn also showed significant inhibitory effects on nitrification activity (Lees 1948; Daif and Van Beusichem 1981; Benbi and Richter 1996; Signor and Cerri 2013; Junejo et al. 2014), and reduced N_2O emissions and NO_3^- leaching. Furthermore, Cu is the cofactor of the enzyme nitrous oxide reductase (*Nos*Z) responsible for conversion of N_2O to N_2 and reduces N_2O emissions by converting it to N_2 (Richardson et al. 2009; Felgate et al. 2012).

In summary, Cu and Zn have shown potential to inhibit soil UA at application rates as low as 32 mg Cu kg^{-1} soil and 33 mg Zn kg^{-1} soil, and for long durations of 8 weeks with 50 mg Cu or Zn kg^{-1} soil and 12 weeks with 200 mg Cu or Zn kg^{-1} soil, with Cu being more effective than Zn. Based on these results, it is possible that these metals could also reduce NH_3 emissions following urine deposition, and their effectiveness may persist for a longer period beyond a single grazing event. However, there are no published data on the effectiveness of Cu and Zn in reducing NH_3 emissions from urine applied to NZ pasture soils. Only a few of overseas studies have evaluated the effect of Cu and Zn on soil UA and NH_3 emissions when applied with urea. Therefore, further research is needed to see the application effect of Cu and Zn on soil UA and NH_3 emissions following cattle urine deposition in pasture soils.

2.6.1.3 Urease inhibition mechanism of Cu and Zn

Copper and Zn ions react with the SH groups of cysteine, essential for the proper functioning of the urease enzyme, on the active site of urease in a similar way to the formation of metal sulfides. Thus, insoluble sulfides forming micronutrients cause inactivation or inhibition of soil UA (Shaw and Raval 1961; Tabatabai 1977; Takishima et al. 1988). This modification of SH groups results in losses of the mobile flap activity and the reaction is disturbed (Krajewska and Zaborska 2007). However, Krajewska (2008) reported that Cu inhibiting UA involved not only binding to SH groups, but also with N - (His) and O - containing (Aspartic and glutamic acids) functional groups. Unlike Zn, Cu inhibits UA by two mechanisms, SH groups blocking and oligomerisation of the enzyme by binding to His residues to form intermolecular His-Cu^{2+}-His bridges (Follmer and Carlini 2005) as presented in Eqn. 2.6 and Eqn. 2.7. The urease inhibition mechanism of non-specific inhibitors Cu and Zn, and specific inhibitors nBTPT and 2-NPT are presented in Fig. 2.1.

$$\text{SH} \ldots + Cu^{2+} \rightarrow \text{CuS} \ldots \rightarrow \text{inhibition of urease} \qquad \text{(Eqn. 2.6)}$$

$$\text{His} \ldots + Cu^{2+} \rightarrow \text{His} - Cu^{2+} - \text{His bridges} \rightarrow \text{inhibition of urease} \qquad \text{(Eqn. 2.7)}$$

The Cu and Zn induced urease inhibition with the modification of the SH groups is reversible, which is evidenced by restoring enzymatic activity fully after treating deactivated enzyme with dithiothreitol, a SH group protectant (Riddles et al. 1983). Dithiothreitol forms specific and stable monomeric and polymeric complexes with Cu and Zn ions, using both of its S donors and checks the formation of metal sulfides in the active site of the enzyme (Krężel et al. 2001). However, Krajewska (2008) reported that

Cu induced inhibition could only be partly reversed as urease inhibition is as a result of binding Cu to N- or O-ligands in addition to SH groups.

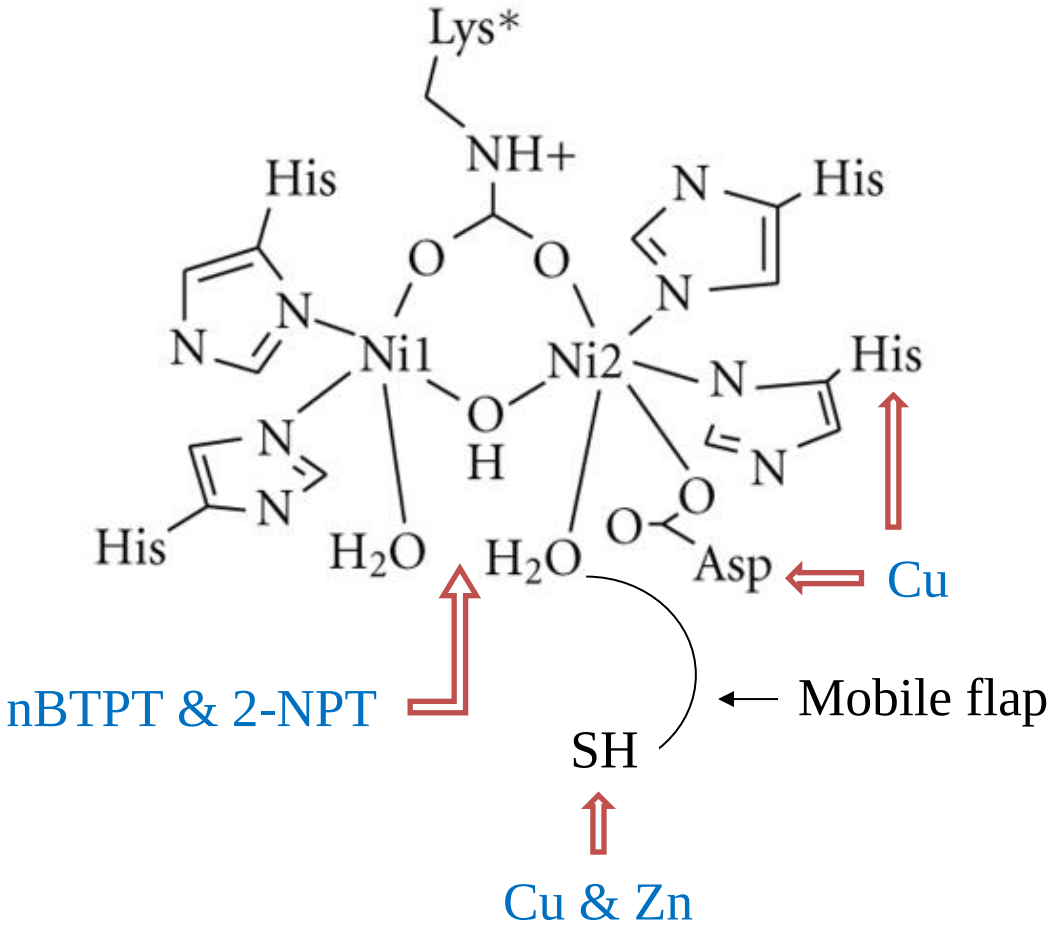

Fig. 2.1 Schematic representation of molecular structure of urease enzyme and proposed urease inhibition mechanisms for urease inhibitors [adapted from Carlsson and Nordlander (2010)]

2.6.1.4 Effectiveness of 2-NPT

A recently introduced UI called 2-NPT has been reported to inhibit soil UA more effectively (65% inhibition after 10 days) and over a longer period (12% inhibition after 30 days) compared to nBTPT (40% inhibition after 10 days) when inhibitors were applied at 0.5% of urea-N (w/w) in an incubation study (Domínguez et

al. 2008). Subsequently, 2-NPT applied at 0.05% of urea-N, resulted in 70% inhibition of UA up to 25 days of application in another incubation study (Hucke et al. 2010). In a recent laboratory study, 2-NPT reduced NH_3 emissions by 89% when applied with urea at 0.075% of urea-N (Ni et al. 2018). In a field study with wheat plants, 2-NPT applied at 0.075% of urea-N decreased NH_3 emissions by 26 - 83% (Ni et al. 2014). In another recent study, Schraml et al. (2016) observed that the application of 2-NPT with fertiliser urea reduced NH_3 emissions by 74 - 84% for a concentration of 0.075 % of total N, by 69 - 88% for a concentration of 0.1% of total N, and by 70 - 100% for a concentration of 0.15 % of total N compared to urea alone. This demonstrated that 2-NPT applied at 0.075 % of total N could be considered an effective level of application, and that there was minimal advantage with using higher rates. Another benefit of 2-NPT is that it has been reported to be more stable during storage than nBTPT. The recovery of inhibitors on urea fertiliser granules after 13 months of storage was more than 95% for 2-NPT while less than 10% for nBTPT (SKW Stickstoffwerke Piesteritz GmbH 2018). Furthermore, it is suggested that 2-NPT does not exert a negative effect on soil microbial activity (Ni et al. 2018).

Overall, studies to date indicate that the effectiveness and longevity of 2-NPT to inhibit soil UA is potentially higher than nBTPT. So, it could be the better option than nBTPT to reduce NH_3 emissions following urine deposition in dairy-grazed pastures. However, 2-NPT has neither been tested for reducing emissions from cattle urine deposited in dairy-grazed pasture soils in NZ nor has there been any comparison between 2-NPT and nBTPT with fertiliser urea or cattle urine. Therefore, further research is needed to determine and compare the effectiveness and longevity of 2-NPT

with nBTPT in reducing NH_3 emissions from cattle urine applied to NZ dairy-grazed pasture soils.

2.6.1.5 Urease inhibition mechanism of 2-NPT

While both the 2-NPT and nBTPT are phosphorodiamidates, they have structural difference of the organic substituent (i.e., -nitrophenyl vs. -butyl groups), and additionally, 2-NPT differs from nBTPT in that an O atom instead of a S atom is bound to the P atom (Modolo et al. 2018). The mechanism of inhibiting soil UA by 2-NPT is the same as nBTPT (Kiss and Simihaian 2002; Domínguez et al. 2008), forming a tridentate ligand with the active site (2 Ni atoms) of urease enzyme in a similar way as nBTPT does, slowing urea hydrolysis (Trenkel 2010).

2.6.2 Factors affecting efficacy of UIs

The efficacy of UIs to inhibit soil UA is not only influenced by its rates of application, but also by several edaphic and environmental factors. The factors affecting efficacy of nBTPT are already described in the published literature (Suter et al. 2011; Saggar et al. 2013; Silva et al. 2017), therefore, this sub-chapter aims to focus more on Cu and Zn, and 2-NPT. With regards to added Cu and Zn, the factors which affect their bioavailability (ionic form) in soil determine the efficacy. This bioavailability is regulated by sorption and desorption of added ionic forms of Cu and Zn in soil. Several soil physical and chemical properties including organic C, clay content, CEC and pH influence these reactions. However, the major drivers that determine the efficacy of 2-NPT are those that affect its persistence in the soil over time. The main factors affecting the efficacy of Cu and Zn, and 2-NPT are discussed below.

2.6.2.1 Soil organic C, clay content and CEC

The effectiveness of Cu largely depends on organic C content of soil because most of the added Cu is immobilized through adsorption and chelation (Arias et al. 2006; Vytopilova et al. 2015), which reduces its bioavailability and vice -versa. As reported by Sauvé et al. (1997), more than 90% of Cu in soil is associated with organic C by binding with humic and fulvic acids present in it (Tipping 2005). Tabatabai (1977) found that the application of Cu at 320 mg kg^{-1} soil inhibited UA by 94% in soil with a C content 3.7%, while inhibition was 51% in a soil with C content of 5.5%. In this study the C content was one of the main differences between the two soils used. The effectiveness of Zn is also negatively correlated with organic C content as its bioavailability is affected by immobilisation with organic C (McBride et al. 1997). Soil textural and mineralogical properties related to organic C such as clay content and CEC also have a role in effectiveness of Cu and Zn in inhibition of soil UA, because higher clay content and CEC possess a greater binding ability and reduced bioavailability of these metals (Kim et al. 2008; Chukwuma et al. 2010). However, there is no information available on the role of organic C, clay content and CEC on the effectiveness of 2-NPT, but it is believed that the effectiveness is inversely proportional to these soil properties because high C, clay content and CEC increases soil UA (Burns et al. 1972; Bremner and Chai 1989; Vahed et al. 2011; Saggar et al. 2013).

2.6.2.2 Soil pH

The effectiveness of added Cu and Zn at inhibiting soil UA could be increased by decreasing soil pH, because at low pH the concentrations of H^+ ions in soil solution is high, which compete with metal cations for binding with humic acids (Tipping 1998). As a result, the concentration of metal ions in soil solution are higher. Soil pH has more

influence in regulating the bioavailability of Zn compared with other soil properties (Degryse et al. 2009). Kappaun et al. (2018) suggested that effectiveness of phosphorodiamidates to inhibit soil UA is less in acidic soils compared to neutral or alkaline soils. The reduction in soil UA by 2-NPT after 30 days of incubation, using a soil with an initial pH 4.5 was 12%, while the reduction was 81% for a soil with a pH 8.4 (Domínguez et al. 2008). However, the effect of differences in initial soil pH on the effectiveness of inhibitors is not likely to be an important factor in NZ dairy-grazed pasture soils because soil pH is typically maintained within a narrow range of 5.8 - 6.0, which is considered as an optimum level.

2.6.2.3 Temperature

None of the studies suggested the direct influence of temperature on Cu and Zn bioavailability, but it could have some impact on their effectiveness because increased temperature enhances soil UA (Makoi and Ndakidemi 2008). Similarly, the effectiveness of 2-NPT could be lower with increasing temperature, as suggested by Ni et al. (2018) because increasing temperature increases soil UA.

2.7 Conclusions

The currently used UI nBTPT has a short duration of effectiveness of 7 - 14 days in reducing NH_3 emissions. While this is adequate for reducing emissions from urea fertiliser because majority of emissions occurs usually within the first week, supressing NH_3 emissions from urine patches will be limited to a single grazing event. In order to reduce the cost of mitigating NH_3 emissions from urine patches, it will be useful to explore alternative mitigation approaches, including other longer lasting UIs, to reduce NH_3 emissions over multiple grazing events. Additions of micronutrients Cu and Zn

have the potential to inhibit soil UA for longer duration up to 12 weeks, with Cu being more effective than Zn. Similarly, a recently introduced UI called 2-NPT has shown more effectiveness and longevity (up to 30 days) than nBTPT to inhibit soil UA. So, the application of Cu, Zn and 2-NPT could be the potential options to mitigate NH_3 emissions following urine deposition for more than a single grazing event in dairy-grazed pastures. However, there is little research information on the effect of these inhibitors at reducing soil UA and NH_3 emissions. Therefore, this research focuses on assessing the effectiveness of Cu, Zn and 2-NPT at reducing NH_3 emissions from cattle urine deposited in dairy-grazed pastures.

Chapter 3

Effect of inherent and added copper and zinc on soil urease activity of dairy-grazed pasture soils

3.1 Introduction

The NH_3 emitted from urine deposited by grazing cattle is one of the major issues contributing to inefficient N use in NZ dairy-grazed pasture soils, as already discussed in the Introduction (Chapter 1). Soil UA influences the rate of urea hydrolysis in the soil environment and the potential for NH_3 emissions to the atmosphere (Gould et al. 1973). Currently, the inhibition of soil UA with UIs is one of the approaches used for reducing NH_3 emissions particularly from fertiliser urea. However, for UIs to be useful for mitigating emissions from urine deposited during grazing by livestock, their effectiveness at inhibition of soil UA need to last longer than a single grazing round (i.e. longer than 25 - 35 days), so that UIs are not required to be applied after each grazing. The effectiveness of the commonly used UI, nBTPT, is relatively short-lived, lasting for a period of 7 - 14 days (Saggar et al. 2009), which will reduce emissions during only for a single grazing event. A field study (Rodriguez 2014) reported the reductions in emissions by 27.6 and 17.5% when nBTPT was applied 5 and 3 days prior to urine application, respectively. It is, therefore, necessary to consider alternative UIs with longer stability and effectiveness in dairy pasture soils to be of practical use at NH_3 emissions from urine patches.

Micronutrients such as Cu and Zn have been shown to inhibit soil UA over a longer duration (8 - 12 weeks). This inhibitory effect has been associated with the level

of Cu and Zn applied; for example, 8 weeks with 50 mg Cu or Zn kg^{-1} soil (Wyszkowska et al. 2006) and 12 weeks with 200 mg Cu or Zn kg^{-1} soil (Hemida et al. 1997). Efficiency of Cu and Zn in inhibiting soil UA is also affected by the physical and chemical properties of the soil because soil properties affect their bioavailability (Tabatabai 1977). The bioavailability of inherent Cu and Zn is influenced by soil pH, organic C content, soil texture and CEC. A large proportion of Cu present in the soil solid phase is immobile because of association with organic C (Ashworth and Alloway 2007), leading to its reduced bioavailability. Bioavailability of Zn is negatively correlated with soil pH, and pH has more influence on bioavailability of Zn compared with other soil properties (Degryse et al. 2009).

Inherent levels of these micronutrients in NZ dairy-grazed pasture soils are in the range of 3 - 71 and 9 - 380 mg kg^{-1} for Cu and Zn respectively with corresponding averages of 16 and 62 mg kg^{-1} (Taylor 2016). These variations in Cu and Zn status of soils or the addition of soluble forms of Cu and Zn could potentially influence differences in soil UA and NH_3 emissions. However, there is minimal NZ and international research information on the influence of inherent soil Cu and Zn, or the application effect of these micronutrients on soil UA and on NH_3 emissions. Therefore, two experiments were conducted in this study with the overall objective of assessing the effectiveness of Cu and Zn in reducing soil UA, for the use of these metals to reduce NH_3 emissions from urine deposited by grazing cattle. The specific objectives of the study reported in this chapter were:

- To assess the relationship between inherent Cu and Zn and soil UA of dairy-grazed pasture soils.
- To determine the effect of Cu and Zn additions on soil UA.

3.2 Materials and methods

3.2.1 Experiment 1: Relationship of inherent Cu and Zn with soil UA

3.2.1.1 Experimental details

Samples of different dairy-grazed pasture soils from 23 sites from Waikato region, NZ, with contrasting inherent Cu and Zn status, and soil carbon C values (Table 3.1) were used to assess the relationship between these soil properties and soil UA. At each site a total of 25 soil cores were collected using a tube auger (diameter 2.5 cm and 0-10 cm soil depth) at 2-m intervals along the 50-m transect line. The individual soil cores collected at each site were bulked together, sieved through a 2-mm sieve and mixed thoroughly in a plastic bag. Soils were classified based on soil order using the Food and Agriculture Organization (FAO) soil classification system (FAO–UNESCO 1998). All chemical analyses were performed with duplicates of composite samples.

3.2.1.2 Measurement of Cu and Zn

Five different extractants of varying degrees of extractability were used to measure extractable Cu and Zn: (i) total acid-extractable following a nitric/hydrochloric acid digestion procedure (Martin et al. 1994); (ii) ethylene-diamine-tetra-acetic acid (EDTA)-extractable; (iii) strontium chloride ($SrCl_2$)-citric acid-extractable; (iv) calcium nitrate ($Ca(NO_3)_2$)-extractable; and (v) calcium chloride ($CaCl_2$)-extractable. The details for EDTA, $SrCl_2$-citric acid, $Ca(NO_3)_2$ and $CaCl_2$ extractions are presented in Table 3.2. The suspension was filtered using Whatman No. 42 filter paper and the extracts were stored in a refrigerator before being analysed for metal content with an Inductively coupled plasma mass spectrometer (PerkinElmer, Waltham, USA) and Microwave plasma atomic emission spectrometer (Agilent Technologies, Yishun Ave, Singapore). The number of soils included in each extraction technique is presented in Table 3.3.

Table 3.1 Characteristics of Waikato dairy-grazed pasture soils studied, urease activity (UA)

Soil order	Soil pH	Soil total C (%)	Soil total N (%)	Total Cu (mg kg^{-1} air-dry soil)	Total Zn (mg kg^{-1} air-dry soil)	UA (mg kg^{-1} air-dry soil hr^{-1})
Podzol	6.2	6.2	0.4	4.7	13.1	36.2
Andosol	5.7	7.8	0.7	5.4	30.0	41.0
Cambisol	6.3	3.7	0.3	8.6	58.0	45.2
Andosol	5.6	8.3	0.8	8.8	33.0	38.5
Andosol	5.8	7.2	0.6	8.8	22.0	28.5
Andosol	6.2	10.8	1.0	16.0	90.0	175.5
Gleysol	5.9	10.7	0.9	16.0	42.0	66.6
Andosol	6.1	7.3	0.7	16.4	73.0	64.0
Gleysol	6.0	9.4	0.8	21.0	56.0	46.8
Fluvisol	6.0	5.8	0.6	23.0	85.0	38.7
Andosol	6.4	9.2	0.9	24.0	120.0	99.9
Gleysol	5.6	6.7	0.9	29.0	125.0	54.9
Gleysol	6.1	6.2	0.7	31.0	102.0	28.0
Allophanic	5.8	10.2	1.1	38.0	129.0	91.2
Andosol	6.4	12.1	1.3	39.0	78.0	158.5
Andosol	5.8	6.0	0.5	5.6	19.3	25.5
Gleysol	6.1	7.9	0.7	14.1	43.0	31.5
Gleysol	6.1	7.9	0.6	19.8	46.0	63.3
Cambisol	6.3	8.7	0.8	9.8	47.0	148.0
Cambisol	6.1	3.6	0.4	12.3	59.0	9.7
Cambisol	5.7	4.3	0.4	12.8	79.0	28.0
Gleysol	6.9	4.3	0.4	20.0	82.0	15.0
Andosol	6.0	8.4	0.8	18.2	106.0	82.5

Table 3.2 Measurement techniques used in the determination of extractable Cu and Zn with end-over-end shaker

Extractant	Soil air-dry weight (g)	Volume of extractant (mL)	Shaking time (hr)	Centrifugation	
				Time (min)	Relative centrifugal force (× g)
0.04 M EDTA	20	25	2.0	15	470
0.02 M $SrCl_2$ + 0.05 M citric acid	3	20	0.5	10	26916
0.05 M $Ca(NO_3)_2$	5	30	2.0	10	26916
0.05 M $CaCl_2$	5	30	2.0	10	2960

Table 3.3 Coefficient of determination (R^2) of soil urease activity (UA) with different methods of determination of soil Cu and Zn status of dairy-grazed pasture soils

Method	No. of soils	Cu	Zn
Total acid-extractable	23	0.1	0.1
EDTA-extractable	22	<0.1	0.3*
$SrCl_2$-citric acid-extractable	16	<0.1	0.4*
$Ca(NO_3)_2$-extractable	15	–	0.1
$CaCl_2$-extractable	15	–	–

Absence of symbol following number indicates no significant relationship; * indicates significant positive correlation ($P < 0.05$)

3.2.1.3 Measurement of soil UA

Soil UA was determined using a slightly modified non-buffer method (Zantua and Bremner 1975; Mulvaney and Bremner 1979). Briefly, 2.5 g of air-dry equivalent moist soil samples were incubated at 20°C for 5 hrs after treating with 2.5 mL of deionised (DI) water containing urea-N, at the concentration of 1000 mg N kg^{-1} air-dry soil. After incubation, the soil was shaken with 25 mL of 2 M KCl, containing 25 µg

mL^{-1} of phenylmercuric acetate (as a UI), on an end-over-end shaker for 60 min and extracted for urea by filtering through Whatman No. 42 filter paper. The amount of urea present in the extract was determined by a colorimetric procedure. Following required dilution, 1 mL of soil extract was added into a glass test tube and mixed with 3 mL of colour reagent (mixture of 5 mL of diacetyl monoxime solution and 3 mL of thiosemicarbazide solution, diluted to 100 mL with acid reagent). The test tubes were placed in a water bath maintained at 85 °C for 30 min. The test tubes were removed from the water bath and cooled for 10 min in a running tap water (12-15°C) to avoid colour loss at warmer temperatures. One mL of deionised (DI) water was added to make the contents to 5 mL and then mixed thoroughly. The colour intensity measurement of the solution was performed by using a Spectrophotometer (Bibby Scientific Ltd, Stone, Staffs, UK) set to a wavelength of 527 nm. Urea N content of the extract was calculated by reference to a calibration graph plotted from the colour intensities obtained with urea N standards. Four reagent blanks were also included in the analyses. Soil UA (mg kg^{-1}air-dry soil hr^{-1}) was measured as the amount of urea hydrolysed (Eqn. 3.1) in soil samples during the incubation period.

$$\text{Soil UA} = \frac{(U_B - U_E) \times V}{W \times D} \qquad \text{(Eqn. 3.1)}$$

where, U_B = average urea present in the reagent blanks (µg mL^{-1}), U_E = urea present in the soil extract (µg mL^{-1}), V = total volume of extract (mL), W = weight of air-dry soil (g), and D = duration of incubation (hr).

3.2.1.4 Analyses

3.2.1.4.1 pH

The soil pH was determined by adding 10 g of 2-mm sieved air-dry soil to 25 mL DI water (1:2.5 soil : water ratio), which was stirred and left for up to 16 hrs before

the solution pH was measured using a pH meter (Hanna Instruments, Romania) (Blakemore et al. 1987).

3.2.1.4.2 Total C and total N

The air-dried soil samples were finely ground using a mortar and pestle, and total C and total N concentrations were measured using a vario MACRO cube CHNS elemental analyser (Elementar Analysensysteme GmbH, Germany), which involves high temperature combustion.

3.2.2 Experiment 2: Effect of Cu and Zn additions on soil UA

3.2.2.1 Experimental details

Four dairy-grazed pasture soils were selected to assess the effect of adding different amounts Cu and Zn to soils on UA, collected from three regions: (i) Manawatu - two soils, Fluvisol (Rangitikei loamy sand, RLS) and Cambisol and Alisol (Tokomaru silt loam,TSL), (ii) Waikato - one soil, Andosol, Horotiu silt loam, HSL and (iii) Taranaki - one soil, Andosol, Egmont black loam, EBL, with contrasting soil organic C, and textural and mineralogical properties (Table 3.4). The Waikato soil used in this experiment was additional to the 23 Waikato soils used in Experiment 1 (Section 3.2.1) as it was selected to represent contrasts in soil organic C and soil texture. At each site a total of 200 soil cores were collected using a soil corer (diameter 2.5 cm and soil depth 0-10 cm) at 2-m intervals (four parallel cores from each point at 30-cm intervals) along the 100-m transect line. The individual soil cores collected at each site were bulked together, sieved through a 2-mm sieve and mixed thoroughly in a plastic bag. All treatments and chemical analyses were performed with four analytical replicates.

Table 3.4 Selected physical and chemical properties of dairy-grazed pasture soils studied

Soil properties	Rangitikei loamy sand (RLS)	Tokomaru silt loam (TSL)	Horotiu silt loam (HSL)	Egmont black loam (EBL)
pH water	5.5	5.5	5.4	5.4
Water holding capacity (%)	17.5	29.1	38.6	53.3
Total C (%)	1.8	3.0	4.6	9.7
Total N (%)	0.2	0.3	0.5	1.0
Sand (%)	60	10	30	40
Silt (%)	30	60	52	40
Clay (%)	10	30	18	20
Exchangeable K (meq 100 g^{-1})	0.3	0.2	1.5	0.6
Exchangeable Ca (meq 100 g^{-1})	5.8	7.1	7.7	12.3
Exchangeable Mg (meq 100 g^{-1})	0.8	1.3	1.5	1.9
Exchangeable Na (meq 100 g^{-1})	0.1	0.2	0.2	0.3
CEC (meq 100 g^{-1})	15.1	20.4	29.1	42.9

3.2.2.2 Treatments and measurements

Standard solutions containing Cu and Zn were prepared in DI water using $CuSO_4.5H_2O$ and $ZnSO_4.7H_2O$ and added to 2.5 g of oven dry equivalent soil samples (all four soil types) to supply 0, 5, 10, and 20 mg Cu kg^{-1} soil, and 5 mg Cu + 5 mg Zn kg^{-1} soil along with urea-N solution. The combination of each metal treatment, urea-N solution and DI water together provided a total solution volume of 2.5 mL added to each soil sample, which achieved a total of 1000 mg N kg^{-1} dry soil for each treatment, and UA was determined immediately after treatment application. Additionally, the same Cu and Zn treatments were added to a single soil (RLS soil) without urea-N solution, and $Ca(NO_3)_2$-extractable Cu and Zn were measured immediately after treatment application. The RLS soil was chosen because it had the lowest organic C, clay content

and CEC of the soils used in this study. Soil UA and $Ca(NO_3)_2$-extractable Cu and Zn were determined as mentioned in section 3.2.1.3 and section 3.2.1.2, respectively.

3.2.2.3 Analyses

3.2.2.3.1 pH, total C, and total N

Soil pH, total C and total N were measured as mentioned in section 3.2.1.4.

3.2.2.3.2 Water holding capacity

The water holding capacity (WHC) of the sieved soil, as used in the soil urease inhibition study, was determined using Pressure plate apparatus (Loveday 1974). Soil samples were saturated with water on a pressure plate and a pressure of 0.3 bar was applied. The assembly was kept undisturbed until the water flow ceased from the plate and then the moisture content was determined gravimetrically by drying the soil at 105°C for 24 hrs. The measured soil moisture content corresponded to the WHC of the soil.

3.2.2.3.3 Cations and CEC

Exchangeable basic cations and CEC were measured following a semi-micro leaching technique (Blakemore et al. 1987). Briefly, the mixture of 1 g of air-dried soil and approximately 3 g of analytical sand was transferred into the leaching tubes blocked with moistened Whatman No. 541 filter paper. The samples were then leached with 1 M ammonium acetate ($NH_4CH_3CO_2$) adjusted to pH 7.00 ± 0.05 for 45 min at a rate of 1 mL min^{-1} using a proportioning pump (Technicon LTD, Ireland). Leachates collected in plastic cups were topped up to 50 mL with 1 M $NH_4CH_3CO_2$ on weight basis and pH was measured using a pH meter. Before being analysed for cations Ca^{2+}, Mg^{2+}, K^+ and

Na^+ using Microwave plasma atomic emission spectrometer, leachates were spiked with 2 mL of 26,000 mg L^{-1} strontium (Sr), cesium (Cs) solution to keep it at 1000 mg L^{-1} Sr, Cs. The total CEC was calculated using the results for pH and cations.

3.2.3 Statistical methods

The data obtained in Experiment 1 were used in a linear regression to determine the relationship of soil UA with inherent Cu and Zn, and total C and N. The data from Experiment 2 were analysed using an Analysis of Variance (ANOVA) to detect any significant difference and different means in the treatments were compared using Tukey's Studentized Range (HSD) Test. All analyses were conducted using Statistical Analysis System software (SAS 9.4, $P < 0.05$).

3.3 Results

3.3.1 Relationship of inherent Cu and Zn with soil UA

Soil total Cu, total Zn, total C, total N and soil UA of the 23 soils studied are presented in Table 3.1 as concentrations on a per kg air dried soil basis. The amounts of Cu and Zn extracted varied with the acidity of these extractants. The total acid-extractable Cu concentrations ranged within 4.7 - 39 mg kg^{-1} and the total acid recoverable Zn levels were 13.1 - 129 mg kg^{-1}. The ranges of EDTA-extractable Cu and Zn values were 1.8 - 14.2 mg kg^{-1} and 5.6 - 35.4 mg kg^{-1}, respectively. The observed levels of $SrCl_2$-citric acid-extractable Cu ranged between 0.2 - 4.3 mg kg^{-1}. Similarly, $SrCl_2$-citric acid-extractable Zn ranged from 12.1 - 37.9 mg kg^{-1}. The concentrations of $Ca(NO_3)_2$-extractable Cu, $CaCl_2$-extractable Cu, and $CaCl_2$-extractable Zn were below detectable levels. However, concentrations of $Ca(NO_3)_2$-extractable Zn were detectable and ranged between 0.5 - 2.9 mg kg^{-1}. The total C contents were 3.6 - 12.1% and the

total N ranged from 0.3 - 1.3%. Soil UA of these soils varied within 9.7 - 175.5 mg kg^{-1} hr^{-1}. There were significant positive correlations of soil UA with soil total C and total N levels (Fig. 3.1).

Among all tested correlations of soil Cu and Zn concentrations with soil UA, measured using different extractants, only EDTA-extractable Zn and $SrCl_2$-citric acid-extractable Zn were positively correlated with UA (Fig. 3.2). The EDTA-extractable Zn and $SrCl_2$-citric acid-extractable Zn were also positively correlated with soil C (Fig. 3.3). No negative correlations were observed for soil Cu and Zn levels with soil UA, determined by the range of extractants (Table 3.3).

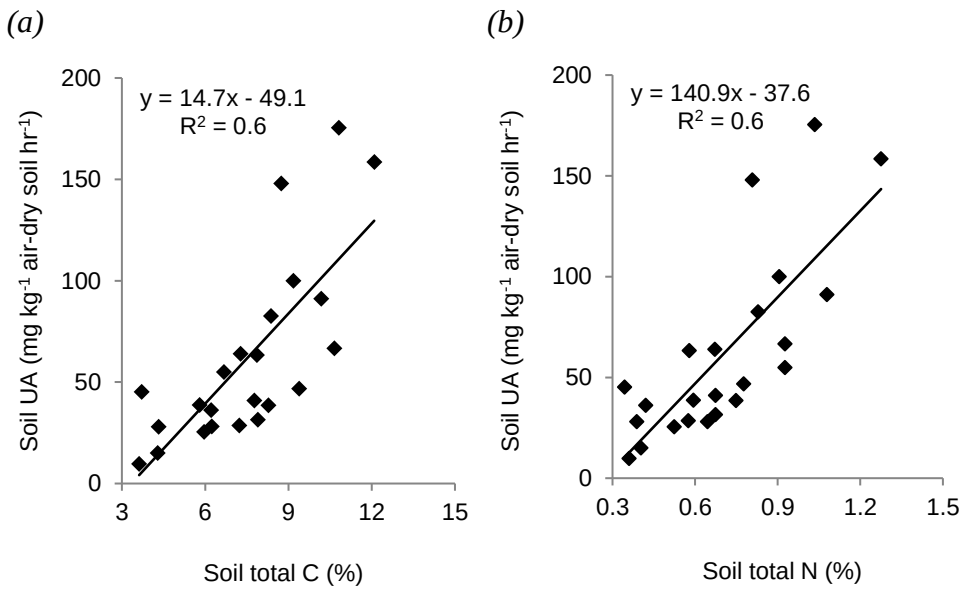

Fig. 3.1 ***(a)*** **Relationship between soil total C level and soil urease activity (UA) of dairy-grazed pasture soils** ***(b)*** **Relationship between soil total N level and soil UA of dairy-grazed pasture soils (P < 0.05)**

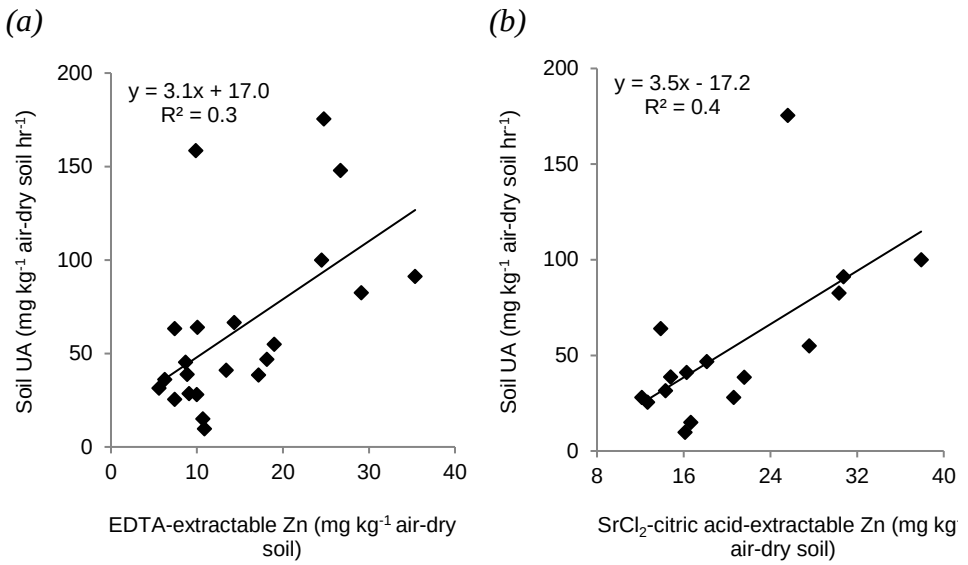

Fig. 3.2 ***(a)*** **Relationship between ethylene-diamine-tetra-acetic acid (EDTA)-extractable Zn and soil urease activity (UA) of dairy-grazed pasture soils** ***(b)*** **Relationship between strontium chloride ($SrCl_2$)-citric acid-extractable Zn and soil UA of dairy-grazed pasture soils ($P < 0.05$)**

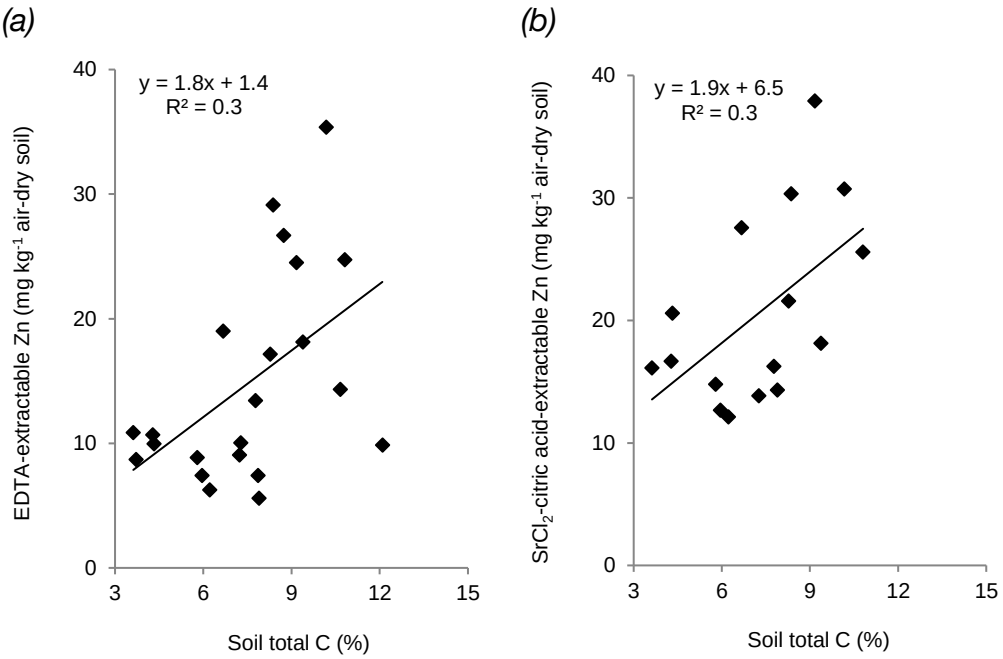

Fig. 3.3 ***(a)*** **Relationship between soil total C level and ethylene-diamine-tetra-acetic acid (EDTA)-extractable Zn of dairy-grazed pasture soils** ***(b)*** **Relationship between soil total C level and strontium chloride ($SrCl_2$)-citric acid-extractable Zn of dairy-grazed pasture soils ($P < 0.05$)**

3.3.2 Effect of Cu and Zn additions on soil UA

The total C, total N, exchangeable bases, CEC values and sand, silt and clay contents for the studied soils are presented in Table 3.4. The total C contents for soils ranged within 1.8 - 9.7% and UA values were 16.8 - 61.5 mg kg^{-1} soil hr^{-1} (Table 3.5). There were no significant reductions in soil UA values from any of the added Cu and Zn treatments. Soil UA values in the control increased with corresponding increase in soil organic C values.

Table 3.5 Effect of Cu and Zn application on soil urease activity (UA) of dairy-grazed pasture soils

Cu (mg kg^{-1} soil)	UA (mg kg^{-1} soil hr^{-1})			
	Rangitikei loamy sand (RLS)	Tokomaru silt loam (TSL)	Horotiu silt loam (HSL)	Egmont black loam (EBL)
0	16.8 [a]	22.2 [a]	51.6 [a]	61.5 [a]
5	16.4 [a]	22.2 [a]	51.6 [a]	62.2 [a]
10	17.1 [a]	21.7 [a]	47.9 [a]	63.3 [a]
20	17.8 [a]	23.1 [a]	48.2 [a]	61.5 [a]
5 + 5 (Cu + Zn)	16.9 [a]	21.3 [a]	49.2 [a]	63.3 [a]

Means followed by different small letters in a column are significantly different ($P < 0.05$)

The $Ca(NO_3)_2$-extractable Cu and Zn values measured immediately after addition of Cu and Zn treatments in RLS soil are reported in Table 3.6. For treatments with Cu, most of the added Cu was not $Ca(NO_3)_2$-extractable. The $Ca(NO_3)_2$-extractable Cu was less than 5% of added Cu even for the highest Cu treatment (20 mg Cu kg^{-1} soil). However, Zn remained highly $Ca(NO_3)_2$-extractable, with 75.2% of Zn added extracted.

Table 3.6 $Ca(NO_3)_2$-extractable Cu and Zn after addition of these metal on the Rangitikei loamy sand (RLS) soil

Cu (mg kg^{-1} soil)	$Ca(NO_3)_2$-extractable metals (mg kg^{-1} soil)	
	Cu	Zn
0	–	0.5
5	0.1	0.8
10	0.2	0.7
20	0.8	0.7
5+5 (Cu+Zn)	0.3	4.3

3.4 Discussion

3.4.1 Relationship of inherent Cu and Zn with soil UA

The total acid-extractable Cu and Zn levels in soils used in this study are similar to the total acid-extractable concentrations of Cu in the range of 3 - 71 mg kg^{-1} and Zn of 9 - 380 mg kg^{-1} in top soil samples from NZ dairy farms reported by Taylor (2016) and were well within the soil limits or environmental guideline values for Cu and Zn in NZ: 100 and 300 mg kg^{-1} soil, respectively (New Zealand Water and Wastes Association 2003). Therefore, the values obtained for the soils used in this study are comparable with results of previous studies.

The significant positive correlations of soil UA with soil total C and total N levels measured in this study are consistent with earlier findings for NZ grazed pastures (Speir et al. 1984) and with international studies (Zantua et al. 1977; Nourbakhsh and Monreal 2004). Growth of microorganisms in soils is associated with availability of C and N. These organisms receive N mainly from intracellular urea hydrolysis, and urease is produced within microbial cells (Dharmakeerthi and Thenabadu 1996). As microbial

cells die, they release urease which is adsorbed on soil colloids as an extracellular enzyme. Thus, a positive correlation between soil UA and soil total C and total N content is generally observed (Vahed et al. 2011) due to higher microbial growth and activity with increasing soil C levels because of soil microbial biomass, normally constituting 1 - 2% of soil organic C levels in NZ pasture soils (Saggar et al. 1999), in soils with higher soil organic C levels.

Although there were significant positive correlations of soil UA with EDTA- and $SrCl_2$-citric acid-extractable Zn, this relationship is likely due to both variables increasing with soil C (Figs. 3.1 *(a)* and 3.3) contents rather than there being causation between them. Results from this study indicated no evidence of soil Cu and Zn levels measured using different extractants having a negative effect on soil UA. Similarly, Bardgett et al. (1994) observed no significant relationship between total Cu (range 25 - 1325 mg kg^{-1} soil) from 10 contaminated NZ pasture soils contrasting in soil C and soil UA. The lack of such a relationship could possibly be attributed to either the inability of these tests to adequately represent the bioavailability of these metals for microorganisms and plants or the observed levels of bioavailable metals having limited effect on soil UA. The total acid-extractable method measures all forms of Cu, except residual. The EDTA potentially extracts mobile portions of metal-ions in soil, including organically complexed metals, but also does not provide a direct measure of ionic forms that could potentially influence soil UA. The $SrCl_2$-citric acid solution may also extract some of the organic pool, as the acid extractants are based on reducing the pH and the consequent solubilization of compounds containing these elements (Bibiso et al. 2016) rather than representing metal bioavailability. Hence, the lack of observed relationships of total acid-, EDTA- and $SrCl_2$-citric acid-extractable measurements with soil UA may

be attributed to the inability of these methods to represent the bioavailability (ionic forms) of Cu and Zn.

The Cu extracted using neutral salt extractants, such as $Ca(NO_3)_2$ and $CaCl_2$, and Zn levels extracted using $CaCl_2$, were below detectable levels. These extractants potentially approximate soluble plus exchangeable metals and provide an estimation of the immediately bioavailable metal (Nolan et al. 2005), but it was not possible to show any correlations of soil UA with these measures because metal concentrations were below detectable levels. The non-detectable levels of extracted metals with the use of these extractants could be attributed to complexation of metals in NZ dairy-grazed pasture soils rich in organic C and reduces the soluble plus exchangeable pool. Although the $Ca(NO_3)_2$-extractable Zn was at a detectable level, the current study showed no significant negative correlation between observed levels of $Ca(NO_3)_2$-extractable Zn and soil UA - indicating that, even when Zn was present in a bioavailable form - soil UA inhibition was not observed.

3.4.2 Effect of Cu and Zn additions on soil UA

No inhibition in UA was observed in all four soils with addition of up to 20 mg Cu kg^{-1} soil and the combination of 5 mg Cu and 5 mg Zn kg^{-1} soil. The ineffectiveness of Cu is likely attributable to the enhanced immobilisation of applied Cu ions with organic C and clay particles through adsorption and chelation in dairy-grazed pasture soils rich in organic C (Arias et al. 2006; Vytopilova et al. 2015). In the current study, the lower proportion (<5%) of Cu added was measured as $Ca(NO_3)_2$-extractable Cu in the RLS soil, supporting low bioavailability of the added Cu being a cause of its ineffectiveness. Even though the bioavailability of added Zn remained high, it was still ineffective at inhibiting UA. This result is in contrast to the literature discussed in the

introduction section for which the applied concentrations of Cu and Zn were higher and soils had different physiochemical properties (organic C content, textural and mineralogical attributes) compared with soils in our study. For example, soil studied by Wyszkowska et al. (2006) had an organic C content of 0.7% and Cu and Zn were applied at 50 mg kg^{-1} soil. Although literature showed successful inhibition of soil UA with high additions of Cu and Zn, we decided not to exceed 20 mg kg^{-1} soil. This decision was based on the practicality of continued application of Cu and Zn with minimum long-term risk and implications of heavy metal pollution by remaining well within the soil limits or environmental guideline values for Cu and Zn of 100 and 300 mg kg^{-1} soil in NZ soils, respectively. The application rates of Cu used in this study were higher than the Cu normally applied to the NZ grazed pastures soils (1 to 5 kg ha^{-1} yr^{-1}) to meet plant/animal demand.

In this study, the measured Cu and Zn levels did not show negative relationships with UA in soils, confirming the inability of soil inherent Cu and Zn to inhibit UA and mitigate NH_3 emissions. Furthermore, Cu and Zn additions to soils were also not effective in inhibiting soil UA. This ineffectiveness of added Cu is attributed to complexation of Cu ions with organic C and clay particles present and reduced bioavailable Cu (ionic form). Although most of the added Zn remained bioavailable, the observed levels of free Zn ions likely had a minimal effect on deactivating the enzyme.

3.5 Conclusions

For the dairy-grazed pasture soils from the Waikato region, there were positive relationships of UA with soil total C and total N levels. Although there were differences in UA between the soils, there was no clear influence of any measure of inherent soil Cu

or Zn, using various methods, on decreasing UA. Potentially bioavailable Cu was undetectable in these soils, likely due to complexation of Cu in organic C-rich soil, and would help explain the ineffectiveness of Cu. When Cu was added to four dairy-grazed pasture soils from three different regions, the bioavailability of this Cu remained low and consequently was not effective at reducing UA. Zinc was not an effective as a UI, even though its bioavailability was sustained in pastoral soils. Therefore, neither metal will be practical UIs for NZ dairy-grazed pasture soils.

Chapter 4

Understanding the ineffectiveness of copper and zinc in reducing soil urease activity in dairy-grazed pasture soils

4.1 Introduction

The results in Chapter 3 indicated that both Cu and Zn are ineffective at reducing soil UA in dairy-grazed pasture soils. The ineffectiveness of Cu was related to its low bioavailability. Although most of the added Zn remained bioavailable, the observed levels had a minimal effect on inhibiting soil UA. Some earlier studies also reported the complexation of Cu with soil organic C (Sauvé et al. 1997), which may reduce their bioavailability and subsequent inhibition of UA and urea hydrolysis. Kim et al. (2008) reported that soil textural and mineralogical properties related to organic C such as clay content and CEC also have a role in regulating the bioavailability of added Cu and Zn and their effectiveness in inhibition of soil UA, because higher clay content and CEC possess a greater binding ability and reduced bioavailability, and vice-versa. However, to our knowledge, no study has evaluated the bioavailability of Cu and Zn in soil solutions. Our assumption was that organic C is the main component for complexation of Cu in soil and soil supernatants may have potentially low C and reduce complexation. The influence of soil organic C, and other properties such as clay content and CEC in inhibition cannot be isolated as these are related, and all these factors together are termed the ‘C factor’ in the subsequent discussion in this thesis. However, there are few studies conducted to assess the role of soil C factor on the effectiveness of Cu and Zn to inhibit urea hydrolysis. Therefore, an experiment was conducted in this study with the overall objective of understanding the processes that regulate Cu and Zn

inhibition of soil UA and provide explanations for their ineffectiveness in dairy pasture soils. The specific objectives of the study reported in this chapter were:

- To investigate the role of soil C factor on the effectiveness of Cu and Zn inhibiting urea hydrolysis.
- To assess the impact of soil C factor on bioavailability of added Cu and Zn.

4.2 Materials and methods

4.2.1 Experimental details

This study investigated the role of soil C factor in the effectiveness of added Cu and Zn in reducing urea hydrolysis, using soil supernatants from two dairy farm soils: RLS, which had the lowest organic C, clay content and CEC; and EBL, which had the highest organic C and CEC and higher clay content. The physical and chemical properties of soils used are described in Chapter 3 (Section 3.2.2.1). About 2400 mL of 0.01 M potassium sulfate (K_2SO_4), representing the ionic strength of soil solution, was added to 1200 g of moist soil (untreated control) pre-incubated for 7 days (80% of WHC, equivalent to 1052.7 and 841.3 g oven-dry soil for the RLS and EBL soils, respectively) and shaken (end-over-end shaker) for 30 min. The mixture was then allowed to settle (2 and 4 hrs for RLS and EBL soils, respectively). Supernatants were collected and stored in two plastic containers separately at room temperature. All subsequent measurements and treatment additions to the supernatants are expressed on a soil oven-dry weight basis for the soil originally extracted. Analytical grade glucose was added to the supernatants as a C source at the rate of 2000 mg C kg^{-1} soil. The supernatants were then pre-incubated for 5 days at 20°C to establish the microbial population and microbial activity and, thereby, also increase UA before treatment application.

4.2.2 Treatments and measurements

The treatments included two additions of urea-N, 120 and 600 mg N kg^{-1} soil, and combinations with and without Cu or Zn to the soil supernatants with glucose:

i) Urea (120 and 600 mg N kg^{-1} soil)

ii) 5 mg Cu kg^{-1} soil + urea (120 and 600 mg N kg^{-1} soil) (5-CuU)

iii) 10 mg Cu kg^{-1} soil + urea (120 and 600 mg N kg^{-1} soil) (10-CuU)

iv) 20 mg Cu kg^{-1} soil + urea (120 and 600 mg N kg^{-1} soil) (20-CuU)

v) 20 mg Zn kg^{-1} soil + urea (120 and 600 mg N kg^{-1} soil) (20-ZnU).

Two additional treatments were also used for comparison, which did not receive Cu or Zn addition. One treatment involved the supernatant without addition of glucose but with only a single addition of urea of 120 mg kg^{-1} soil and the other treatment was a supernatant with glucose addition but without urea.

Supernatants were incubated at 20°C for 32 days and during this period subsamples were taken and analysed for urea hydrolysis which was determined from the amount of NH_4^+-N, and NO_3^--N present (Blakemore et al. 1987), using a Technicon AutoAnalyzer (Technicon LTD, Ireland). In addition, $Ca(NO_3)_2$-extractable Cu and $Ca(NO_3)_2$-extractable Zn (measured as mentioned in Chapter 3, section 3.2.1.2) were measured on day 1, 2, 4, 8,16, and 32 following treatment application. Four analytical replicates were established prior to treatment application. The percentage reduction in urea hydrolysis with the addition of Cu and Zn treatments to supernatants were calculated as in Eqn. 4.1.

$$\text{Reduction in urea hydrolysis (\%)} = \frac{(A_U - A_M)}{A_U} \times 100\ \% \qquad \text{(Eqn. 4.1)}$$

where, A_U = cumulative ammonium present in urea-only treatment (mg kg^{-1} soil) and A_M = cumulative ammonium present in treatment with metal (mg kg^{-1} soil).

4.2.3 Soluble C analysis

Following pre-incubation and before treatment application, soluble C contents in the supernatants were measured using a TOC Analyser (Elementar, Langenselbold, Germany).

4.2.4 Statistical methods

The data for urea hydrolysis, NO_3^--N levels and $Ca(NO_3)_2$)-extractable Cu and Zn levels were analysed using an ANOVA to detect any significant difference and different means in the treatments were compared using Tukey's Studentized Range (HSD) Test. Additionally, data for urea hydrolysis and NO_3^--N levels were analysed using a Kruskal–Wallis test where the data sets did not meet normality requirement even after transformations, to detect any significant difference and different means in the treatments were compared using Bonferroni (Dunn) *t*-test. All analyses were conducted using Statistical Analysis System software (SAS 9.4, $P < 0.05$).

4.3 Results

The addition of Cu to soil supernatants was effective at reducing urea hydrolysis, but the effect varied with Cu addition and soil type. The effect of Zn treatment on reducing urea hydrolysis was small and short lived compared with the higher Cu treatments. Among the three Cu additions, the highest Cu treatment (20-CuU) was the most effective with both supernatants. The effectiveness of Cu was sustained

longer in the RLS supernatant (Fig. 4.1) compared with the EBL supernatant (Fig. 4.2). With the RLS supernatant, all three Cu treatments were effective at reducing urea hydrolysis. During the first 8 days, all Cu treatments reduced urea hydrolysis by 52.1% or greater at both N rates (120 and 600 mg N kg^{-1} soil). More reductions in urea hydrolysis occurred for 20-CuU than 10-CuU and 5-CuU treatments at both N rates. Reductions in urea hydrolysis decreased during days 8 - 32, with the greatest decline measured in the 5-CuU treatment. The Zn treatment (20-ZnU) had minimal effect on reducing urea hydrolysis at the lower N addition; however, at the higher N addition the urea hydrolysis was reduced by 11.1 - 23.7% over the first 8 days.

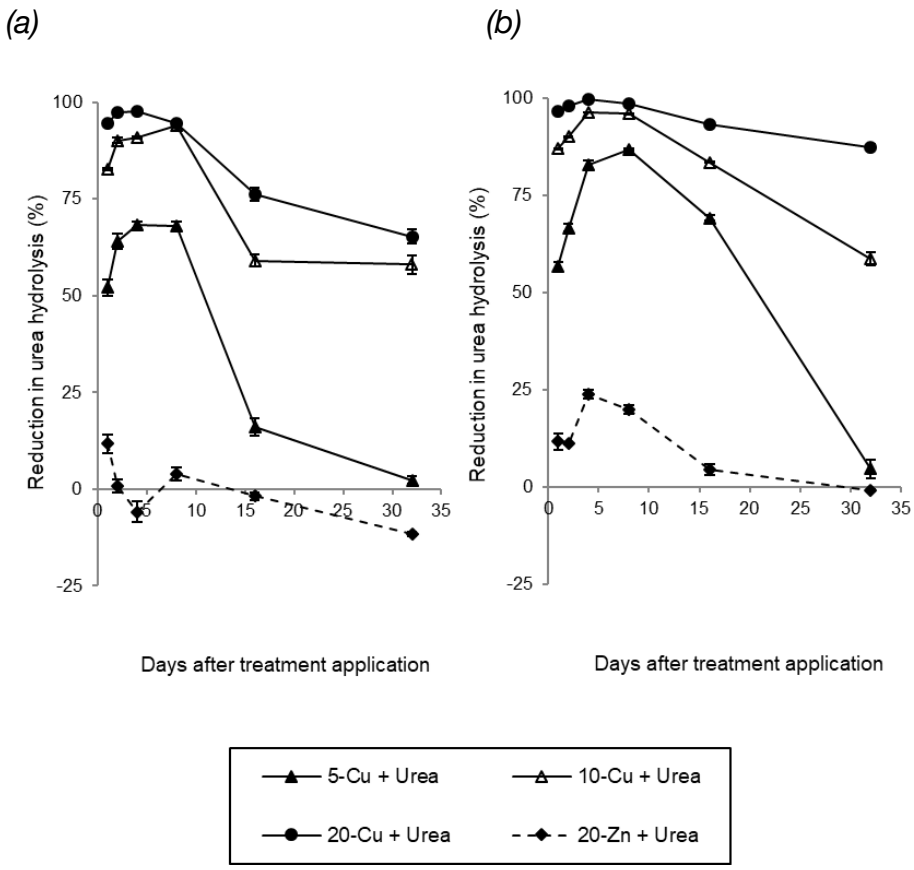

Fig. 4.1 Effect of Cu and Zn on mean reduction in urea hydrolysis on Rangitikei loamy sand (RLS) soil supernatant as affected by urea for *(a)* 120 and *(b)* 600 mg N kg^{-1} soil application; vertical bars indicate standard error values

In the EBL supernatant, the 20-CuU treatment significantly reduced urea hydrolysis for the first 16 days after treatment application for both N additions (Fig. 4.2). The 10-CuU treatment was effective at reducing urea hydrolysis for 8 days at the 120 mg N kg^{-1} soil N addition. At the higher N addition, 10-CuU was not effective at reducing urea hydrolysis at day 1, but reductions during days 2 - 16 were effective. The 5-CuU treatment was not effective at reducing urea hydrolysis at day 1 for both N additions. However, reductions during days 4 - 8 were effective for both N additions. The 20-ZnU treatment did not reduce urea hydrolysis effectively over the first 4 days for both N additions. However, by day 8 the 20-ZnU treatment was achieving reductions in urea hydrolysis of 14.3% at the higher N addition.

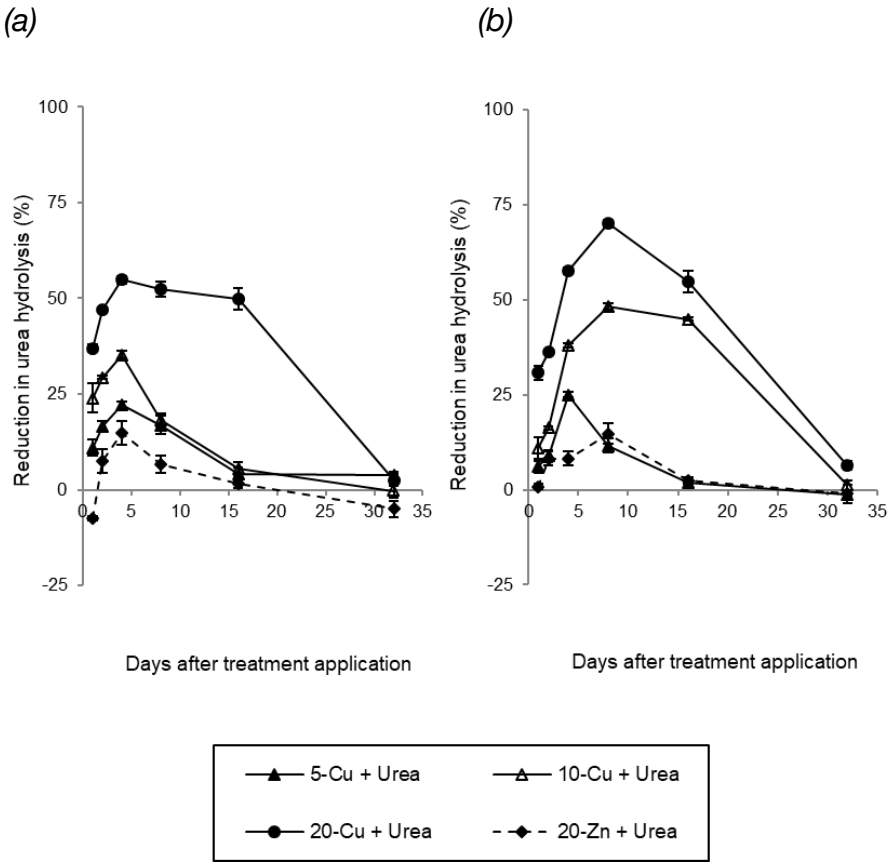

Fig. 4.2 Effect of Cu and Zn on mean reduction in urea hydrolysis on Egmont black loam (EBL) soil supernatant as affected by urea for *(a)* 120 and (*b*) 600 mg N kg^{-1} soil application; vertical bars indicate standard error values

The NO_3^--N levels in supernatants at the 120 mg N kg^{-1} soil N addition are presented in Table 4.1. The NO_3^--N levels were mostly only detectable for the first 4 days after treatment application in both supernatants, and showed a decreasing trend over this period. Only NO_3^--N in EBL supernatant for the 20-CuU treatment was still detectable at day 8 (3.4 mg kg^{-1} soil, data not presented). There was a general trend of lower NO_3^--N levels in the RLS relative to EBL supernatant during this period. For the RLS supernatant, NO_3^--N levels were significantly greater in all Cu treatments compared with the urea-only and 20-ZnU, at the 120 mg N kg^{-1} soil N addition. The 20-CuU and 10-CuU treatments resulted in significantly higher NO_3^--N levels compared with the 5-CuU treatment during days 2 - 4. There were significantly higher NO_3^--N levels in the 20-ZnU treatment compared with urea-only for the first 2 days. In the EBL supernatant, there was no significant difference in NO_3^--N between all three Cu treatments and urea-only treatment for the first 2 days. By day 4, NO_3^--N in the 20-CuU and 10-CuU treatments were significantly higher relative to urea-only. There was no significant difference in NO_3^--N between the 20-ZnU and urea-only treatments for the first 4 days.

The NO_3^--N results for the 600 mg N kg^{-1} soil N addition (Table 4.2) were similar to those for the lower levels of N addition and were only detectable for the first 4 days after treatment application, showing a decreasing trend over this period. In the RLS supernatant, the Cu treatments achieved significantly greater NO_3^--N levels relative to urea-only. By day 2, NO_3^--N in the 20-CuU and 10-CuU treatments were significantly greater compared with the 5-CuU treatment. There was no significant difference in NO_3^--N between the 20-ZnU and urea-only treatments for day 1 and day 4. With the EBL supernatant, all three Cu treatments and the 20-ZnU treatment resulted in

significantly greater NO_3^--N levels relative to urea-only by day 2. Similarly, NO_3^--N were significantly higher in the 20-CuU and 10-CuU treatments compared with the 5-CuU treatment by day 4.

Table 4.1 Effect of Cu and Zn on mean NO_3^--N concentrations in the Rangitikei loamy sand (RLS) and Egmont black loam (EBL) soil supernatants for urea applied at a rate equivalent to 120 mg kg^{-1} soil to all treatments (NO_3^--N was mostly not detectable after day 4)

	NO_3^--N (mg kg^{-1} soil)					
	RLS			EBL		
Treatments	Day 1	Day 2	Day 4	*Day 1	Day 2	Day 4
Urea	10.0 ^{c}	1.5 ^{d}	1.1 ^{c}	88.7 ^{a}	67.9 ^{ab}	13.1 ^{cd}
5-CuU	22.4 ^{a}	14.7 ^{b}	7.3 ^{b}	85.7 ^{a}	70.4 ^{ab}	17.9 ^{c}
10-CuU	25.6 ^{a}	18.8 ^{a}	12.2 ^{a}	86.1 ^{a}	74.9 ^{a}	43.5 ^{b}
20-CuU	25.6 ^{a}	19.8 ^{a}	11.8 ^{a}	86.9 ^{a}	76.1 ^{a}	58.0 ^{a}
20-ZnU	18.5 ^{b}	6.8 ^{c}	1.5 ^{c}	85.1 ^{a}	66.4 ^{b}	10.4 ^{d}

Means followed by different lower case letters in a column are significantly different by Tukey's Studentized Range Test ($P < 0.05$); * indicates means followed by different small letters in a column are significantly different by Bonferroni (Dunn) *t*-test ($P < 0.05$)

The $Ca(NO_3)_2$)-extractable Cu levels in Cu treatments were sustained longer in the RLS compared with the EBL supernatant, at the 120 mg N kg^{-1} soil N addition (Table 4.3). The Cu in treatments without Cu (urea and 20-ZnU) was not detectable for either supernatant. In the RLS supernatant, Cu was sustained over the 32 days for the 20-CuU and 10-CuU treatments. For the 5-CuU treatment, Cu was at a detectable level for the first 16 days. For the EBL supernatant, $Ca(NO_3)_2$-extractable Cu was sustained over the first 16 days for the 20-CuU treatment, but only the first 4 days for the 10-CuU and 5-CuU treatments.

Table 4.2 Effect of Cu and Zn on mean NO_3-N concentrations in the Rangitikei loamy sand (RLS) and Egmont black loam (EBL) soil supernatants for urea applied at a rate equivalent to 600 mg kg^{-1} soil to all treatments (NO_3^--N was not detectable after day 4)

	NO_3^--N (mg kg^{-1} soil)					
	RLS			EBL		
Treatments	*Day 1	Day 2	Day 4	Day 1	Day 2	Day 4
Urea	14.2 ^{c}	2.5 ^{e}	1.2 ^{c}	84.0 ^{a}	41.1 ^{c}	2.0 ^{d}
5-CuU	25.6 ^{ab}	16.1 ^{c}	9.5 ^{b}	84.1 ^{a}	61.1 ^{b}	5.2 ^{c}
10-CuU	27.8 ^{a}	19.0 ^{b}	13.0 ^{a}	84.8 ^{a}	64.0 ^{b}	10.6 ^{b}
20-CuU	27.2 ^{a}	20.1 ^{a}	12.3 ^{a}	83.3 ^{a}	74.8 ^{a}	31.7 ^{a}
20-ZnU	23.7 ^{bc}	9.9 ^{d}	0.9 ^{c}	85.8 ^{a}	61.2 ^{b}	5.3 ^{c}

Means followed by different lower case letters in a column are significantly different by Tukey's Studentized Range Test ($P < 0.05$); * indicates means followed by different small letters in a column are significantly different by Bonferroni (Dunn) *t*-test ($P < 0.05$)

Table 4.3 Effect of Cu and Zn on mean $Ca(NO_3)_2$-extractable Cu concentration in the Rangitikei loamy sand (RLS) and Egmont black loam (EBL) soil supernatants for urea applied at a rate equivalent to 120 mg kg^{-1} soil to all treatments (Cu levels for urea and 20-ZnU were not detectable)

	$Ca(NO_3)_2$-extractable Cu (mg kg^{-1} soil)						
	Treatments	Day 1	Day 2	Day 4	Day 8	Day 16	Day 32
RLS	5-CuU	1.3 ^{b}	1.8 ^{b}	1.3 ^{c}	1.7 ^{b}	1.1 ^{b}	–
	10-CuU	2.4 ^{a}	2.6 ^{a}	2.2 ^{b}	3.0 ^{a}	2.8 ^{a}	1.5 ^{b}
	20-CuU	2.8 ^{a}	3.3 ^{a}	3.7 ^{a}	3.3 ^{a}	3.0 ^{a}	3.7 ^{a}
EBL	5-CuU	1.6 ^{c}	2.1 ^{c}	0.9 ^{b}	–	–	–
	10-CuU	6.5 ^{b}	6.8 ^{b}	1.7 ^{b}	–	–	–
	20-CuU	10.1 ^{a}	9.5 ^{a}	6.9 ^{a}	1.4	1.1	–

Means followed by different lower case letters in a column are significantly different by Tukey's Studentized Range Test ($P < 0.05$)

The $Ca(NO_3)_2$-extractable Cu levels at the 600 mg N kg^{-1} soil N addition (Table 4.4) followed a similar trend to those for the lower levels of N addition, which sustained longer in the RLS supernatant relative to the EBL supernatant. For the RLS supernatant, Cu levels were detectable over the 32 days for the 20-CuU and 10-CuU treatments, and for the first 16 days for the 5-CuU treatment. In the EBL supernatant, Cu was at a detectable level during the first 8 days for the 20-CuU and 10-CuU treatments, but only for the first 2 days with the 5-CuU treatment.

Table 4.4 Effect of Cu and Zn on mean $Ca(NO_3)_2$-extractable Cu concentrations in the Rangitikei loamy sand (RLS) and Egmont black loam (EBL) soil supernatants for urea applied at a rate equivalent to 600 mg kg^{-1} soil to all treatments (Cu levels for urea and 20-ZnU were not detectable)

		$Ca(NO_3)_2$-extractable Cu (mg kg^{-1} soil)					
	Treatments	Day 1	Day 2	Day 4	Day 8	Day 16	Day 32
RLS	5-CuU	0.9 ^{c}	1.0 ^{c}	1.0 ^{c}	1.1 ^{a}	0.5 ^{b}	–
	10-CuU	1.5 ^{b}	1.7 ^{b}	2.3 ^{b}	2.3 ^{a}	2.2 ^{a}	0.2 ^{b}
	20-CuU	3.5 ^{a}	4.0 ^{a}	4.1 ^{a}	2.1 ^{a}	2.1 ^{a}	1.4 ^{a}
EBL	5-CuU	2.5 ^{c}	2.7 ^{c}	–	–	–	–
	10-CuU	5.1 ^{b}	4.4 ^{b}	1.0 ^{a}	0.3 ^{a}	–	–
	20-CuU	8.3 ^{a}	7.9 ^{a}	1.6 ^{a}	0.6 ^{a}	–	–

Means followed by different lower case letters in a column are significantly different by Tukey's Studentized Range Test ($P < 0.05$)

The $Ca(NO_3)_2$-extractable Zn levels in the 20-ZnU treatment were sustained for 32 days in both supernatants, at the 120 mg N kg^{-1} soil N addition (Table 4.5). Zinc levels were significantly greater in 20-ZnU treatment compared with all three Cu treatments and urea-only for 32 days in both supernatants. The $Ca(NO_3)_2$-extractable Zn

results at the 600 mg N kg^{-1} soil N addition were similar to those for the lower levels of N addition (Table 4.6). The Zn levels in the 20-ZnU treatment were significantly higher relative to all three Cu treatments and the urea-only for first 16 days in RLS supernatant, but for the first 8 days in EBL supernatant.

Table 4.5 Effect of Cu and Zn on mean $Ca(NO_3)_2$-extractable Zn concentration in the Rangitikei loamy sand (RLS) and Egmont black loam (EBL) soil supernatants for urea applied at a rate equivalent to 120 mg kg^{-1} soil to all treatments

		$Ca(NO_3)_2$-extractable Zn (mg kg^{-1} soil)					
	Treatments	Day 1	Day 2	Day 4	Day 8	Day 16	Day 32
RLS	Urea	1.7 ^{b}	2.0 ^{b}	0.8 ^{c}	0.8 ^{c}	1.6 ^{b}	–
	5-CuU	1.7 ^{b}	2.1 ^{b}	1.6 ^{bc}	2.6 ^{b}	2.0 ^{b}	–
	10-CuU	1.9 ^{b}	1.7 ^{b}	2.4 ^{b}	2.5 ^{b}	2.8 ^{b}	1.0 ^{b}
	20-CuU	1.9 ^{b}	1.5 ^{b}	1.8 ^{bc}	1.7 ^{bc}	2.2 ^{b}	1.5 ^{b}
	20-ZnU	13.2 ^{a}	14.5 ^{a}	13.0 ^{a}	15.5 ^{a}	7.9 ^{a}	2.9 ^{a}
EBL	Urea	1.3 ^{c}	1.5 ^{c}	1.2 ^{b}	2.6 ^{b}	2.2 ^{b}	0.5 ^{b}
	5-CuU	1.9 ^{b}	2.4^{b}	1.8 ^{b}	2.7 ^{b}	2.0 ^{b}	0.5 ^{b}
	10-CuU	2.0 ^{b}	2.7 ^{b}	2.5 ^{b}	2.1 ^{b}	2.7 ^{b}	0.6 ^{b}
	20-CuU	2.1 ^{b}	2.7 ^{b}	2.6 ^{b}	2.3 ^{b}	2.9 ^{b}	0.4 ^{b}
	20-ZnU	21.3 ^{a}	19.9 ^{a}	18.8 ^{a}	17.8 ^{a}	10.6 ^{a}	1.2 ^{a}

Means followed by different lower case letters in a column are significantly different by Tukey's Studentized Range Test ($P < 0.05$)

Table 4.6 Effect of Cu and Zn on mean $Ca(NO_3)_2$-extractable Zn concentration in the Rangitikei loamy sand (RLS) and Egmont black loam (EBL) soil supernatants for urea applied at a rate equivalent to 600 mg kg^{-1} soil to all treatments

		$Ca(NO_3)_2$-extractable Zn (mg kg^{-1} soil)					
	Treatments	Day 1	Day 2	Day 4	Day 8	Day 16	Day 32
RLS	Urea	1.1 ^{c}	1.0 ^{c}	0.5 ^{c}	1.3 ^{b}	–	–
	5-CuU	1.5 ^{bc}	1.9 ^{b}	2.2 ^{b}	2.1 ^{b}	–	–
	10-CuU	1.8 ^{b}	1.9 ^{b}	2.1 ^{b}	2.5 ^{b}	1.4 ^{b}	0.3 ^{a}
	20-CuU	2.1 ^{b}	2.0 ^{b}	1.8 ^{b}	2.8 ^{b}	1.9 ^{b}	0.4 ^{a}
	20-ZnU	5.3 ^{a}	5.7 ^{a}	5.1 ^{a}	5.5 ^{a}	3.1 ^{a}	0.3 ^{a}
EBL	Urea	1.8 ^{bc}	1.6 ^{c}	1.1 ^{b}	1.7 ^{b}	1.3 ^{b}	–
	5-CuU	1.5 ^{c}	2.2 ^{bc}	1.4 ^{b}	1.5 ^{b}	1.7 ^{ab}	–
	10-CuU	2.2 ^{bc}	2.8 ^{b}	1.4 ^{b}	1.7 ^{b}	1.8 ^{ab}	–
	20-CuU	2.8 ^{b}	2.7 ^{bc}	1.2 ^{b}	1.5 ^{b}	1.7 ^{ab}	–
	20-ZnU	21.0 ^{a}	18.5 ^{a}	13.6 ^{a}	5.8 ^{a}	3.0 ^{a}	0.6

Means followed by different lower case letters in a column are significantly different by Tukey's Studentized Range Test ($P < 0.05$)

At the 120 mg N kg^{-1} soil N addition, the cumulative NH_4^+ concentrations in the RLS and EBL supernatants without the addition of glucose were 44.8 and 70.3 mg kg^{-1} soil, which were only 51.4 and 63.7% of urea-only treatment over the 32 day period, respectively (Table 4.7). The NO_3^--N levels for this treatment were greater, compared to all other treatments, for both supernatants and sustained similar levels over the 32 day period. For the supernatant with glucose only, NO_3^--N concentrations were at detectable levels for the first 4 days in both supernatants (Table 4.8). For the N addition at 120 mg kg^{-1} soil without addition of glucose treatment and the supernatant with glucose only treatment, the $Ca(NO_3)_2$-extractable Zn levels were sustained over the 32 day period in

EBL supernatant, but only for the first 16 days in RLS supernatant. However, $Ca(NO_3)_2$-extractable Cu concentrations were not at detectable levels for the both treatments.

Table 4.7 The concentrations of NH_4^+-N, NO_3^--N and $Ca(NO_3)_2$-extractable Zn in the Rangitikei loamy sand (RLS) and Egmont black loam (EBL) soil supernatants *(a)* urea applied at 120 mg kg^{-1} soil without addition of glucose *(b)* urea applied at 120 mg kg^{-1} soil with addition of glucose

	Treatments	Day 1	Day 2	Day 4	Day 8	Day 16	Day 32
				NH_4^+-N (mg kg^{-1} soil)			
RLS	*(a)*	1.0	2.6	4.8	12.4	26.2	44.8
	(b)	28.6	44.3	50.8	77.0	87.5	87.1
EBL	*(a)*	3.0	9.1	20.2	46.3	85.1	70.3
	(b)	9.4	25.1	66.0	103.0	115.7	110.2
				NO_3^--N (mg kg^{-1} soil)			
RLS	*(a)*	60.3	58.7	61.8	54.5	59.2	62.9
	(b)	10.0	1.5	1.1	–	–	–
EBL	*(a)*	126.1	116.8	121.7	119.0	125.3	135.3
	(b)	88.7	67.9	13.1	–	–	–
				$Ca(NO_3)_2$-extractable Zn (mg kg^{-1} soil)			
RLS	*(a)*	2.2	2.1	1.7	1.0	1.0	–
	(b)	1.7	2.0	0.8	0.8	1.6	–
EBL	*(a)*	3.0	3.2	3.2	3.7	2.2	2.4
	(b)	1.3	1.5	1.2	2.6	2.2	0.5

Table 4.8 The concentrations of NH_4^+-N, NO_3^--N and $Ca(NO_3)_2$-extractable Zn in the Rangitikei loamy sand (RLS) and Egmont black loam (EBL) soil supernatants with glucose only

	Day 1	Day 2	Day 4	Day 8	Day 16	Day 32
			NH_4^+-N (mg kg^{-1} soil)			
RLS	0.1	0.5	0.3	0.2	0.2	0.4
EBL	3.0	3.8	2.4	0.6	0.6	1.0
			NO_3^--N (mg kg^{-1} soil)			
RLS	10.8	1.0	1.1	–	–	–
EBL	89.0	78.5	17.9	–	–	–
			$Ca(NO_3)_2$-extractable Zn (mg kg^{-1} soil)			
RLS	2.3	2.2	1.8	1.6	2.1	–
EBL	2.5	2.6	2.1	2.1	3.0	0.9

4.4 Discussion

The use of soil supernatants with potentially low organic C, clay particles and cation exchangeable bases reduced the complexation of Cu and enhanced Cu bioavailability. This resulted in the Cu treatments reducing the urea hydrolysis for both the RLS and EBL supernatants. The effectiveness of Cu for reducing urea hydrolysis was sustained longer in the RLS, compared to the EBL supernatant. This difference between the supernatant performances occurred despite the added glucose resulting in similar soluble C values, following pre-incubation and before treatment application: 1580.7 and 1551.2 mg kg^{-1} soil for the RLS and EBL supernatants respectively. This could be attributed partly to the faster Cu immobilisation rates in EBL relative to RLS supernatant (Tables 4.3 and 4.4) and partly to higher NO_3^--N levels in the EBL supernatant (Tables 4.1 and 4.2), which could enhance the rate of microbial growth. The

greater effectiveness and longevity of effectiveness of higher Cu (20-CuU and 10-CuU) compared to lower Cu treatment (5-CuU) at reducing urea hydrolysis for both supernatants is accredited to higher and more sustained Cu levels in treatments receiving higher Cu. The percentage reductions in urea hydrolysis by Cu treatments were not influenced by the concentrations of the substrate (urea-N addition) and were similar for both N additions, suggesting that Cu behaved as a non-competitive UI as observed by Shaw and Raval (1961).

In the current study, Cu was more effective than Zn at inhibiting urea hydrolysis despite sustained Zn bioavailability over the 32 days. Similar observations were also reported in previous studies (Tabatabai 1977; Hemida et al. 1997), suggesting Cu has a higher affinity for amino groups that occur in both urea and urease (Daif and Van Beusichem 1981). Copper ion forms a more stable and insoluble metal sulphide with the SH group of urease than the Zn ion and, therefore, is a stronger UI (Hughes et al. 1969; Hughes and Poole 1989; Zaborska et al. 2004).

Cumulative urea hydrolysis in the supernatants without addition of glucose was 48.6 and 36.3% smaller than the urea-only treatment over the 32 days, for RLS and EBL supernatants, respectively, at the 120 mg N kg^{-1} soil N addition. This suggests that urea hydrolysis in the supernatants with added glucose was the cumulative effect of extracellular soil UA and increasing microbial urease. This is likely due to higher microbial growth in supernatants as a result of higher availability of labile C coming from added glucose, and microbial urease is directly linked with microbial growth (Paulson and Kurtz 1969). There was higher reduction in NO_3^--N levels in the urea-only treatment than the supernatants without addition of glucose over the 32 days, at the 120

mg N kg^{-1} soil N addition. This could be associated with increased population of denitrifiers in anaerobic conditions when availability of labile C was increased.

Our results showed that when Cu was added to soil supernatants, which potentially had low organic C, clay particles and cation exchangeable bases, the complexation of Cu was minimised and Cu bioavailability was enhanced. This resulted in Cu treatments significantly reducing urea hydrolysis in soil supernatants. Zinc had little influence at inhibiting urea hydrolysis in the soil supernatants even though its bioavailability remained high.

4.5 Conclusions

With the soil supernatants, inhibition in urea hydrolysis by the addition of Cu occurred when its bioavailability was increased by reducing soil organic C, clay particles and cation exchangeable bases. This supports that Cu does have a urease inhibitory effect, however, it is not effective as an inhibitor for use in pastoral soils, which typically have high C contents. Zinc was not effective in reducing urea hydrolysis in the soil supernatants although its bioavailability was sustained, suggesting its weaker inhibitory effect on soil UA.

Chapter 5

Understanding the performance of 2-NPT and nBTPT in reducing ammonia emissions from cattle urine applied to dairy-grazed pasture soils

5.1 Introduction

The results from the studies reported in Chapters 3 and 4 evidently suggest that both micronutrients Cu and Zn cannot be used as UIs in reducing NH_3 emissions from urine patches in NZ dairy-grazed pasture soils. A recently introduced UI called 2-NPT has been reported to inhibit soil UA more effectively (10 days, 65% inhibition) compared to commonly used nBTPT (10 days, 40% inhibition) (Domínguez et al. 2008). In addition, 2-NPT has shown a sustained ability to inhibit UA, being effective even at 30 days, although at a reduced level of inhibition (12% inhibition). Although 2-NPT appears promising to mitigate NH_3 emissions from freshly deposited urine patches, it has never been evaluated using cattle urine deposited in dairy pasture soils. Also, no comparative study has been conducted using both 2-NPT and nBTPT at reducing emissions from both fertiliser urea and cattle urine. It is hypothesized that 2-NPT will be more effective and have greater longevity than nBTPT at inhibiting UA and NH_3 emissions from cattle urine, and be effect for more than a single grazing event. Although the probability of overlapping urine patches in consecutive grazing events is low, given that urine patches only cover approximately 2 - 3% of the grazed pasture area at each grazing (Haynes and Williams 1993; Jarvis et al. 1995), there will be instances where N is added as effluent or fertiliser to the same areas where there are urine patches. Hence, it is also useful to explore the efficiency of UIs to reduce emissions from multiple additions of N to the same area of pasture. The series of

experiments were conducted in this study with the overall objective of understanding both the effectiveness and longevity of 2-NPT in comparison with commonly used UI nBTPT in reducing NH_3 emissions after the deposition of cattle urine in pasture soils during a single or multiple grazing events. The specific objectives of the study reported in this chapter were:

- To determine and compare the effectiveness and longevity of 2-NPT with nBTPT in reducing NH_3 emissions from cattle urine added to dairy-grazed pasture soils.
- To assess the efficiency of 2-NPT and nBTPT in reducing NH_3 emissions from reapplication of cattle urine to same pasture soils after inhibitor application.
- To evaluate the effect of 2-NPT and nBTPT on pH changes of pasture soils for understanding role of UIs on UA dynamics following cattle urine deposition.
- To investigate the impact of 2-NPT and nBTPT on microbial biomass of pasture soils for understanding specificity of UIs on UA following cattle urine deposition.

5.2 Materials and methods

5.2.1 Experimental details

Two pasture soils (RLS and EBL) of contrasting soil organic C levels and UA, and which were previously used in studies on the performance of Cu and Zn in reducing soil UA and urea hydrolysis in soil supernatants (Chapters 3 and 4), were used in an incubation study. Information on the collection and preparation of soil samples and their physico-chemical properties are reported in Chapter 3 (Section 3.2.2.1). The experiments were conducted in an incubator room at 20°C. The moisture contents of air

dried soils used for the study were 1.4 and 16.3% for RLS and EBL soil, respectively. The dry soils were chosen to create favourable condition for high NH_3 emissions.

5.2.2 Treatments

The five treatments applied at the start of experiment were: i) No-inhibitor, ii) nBTPT-low, iii) 2-NPT-low, iv) 2-NPT-medium, and v) 2-NPT-high. There were four replicates of each treatment. Cattle urine was applied at four stages to all the treatments: immediately before inhibitor application (Stage-A), 29 days after inhibitor application (Stage-B), 56 days after inhibitor application (Stage-C), and 29 days after (Stage-B) and again 60 days after inhibitor application (Stage-D); and NH_3 emissions were measured following each urine application. So the total number of experimental units established were 120 (2 soils × 5 treatments × 4 replicates = 40, 40 ×3 stages = 120, experimental units for Stages B and D are same). The initial inhibitor application rates for all stages were the same and based on the percentage of total urine-N applied at Stage-A. But urine N concentrations differed, because the urine used for different stages were collected at different timings, and application depths (urine volume) were also not the same at each stage. This resulted in variations in the rate of inhibitors relative to the amount of N added for equivalent treatments at the different stages (Table 5.1).

The air-dried soil samples (138 and 86 g of oven-dry equivalent soil for RLS and EBL soil, respectively) were weighed and packed to a depth of 78 mm in plastic containers (42 mm diameter), resulting in bulk densities of 1.3 and 0.8 g cm^{-3} for RLS and EBL soil, respectively. Each container of soil was placed inside a 1 L Agee jar. The standard solutions containing of nBTPT (20.5 mg L^{-1}) and 2-NPT (20.5, 41.1 and 61.6 mg L^{-1} for low, medium and high inhibitor treatment, respectively) were prepared in DI

water using Agrotain® [liquid form, 10% active ingredient (w/w)] and 2-NPT [powder form, 96% active ingredient (w/w)], respectively. The solution volume of 1 mL with the required amount of the inhibitors was sprayed on to the surface of the soil samples using a 5 mL syringe connected to the lid of 50 mL plastic atomizer. Agee jars without soil and with filter papers only, were also included in each stage to measure background NH_3 levels. Urine was applied uniformly to the soils packed in the plastic containers using a pipette. The volume of urine applied was 13.9 mL for the first 3 stages (Stages A, B and C) and 10 mL for Stage-D, to provide application depths of 10 and 7.2 mm to soil surfaces, respectively. An application depth of 10 mm was selected for the first 3 stages to provide a similar application depth to a typical cattle urination volume of 2·L (Doak 1952) onto an average urine patch area of 0·2 m^2 (Haynes and Williams 1993). During the first 3 stages the urine was applied to air-dried soils, however, at Stage-D it was applied to moist soil, which meant that a lower application depth of 7.2 mm was applied to avoid over wetting the soil samples. Soil moisture content immediately after urine application was recorded by weighing the plastic container when the soil was at 70 and 63% of WHC for RLS and EBL soil, respectively at the first 3 stages. This moisture content was maintained by adding DI water. However, moisture content immediately after urine application at Stage-D was at 19% (slightly more than soil WHC of 18%) for the RLS soil and 85% of soil WHC for EBL soil, and these moisture levels were maintained throughout the experiment. In soils below 100% WHC the soil moisture does vary with depth, being higher in the surface soil. The moisture contents presented are the average for the amount of soils used.

Table 5.1 The application rates of inhibitors to treatments at different stages of urine application, inhibitor rate for Stage-D is for the second urine application

Stage	Urine application time	Inhibitor rates to treatments (% of urine N applied)				
		No-inhibitor	nBTPT-low	2-NPT-low	2-NPT-medium	2-NPT-high
A)	immediately before inhibitor application	–	0.025	0.025	0.050	0.075
B)	29 days after inhibitor application	–	0.026	0.026	0.051	0.077
C)	56 days after inhibitor application	–	0.013	0.013	0.026	0.040
D)	29 days after (Stage-B) and again 60 days after inhibitor application.	–	0.018	0.018	0.037	0.055

5.2.3 Ammonia emission

5.2.3.1 Evaluation of passive sampling for NH_3 measurement

Ammonia emissions were measured using a passive sampling technique in which the diffusion of gas inside a vessel is trapped by an absorbent. The technique used in this study was a modification of the method reported by Shigaki and Dell (2015). This procedure involves placing an air dried glass microfiber borosilicate filter paper (42 mm diameter, 0.6 µm pore size and 0.21 mm thickness), which is pre-soaked in 0.5 M sulphuric acid (H_2SO_4) to trap emitted NH_3, inside an Agee jar that is sealed with an air tight lid supplied with rubber septum. A known volume of air was sucked from each jar and the same volume of gas containing a known amount of NH_3 was introduced via the rubber septum using a syringe. The levels of NH_3-N introduced ranged from 1.8 - 80.9 µg with triplicates for each level. The Agee jars were kept closed

for 2 hrs and then each filter paper was removed and placed in a plastic container containing 10 mL of DI water. The plastic containers were then shaken for 20 min at 220 rpm with a horizontal shaker to elute the NH_3 trapped in filter paper as NH_4^+-N. The concentration of NH_4^+-N in the extract was determined using a Technicon AutoAnalyzer. There was a highly significant positive linear relationship ($R^2 = 1$) between the amounts of NH_3 introduced in the Agee jars and the amounts recovered. An average of 95% of the NH_3 introduced into the jars was recovered by the filter papers (Fig. 5.1).

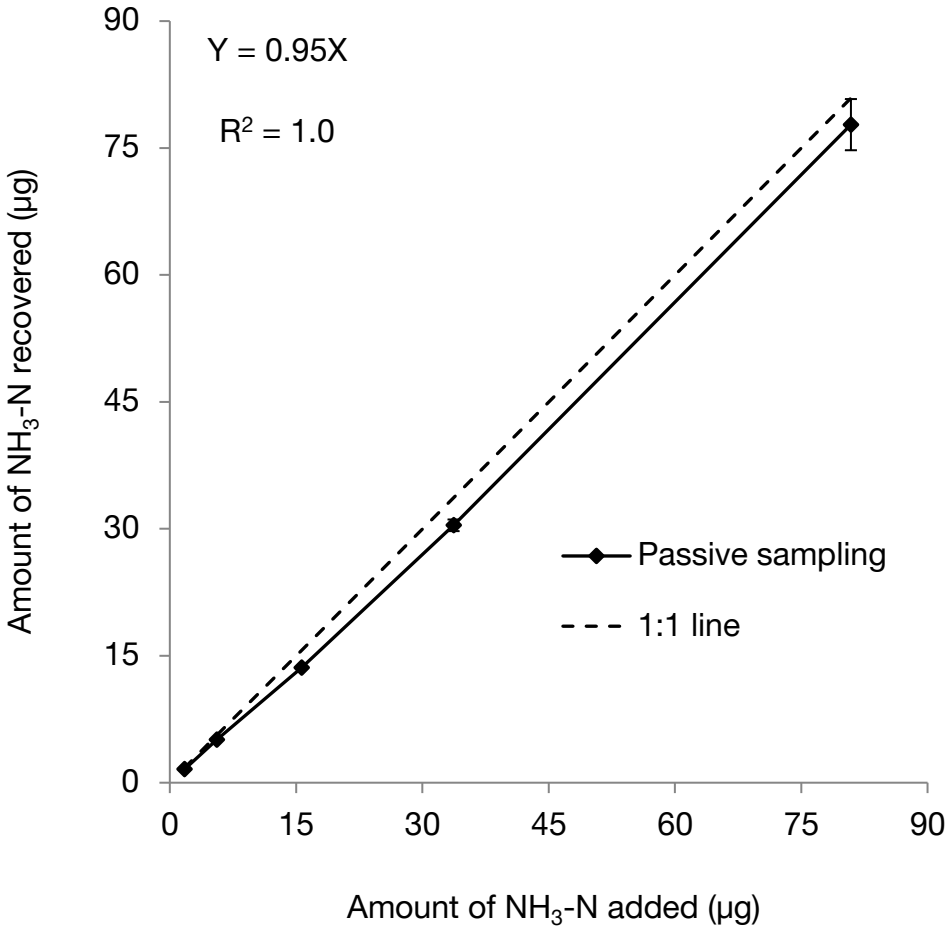

Fig. 5.1 Relationship between amounts of NH_3-N added and recovered in the passive sampling technique, vertical bars indicate standard error values ($P < 0.05$)

5.2.3.2 Ammonia emissions under laboratory conditions: passive sampling in the absence of plants

Passive sampling technique evaluated in the previous section (Section 5.2.3.1) was used for measuring NH_3 emissions from the treated soils in this study (Fig. 5.2). Measurements of NH_3 were performed daily for the first 7 days (by replacing the filter paper) and then periodically up to day 31 after each urine application. The NH_3 trapped in filter papers was extracted as described in the previous Section 5.2.3.1. Extracts were stored at 4°C until they were analysed for NH_4^+-N concentration using a Technicon AutoAnalyzer. The NH_3-N flux (mg m^{-2} d^{-1}) is calculated by using Eqn. 5.1.

$$NH_3\text{-}N\ flux = \frac{C \times V}{a \times D} \qquad Eqn.\ 5.1$$

where, C = NH_3 concentration in the extract (mg L^{-1}); V = the total volume of solution in the extract (L); a = total cross sectional area (m^2) of the plastic container with soil in the Agee jar; D = duration (days) of each sampling.

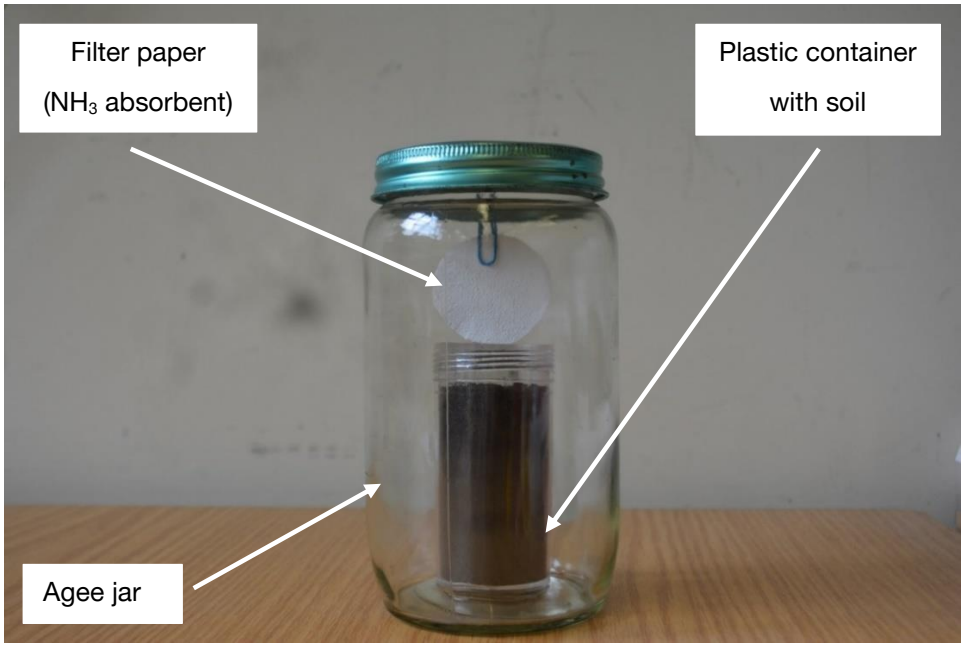

Fig. 5.2 Schematic representation of passive sampling technique used to measure NH_3 emissions

The percentage reduction in total NH_3-N flux with the application of inhibitors relative to the No- inhibitor treatment was calculated using Eqn. 5.2.

$$\%\, reduction\ in\ NH_3 - N\ flux = \frac{C - A}{C} \times 100\% \qquad Eqn.\,5.2$$

where, C = total NH_3-N emitted from No-inhibitor treatment; A = total NH_3-N emitted from inhibitor treatment.

5.2.4 Soil pH

A parallel experiment was set up to measure the changes in surface soil pH, after applying urine immediately before inhibitor application (Stage-A). This experiment used 90 and 56 g (oven-dry equivalent) air-dry soil held in plastic containers (50 mm soil depth) for the RLS and EBL soil, respectively. The soil depth chosen for pH measurement was lower than for the NH_3 measurement experiment (78 mm depth), because most of the changes in NH_3 emissions are explained by changes in surface soil pH. The plastic container was then placed inside an Agee jar after applying urine at an application depth of 10 mm and then the same inhibitor treatments as mentioned in Section 5.2.2. The Agee jar was sealed with an air tight lid and filter paper was changed periodically to create similar conditions as in the NH_3 measurement experiment. Soil pH was measured using a HI99121 Direct Soil Measurement pH Portable Meter (Hanna Instruments, Italy) up to day 31 by inserting the electrode into the soil, ensuring proper contact between reference junction and soil as shown in Fig. 5.3. The background pH level on day 1 was also measured for both soils with the application of DI water only.

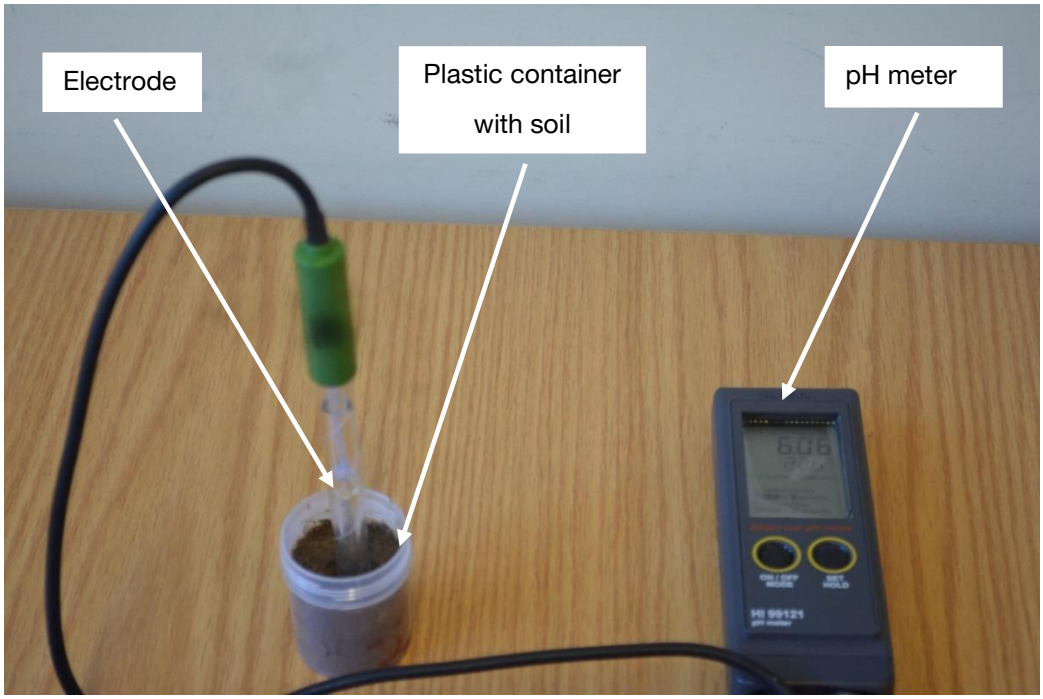

Fig. 5.3 Schematic representation of soil pH measurement using Direct Soil pH Meter

5.2.5 Analyses

5.2.5.1 Urine

Urine was collected from dairy cattle at the Massey University No.1 and No. 4 Dairy Farms while they were being milked. The fresh urine samples that were collected from individual cattle were bulked together and stored for up to five days at 4^{o}C to avoid urea hydrolysis prior to application. Urine samples were analysed for total N, urea-N and pH before application. The total N was determined following Kjeldahl digestion method (McKenzie and Wallace 1954). Briefly, 4 mL of digest mixture (250 g K_2SO_4 + 2.5 g selenium + 2.5 L conc. H_2SO_4) was added to 1 mL of urine sample in a digestion tube with a 50 mL mark and heated at 350^{o}C for 4 hrs in aluminium block. The digested solution was then allowed to cool down and diluted to 50 mL with DI water. Before being analysed for total N using the Technicon AutoAnalyzer, diluted sample was mixed thoroughly and left to settle. The amount of urea present in the urine

samples was determined by a colorimetric procedure using a Spectrophotometer, set to a 527 nm wavelength (Mulvaney and Bremner 1979), and pH was measured using pH meter. The chemical properties and application rates of urine, expressed on a total N in g m^{-2}, used in all of the experiments are presented in Table 5.2. The chemical properties and application rates of urine used in the soil pH experiment was the same as used in Stage-B of the NH_3 experiment.

Table 5.2 Chemical properties of cattle urine and application rates used in the study

Stage	Urine application time	pH	Urea-N (g L^{-1})	Total N (g L^{-1})	Application depth (mm)	N applied (g m^{-2})
A)	immediately before inhibitor application	8.2	4.7	5.9	10.0	59.3
B)	29 days after inhibitor application	8.4	4.4	5.8	10.0	58.0
C)	56 days after inhibitor application	8.3	9.8	11.2	10.0	112.0
D)	29 days after (Stage-B) and again 60 days after inhibitor application.	8.3	9.8	11.2	7.2	80.9

5.2.5.2 Microbial biomass C

The soil samples from Stage-A of the NH_3 emissions experiment were analysed for soil microbial biomass C at the end of the measurement period. From each container a total of 3 soil cores were collected using a soil corer (diameter 1 cm and soil depth 0-5 cm). The individual soil cores collected from each container were bulked together, sieved through a 2-mm sieve and mixed thoroughly. The chloroform fumigation extraction method (Vance et al. 1987; Solaiman 2007) was followed for the extraction

of soluble C. This method involved weighing 5 g oven dry equivalent soil samples in to a centrifuge tube (not fumigated) and 50 mL glass beaker (to be fumigated). Twenty-five mL of 0.5 M K_2SO_4 was added into each centrifuge tube and then was shaken on an end-over-end shaker for 30 min. Four reagent blanks were also included. The suspension was filtered using Whatman No. 42 filter paper and the extracts were stored in a freezer before analysis. Samples in the glass beakers (to be fumigated) were placed into the desiccator lined with wet filter paper (to maintain humidity and to stop soil drying out), and then a 50 mL glass beaker containing 30 mL ethanol free chloroform and some anti-bumping granules was added. The desiccator was then sealed and a vacuum was applied to allow the chloroform to boil for about 2 min. The vacuum was re-applied after 5-10 min to provide a couple more minutes of boiling (to ensure adequate fumigation). The sealed desiccator was stored in the dark (covered with a towel) at room temperature for 24 hrs. After 24 hrs, the beaker of chloroform was removed from the desiccator and the residual chloroform vapour in the soil was removed by frequent evacuation before extraction. Similar to the unfumigated soil samples, each fumigated soil sample was transferred into a centrifuge tube and extracted with 25 mL of 0.5 M K_2SO_4, then the extracts were stored in a freezer before analysis.

The total soluble C in the extract was measured with some modification of dichromate semi-automated method (O'Dell 1993). Briefly, 10 mL of the extract was transferred into a digestion tube with a 25 mL mark. Two grains of anti-bumping granules, 1.5 mL of digestion solution [5.1 g potassium dichromate + 84 mL conc. H_2SO4 + 16.7 g mercury sulphate + 500 mL DI water] and 10 mL of catalyst solution (5 g silver sulphate) + 500 mL conc. H_2SO4) were added in sequence to the tube. The solution was then mixed using a vortex mixer and a glass funnel was placed on the top

of the tube. The tube was placed into a digestion block at 150°C for 2 hrs. The digested solution was cooled at room temperature and DI water was added to make the content to 25 mL by rinsing the inside of the tube. The solution was then mixed with a vortex mixer, before colour intensity measurement was performed using a Spectrophotometer at a wavelength of 420 nm. Soluble C content in the extract was calculated by reference to a calibration graph plotted from the colour intensities obtained with potassium hydrogen phthalate as a standard. The soil microbial biomass C was calculated using Eqn. 5.3.

$$Microbial\ biomass\ C = \frac{(Cf\ - Cnf)}{K_{EC}} \qquad Eqn.\ 5.3$$

where, Cf = soluble C in the fumigated extract; Cnf = soluble C in the unfumigated extract; K_{EC} = proportion of the microbial C that is extracted from the soil and is soil-specific. A K_{EC} value of 0.41 (Sparling et al. 1990; Environmental Chemistry Laboratory - Landcare Research 2015) was used.

5.2.6 Statistical methods

The data for NH_3-N emissions, soil pH, and soil microbial biomass C were analysed using ANOVA to detect any significant difference and treatment means were compared using Tukey's Studentized Range (HSD) Test. All of the analyses were conducted using the Statistical Analysis System software (SAS 9.4, $P < 0.05$).

5.3 Results

5.3.1 Ammonia emission under laboratory conditions: passive sampling in the absence of plants

Total NH_3 emitted from the No-inhibitor treatment (urine alone) was between 14.2 - 50.5 % of the total N applied (Table 5.3), with significantly higher emissions measured from RLS soil (35.8 - 50.5%) compared to the EBL soil (14.2 - 26.7%).

When urine was applied immediately before inhibitor application, the NH_3 flux from the No-inhibitor treatment exhibited the maximum values on day 1, which were 3304 and 925 mg NH_3-N m^{-2} d^{-1} for RLS and EBL soils, respectively (Fig. 5.4*(a)*). For the RLS soil, the NH_3 fluxes from the No-inhibitor treatment remained high for the first two days and then decreased to similar levels as those of the two inhibitor treatments by 4 - 5 days after urine application. Whereas, the values for the inhibitor treatments increased from an average of 420.5 mg NH_3-N m^{-2} d^{-1} at day 1 to 1307.7 mg NH_3-N m^{-2} d^{-1} by day 5. Over the subsequent 26 days the NH_3 fluxes decreased gradually to an average of 35.3 mg NH_3-N m^{-2} d^{-1} for all treatments. With the EBL soil, similar to the RLS soil, the NH_3 fluxes from the No-inhibitor treatment were high for the first two days and then declined to similar levels as those of inhibitor treatments by 4 - 5 days after urine application. The values for the inhibitor treatments increased from an average of 45.9 mg NH_3-N m^{-2} d^{-1} at day 1 to 388 mg NH_3-N m^{-2} d^{-1} by day 5. Over the subsequent 26 days the NH_3 fluxes decreased gradually to 75.7 mg NH_3-N m^{-2} d^{-1} for all treatments on average. The reduction in NH_3 emissions from the inhibitor treatments ranged 23.7 - 27.3 % for the RLS soil and 20.6 - 27.2% for the EBL soil (Fig. 5.4*(a)* and Table 5.3), compared to the No-inhibitor treatment. Although there was a general trend of lower emissions from 2-NPT treatments compared to nBTPT-low treatment for the first few days, the difference between inhibitors in total reduction was not significant. Similarly, emissions from treatments 2-NPT-medium and 2-NPT-high, was lower compared to 2-NPT-low treatment for the first few days, but the difference between the rates was also not significant in terms of total reduction.

Table 5.3 Effect of inhibitors on NH_3-N emissions from urine-N added to two pasture soils [Rangitikei loamy sand (RLS) and Egmont black loam (EBL)] at different Stages; A) immediately before, B) 29 days after, C) 56 days after, and D) 29 days after (B) and again 60 days after inhibitor application [mean (standard error)]

	Stage-A		Stage-B		Stage-C		Stage-D	
N added (g m^{-2})	59.3		58.0		112.0		80.9	
	RLS	EBL	RLS	EBL	RLS	EBL	RLS	EBL
Treatments	Cumulative NH_3-N emissions (g m^{-2})							
No-inhibitor	$21.2^{(0.3)a}$	$8.4^{(0.2)a}$	$25.4^{(0.3)a}$	$11.8^{(0.2)a}$	$56.5^{(0.4)a}$	$29.9^{(0.2)a}$	$38.3^{(0.6)a}$	$14.1^{(0.3)a}$
nBTPT-low	$15.9^{(0.1)b}$	$6.7^{(0.1)b}$	$24.3^{(0.2)b}$	$12.2^{(0.1)a}$	$56.2^{(0.4)a}$	$30.9^{(0.2)a}$	$39.9^{(0.8)a}$	$13.8^{(0.3)a}$
2-NPT-low	$16.2^{(0.1)b}$	$6.4^{(0.1)b}$	$22.7^{(0.2)c}$	$11.6^{(0.2)a}$	$53.4^{(0.1)b}$	$30.3^{(0.3)a}$	$40.4^{(0.6)a}$	$13.9^{(0.2)a}$
2-NPT-medium	$15.8^{(0.2)b}$	$6.1^{(0.1)b}$	$22.6^{(0.2)c}$	$11.6^{(0.1)a}$	$53.1^{(0.3)b}$	$30.3^{(0.2)a}$	$40.3^{(1.0)a}$	$13.9^{(0.2)a}$
2-NPT-high	$15.4^{(0.1)b}$	$6.3^{(0.1)b}$	$22.0^{(0.1)c}$	$11.7^{(0.1)a}$	$52.3^{(0.3)b}$	$29.9^{(0.2)a}$	$39.8^{(0.7)a}$	$13.9^{(0.4)a}$
	% change in cumulative NH_3-N relative to No-inhibitor treatment							
nBTPT-low	-25.1	-20.6	-4.2	+3.9	-0.6	+3.3	+4.1	-1.8
2-NPT-low	-23.7	-24.4	-10.5	-1.7	-5.6	+1.2	+5.5	-1.2
2-NPT-medium	-25.3	-27.2	-11.1	-1.3	-6.0	+1.5	+5.3	-1.0
2-NPT-high	-27.3	-25.8	-13.4	-1.0	-7.4	-0.1	+3.9	-0.9
	% N emitted as NH_3 of the applied N							
No-inhibitor	35.8	14.2	43.7	20.3	50.5	26.7	47.4	17.4
nBTPT-low	26.8	11.3	41.9	21.1	50.2	27.6	49.3	17.1
2-NPT-low	27.3	10.8	39.2	19.9	47.6	27.0	50.0	17.2
2-NPT-medium	26.7	10.4	38.9	20.0	47.4	27.1	49.9	17.2
2-NPT-high	26.0	10.6	37.9	20.1	46.7	26.7	49.2	17.2

Means followed by different small letters in a column are significantly different ($P < 0.05$)

With the cattle urine applied 29 days after UIs application, NH_3 flux from the No-inhibitor treatment possessed the highest value on day 1 of 3571 mg NH_3-N m^{-2} d^{-1} for RLS soil and 1151 mg NH_3-N m^{-2} d^{-1} for EBL soil (Fig. 5.4*(b)*). The NH_3 flux for the nBTPT-low treatment had lower values than the No-inhibitor treatment but higher

than 2-NPT treatments for the first 2 days in RLS soil. However, with the EBL soil, nBTPT-low and No-inhibitor treatment had similar fluxes on day 1. 2-NPT treatments started on day 1, on an average of 1314.1 and 872.5 mg NH_3-N m^{-2} d^{-1}, for the RLS and EBL soils respectively, and increased to an average of 2331.9 and 992.6 mg NH_3-N m^{-2} d^{-1} by day 3. The inhibitors significantly reduced total NH_3 emissions compared to No-inhibitor treatment by 4.2 to 13.4% to the RLS soil being a significantly greater reduction from using 2-NPT compared to nBTPT (Fig. 5.4*(b)* and Table 5.3). Although there was reduction in emissions by 2-NPT treatments compared to No-inhibitor treatment and nBTPT-low treatment for the first 2 days, treatment effects were not significant in the EBL soil in terms of total reduction (Fig. 5.4*(b)* and Table 5.3).

Following urine application at 56 days after inhibitor application, the maximum values of NH_3 flux from No-inhibitor treatment were exhibited on day 1 with 6573 and 3096 mg NH_3-N m^{-2} d^{-1} for RLS and EBL soils, respectively (Fig. 5.4*(c)*). In RLS soil, NH_3 fluxes from No-inhibitor treatment were higher relative to 2-NPT treatments for 1 - 2 days but not with the nBTPT-low. The NH_3 fluxes for different rates of 2-NPT were different on day 1 with 3743.7, 3216.4 and 2609.9 mg NH_3-N m^{-2} d^{-1} for 2-NPT low, 2-NPT medium and 2-NPT high, respectively. With the EBL soil, NH_3 fluxes from No-inhibitor treatment were similar to the inhibitor treatments on day 1. The 2-NPT significantly reduced total NH_3 emissions in the measurement period on the RLS soil with a reduction of 5.6 - 7.4% (Fig. 5.4*(c)* and Table 5.3) compared to those from No-inhibitor treatment, but not with nBTPT. Treatment effects were not significant with the EBL soil at this time either. When urine was applied at 29 days after inhibitor application and reapplied at 60 days, there was no difference in total NH_3 emissions from the second urine application between the treatments in both soils (Fig. 5.4*(d)* and Table 5.3).

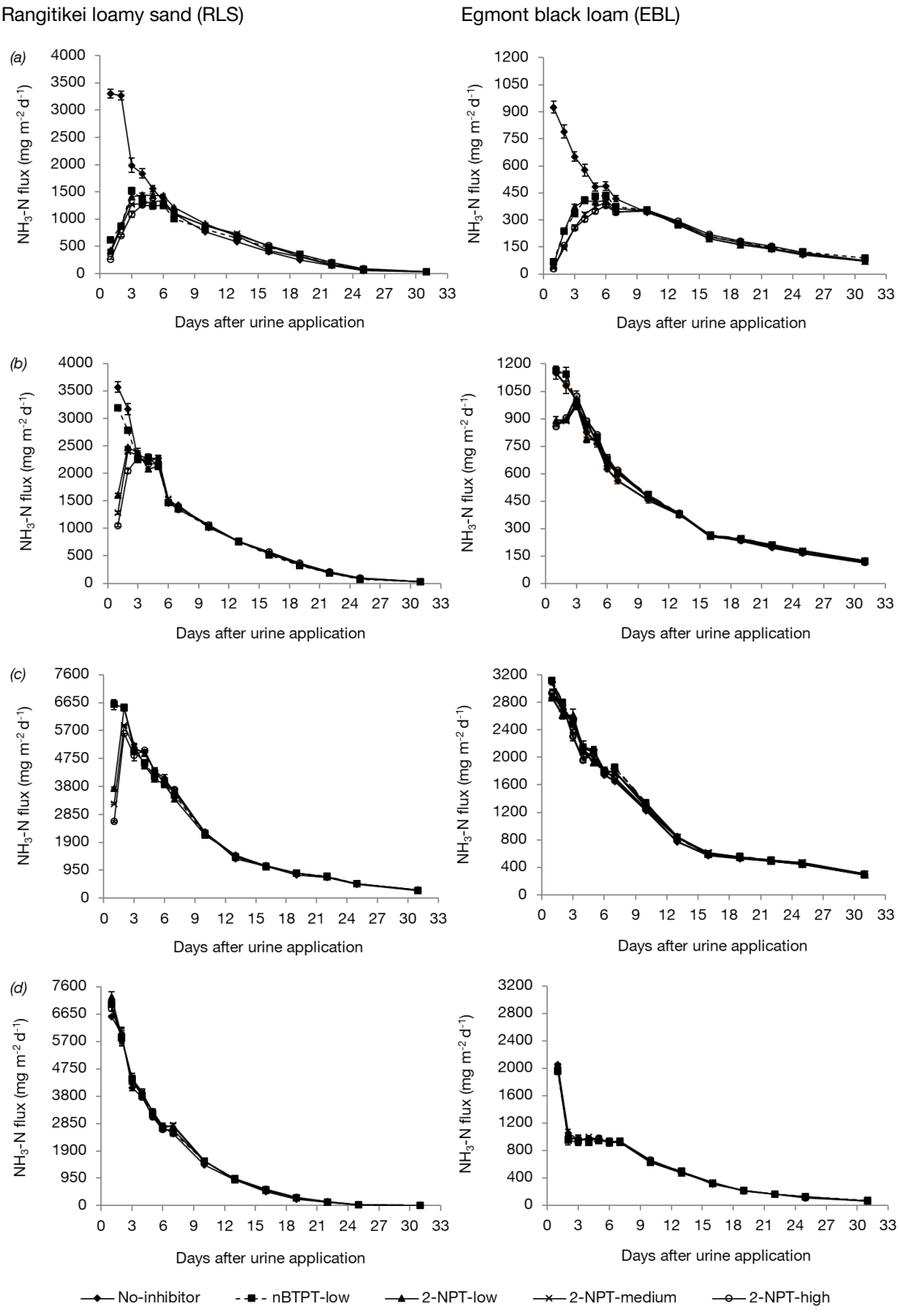

Fig. 5.4 Effect of applying cattle urine *(a)* immediately before, *(b)* 29 days after, *(c)* 56 days after and *(d)* 29 days after *(b)* and again at 60 days after inhibitor application on mean NH_3-N emissions from dairy-grazed pasture soils, vertical bars indicate standard error values

5.3.2 Soil pH

The changes in soil pH measured in a parallel experiment, following urine applied immediately before inhibitor application (Stage-A) to the RLS and EBL soils are shown in Fig. 5.5. The changes in surface soil pH were greater in RLS soil compared to EBL soil and reached a maximum of 8.3 on day 1 for the No-inhibitor treatment and remained higher than in the inhibitor treatments for the first 3 days (Fig. 5.5*(a)*). While with the EBL soil, pH reached a maximum of 7.5 on day 1 for the No-inhibitor treatment and also remained higher than for the inhibitor treatments for the first 3 days (Fig. 5.5*(b)*). By day 31, soil pH had almost returned to the background level of 5.6 with the RLS soil for all the treatments. However, for EBL soil the pH was still higher than background level of 5.7 by day 31 with the all treatments.

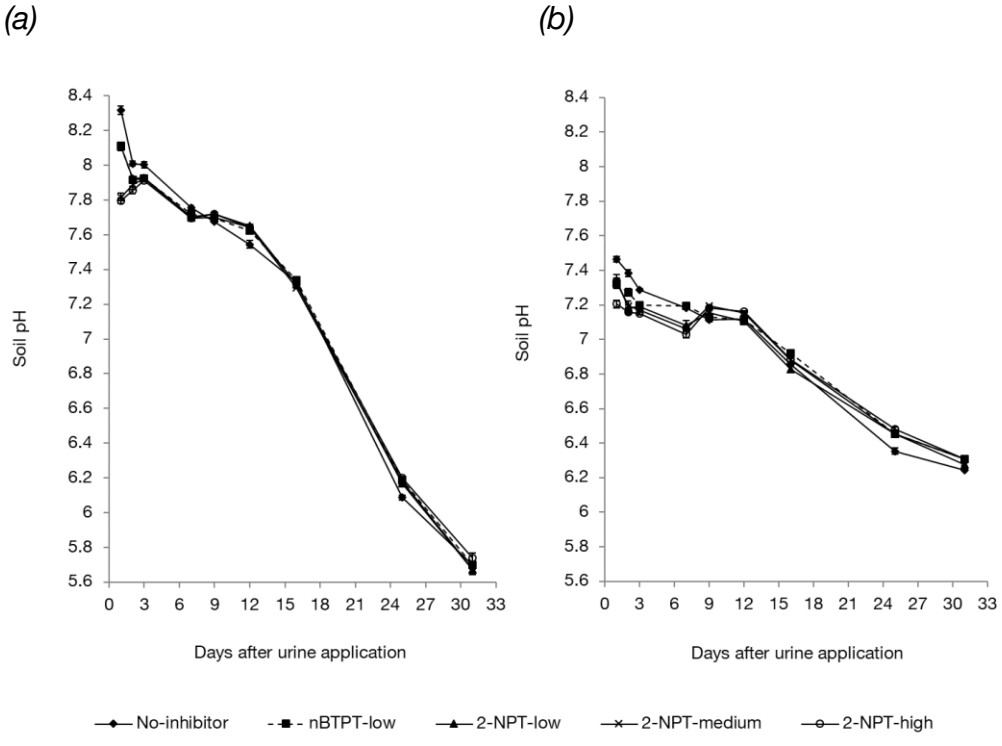

Fig. 5.5 Effect of applying cattle urine immediately before inhibitor application on mean soil pH of dairy-grazed pasture soils *(a)* Rangitikei loamy sand (RLS) and *(b)* Egmont black loam (EBL), vertical bars indicate standard error values

5.3.3 Microbial biomass C

Soil microbial biomass C did not differ among the No-inhibitor and inhibitor treatments during Stage-A (Table 5.4), thus, no further measurements were carried out for the remaining urine application stages.

Table 5.4 Effect of applying cattle urine immediately before inhibitor application (Stage-A) on mean soil microbial biomass C measured at the end of experimental period for dairy-grazed pasture soils, Rangitikei loamy sand (RLS) and Egmont black loam (EBL)

	Soil microbial biomass C (mg kg^{-1} soil)				
Soils/Treatments	No-inhibitor	nBTPT-low	2-NPT-low	2-NPT-medium	2-NPT-high
RLS	780.3 [a]	763.5 [a]	673.4 [a]	714.6 [a]	681.1 [a]
EBL	1469.7 [a]	1399.6 [a]	1455.7 [a]	1413.7 [a]	1557.7 [a]

Means followed by different lower case letters in a row are significantly different ($P < 0.05$)

5.4 Discussion

The total NH_3 emissions from the No-inhibitor treatment (urine alone) were influenced strongly by soil type during this laboratory incubation study. The percentage of urine-N that was emitted as NH_3 ranged from 35.8 - 50.5% for the RLS soil, which are about double the losses compared to the EBL soil. These losses are high compared to those typically measured in field studies (Zaman and Blennerhassett 2010; Zaman and Nguyen 2012), however, there are examples of similarly high losses under field conditions (Carran et al. 1982; Ball and Ryden 1984; Petersen et al. 1998). For example, a NZ study on pasture soil (Carran et al. 1982) reported that 36% of the applied N emitted as NH_3, when cattle urine was applied to soil that was near wilting point during autumn. For the present incubation study, the dry soil conditions and absence of plants to take up exchangeable NH_4^+-N, which would have resulted in high and prolonged

increases in surface NH_4^+-N concentration, are likely to have favoured higher emissions. The significantly higher total NH_3 emitted from the RLS soil, compared to the EBL soil, from the No-inhibitor treatment is attributed to lower pH buffering capacity and higher changes in the pH of the RLS soil (up to pH 8.3), compared with the EBL soil (up to pH 7.5). Soil pH influences NH_3 emissions by facilitating the dissociation of NH_4^+ to gaseous NH_3 in soil solution, which is ultimately emitted into the atmosphere (Saggar et al. 2004). Furthermore, NH_3 concentration in soil solution is influenced by soil CEC via the reaction of NH_4^+ with the negatively charged cation exchange sites (Bolan et al. 2004; Saggar et al. 2004). The lower CEC in the RLS soil resulted in increased NH_4^+ concentration in soil solution, contributing to higher NH_3 emissions.

Results from this study indicated that the addition of both the inhibitors immediately after urine application significantly reduced total NH_3 emissions by up to about 27%, compared to No-inhibitor treatment with no significant difference in reduction between inhibitors. This reduction occurred mostly during the first 5 days after urine application, which is the period of the highest emissions. However, following this initial period of inhibition, emissions continued to be measured up to about 30 days after urine application, which explains why the inhibitors were not able to prevent the majority of the emissions. When urine was applied 29 days after inhibitor application, 2-NPT reduced emissions showing a significantly greater effect than nBTPT and only for the RLS soil. However, the emission reduction was only about half of that compared to when the urine was applied directly before inhibitor application. The 2-NPT only continued to reduce emissions from urine applied up to 56 days after inhibitor application in RLS soil. Domínguez et al. (2008) reported a similar result, showing a

12% inhibition of UA in soil incubated with 2-NPT for 30 days, however, no reduction in UA with nBTPT. These results support that applying 2-NPT to a paddock following a grazing event may have a carry-over effect of also reducing emissions from up to two subsequent grazing, especially when the grazing cycle is short (i.e. ≤28 days). However, the benefit will be soil dependent, with a longer effectiveness for soils with low microbial biomass C (RLS soil), because microbial degradation greatly affects the persistency of phosphorodiamidates (2-NPT) in soil (Kappaun et al. 2018). In addition, soils like the RLS soil, with lower background UA, may exhaust the activity of the inhibitor more slowly and so enhance longevity (Saggar et al. 2013). The absence of an effect of inhibitor treatments on NH_3 emissions at Stage-D (following the second urine application) is likely to be due to the activity of the inhibitors being exhausted by the first urine application.

The significant reduction in NH_3 emissions from the treatment 2-NPT-low at Stage-C (56 days after application of 2-NPT at 0.013% of total urine N) in the RLS soil, reflects that even the lower rate of 2-NPT, than that of 0.025% of total N (standard application rate for nBTPT), has the potential to reduce emissions. A similar result has been reported in an incubation study in soil-water suspension (Phillip et al. 2015) indicating 2-NPT at 0.0125% of total urea N significantly inhibited urea hydrolysis by 72% after 17 days of application. Another study (Hucke et al. 2010) also suggested that 2-NPT could be used at concentrations ranging from 0.001 to 10% (w/w) with livestock effluent to inhibit UA.

The additional reductions achieved by the 2-NPT treatments, compared to the nBTPT-low treatment, occurred during the first few days after urine application, when

urine was applied immediately before inhibitor application. However, there were no significant differences between the inhibitor treatments on total reductions over the entire experimental period for either soil. Similarly, there was no effect of 2-NPT treatments in reducing total emissions from the EBL soil at Stage-B despite successful inhibition for the first two days. Because the additional benefit from using 2-NPT as an inhibitor was mostly observed within the first 2 - 3 days after urine application, its relative effectiveness may be greater where the majority of emissions occur over a shorter period to that seen in this study. This may be the case in field studies where the majority of emission (>90%) occur within two weeks (Zaman and Blennerhassett 2010), due to the uptake of NH_4^+ions by plants contributing to reduce accumulation in surface soil.

The maximum NH_3-N flux occurred within the first day of urine application for the No-inhibitor treatment was probably because of quick urea hydrolysis and the sharp rise in soil pH (Fig. 5.5). In addition to soil UA, hippuric acid present in cattle urine and the high urine pH (>8) stimulated the hydrolysis of urea and resulted in fast urea hydrolysis (Tabatabai and Bremner 1972; Whitehead et al. 1989). These NH_3-N fluxes decreased afterwards as pH decreased, which was also observed in a glasshouse study with pasture soil cores receiving cattle urine (Singh et al. 2013). Wherever NH_3-N fluxes were lower for inhibitor treatments compared to the No-inhibitor treatment in this study, this could be attributed to reduced soil UA by nBTPT and 2-NPT, and, thereby, decreasing urea hydrolysis and minimising the rise in soil pH (Zaman et al. 2009; Schraml et al. 2016) .

There were no significant effects of inhibitor treatments on soil microbial biomass C, compared to the No-inhibitor treatment measured during Stage-A, which could be due to both nBTPT and 2-NPT only specifically blocking enzymatic reactions (i.e. delaying the activity of the urease enzyme) and are not broad biocidal on soil microbial biomass. This is consistent with the findings of an earlier studies suggesting no significant effect of added nBTPT (Zaman et al. 2009) and 2-NPT (Ni et al. 2018) on soil microbial biomass.

In this study, both of the inhibitors, 2-NPT and nBTPT, showed effectiveness in reducing NH_3 emissions from both soils with the application of inhibitors immediately after urine application. The 2-NPT also reduced emissions from urine applied up to 56 days after inhibitor application, but only for the RLS soil. The results of the study showed that the longevity of 2-NPT in reducing emissions is greater than nBTPT and is strongly influenced by soil type, exhibiting longer effectiveness in RLS soil, which had the lower microbial biomass C and UA.

5.5 Conclusions

Addition of both of 2-NPT and nBTPT immediately after urine application significantly reduced total NH_3 emissions by slowing down urea hydrolysis and lowering soil pH from both soils. The inhibitor 2-NPT also reduced emissions from urine applied up to 56 days after inhibitor application, but only on the soil with lower microbial C and UA. These results suggest that the UI 2-NPT applied following a grazing event may reduce emissions from up to two subsequent grazing especially when the grazing cycle is short (i.e. $\leq$28 days), but this effect will be soil specific. Therefore, from this study it can be concluded that the longevity of 2-NPT in inhibiting of soil UA

and NH_3 emissions is slightly greater compared to the currently used inhibitor nBTPT. Further research is required to assess whether these differences between the inhibitors are also achieved under field conditions. Results from this study also indicated that neither inhibitors possess a negative effect on soil microbial biomass C.

Chapter 6

Field evaluation of 2-NPT and nBTPT in reducing ammonia emissions from cattle urine applied to pasture soils

6.1 Introduction

Chapter 5 reports the result of a laboratory study that involved applying UIs, 2-NPT and nBTPT, to air-dried and sieved soil samples, immediately after or before the addition of cattle urine. In this study both UIs reduced NH_3 emissions when inhibitors were applied immediately after urine, however, 2-NPT showed slightly greater longevity than nBTPT. Therefore, 2-NPT could potentially mitigate emissions from cattle urine deposited from more than one grazing in some pasture soils, especially for soils with lower microbial biomass C and UA as observed in the laboratory study. However, no field study has evaluated the effectiveness and longevity of 2-NPT and nBTPT in reducing emissions from cattle urine applied to pasture soils. Therefore, field testing is needed to assess the differences in longevity between 2-NPT and nBTPT. The effectiveness of UIs applied to freshly deposited urine could be influenced by soil and environmental conditions, such as soil moisture content and temperature, and timing of their application by regulating the ability of inhibitors to interact with deposited urine. Hence, for optimising application management, it is also useful to explore the effect of these factors on the effectiveness of UIs applied following urine application. This chapter reports the results of two field plot experiments evaluating both the effectiveness and longevity of 2-NPT and nBTPT in reducing NH_3 emissions from cattle urine deposited in pasture soils, simulating one or more grazing events. The specific objectives of the study were:

- To measure and compare the effectiveness and longevity of 2-NPT with nBTPT in reducing NH_3 emissions from cattle urine applied to pasture soils.
- To assess the effect of soil moisture content and temperature, and timing of inhibitor application on the efficiency of 2-NPT and nBTPT applied following urine application in reducing NH_3 emissions.
- To examine the effect of 2-NPT and nBTPT on soil pH and urine-N transformations following cattle urine application in a pasture soil.
- To determine the impact of 2-NPT and nBTPT on pasture dry matter production and N uptake following cattle urine application.

6.2 Materials and methods

6.2.1 Experimental site

Two experiments were conducted at the Massey University No.1 Dairy Farm, Palmerston North, New Zealand. The pasture consisted predominantly mixture of perennial ryegrass (*Lolium perenne*) and white clover on Manawatu fine sandy loam soil. The first experiment was initiated on 23rd of November 2017 and the second experiment was initiated on 1st of May 2018, and hereafter referred as 'summer experiment' and 'autumn experiment' (southern hemisphere) in the subsequent discussion in this thesis, respectively. The free-draining soil of the site used is classified as Eutric Fluvisol (FAO–UNESCO 1998). The site was grazed by dairy cattle up to 2008 and subsequently used for a mixture of cropping and sheep grazing. More recently it was used for research trials (Hoogendoorn et al. 2017). The selected area for the experiment was not grazed and fertilised for 8 months before the establishment of field plots to minimise the effect of previous livestock excreta/fertiliser inputs.

The physio-chemical properties of the soil at the site at the beginning of summer experiment are presented in Table 6.1.

Table 6.1 Initial physio-chemical properties of the site soil from 0-5 and 5-10 cm depths (November, 2017)

Depth (cm)	pH water	Total C (%)	Total N (%)	CEC (meq 100 g^{-1})	Soil UA (mg kg^{-1} soil hr^{-1})	Field capacity (%)	Bulk density (Mg m^{-3})
0-5	5.5	2.8	0.3	18.5	37	37	1.2
5-10	5.5	2.2	0.3	16.5	21	33	1.4

6.2.2 Experimental design and treatments

Both summer and autumn experiments consisted of a completely randomised block design using four replicates of each treatment. In the summer experiment, the treatments were i) Control (water only), ii) Urine, iii) Urine + nBTPT, and iv) Urine + 2-NPT. Treatments were repeated, using separate plots, for three urine application timings: 3 hrs before inhibitor application (Stage-A), 28 days after inhibitor application (Stage-B), and 68 days after inhibitor application (Stage-C); and NH_3 emissions measurement and soil sampling were performed following each urine application. The separate sampling areas were established for NH_3 emissions measurements and soil sampling (Fig. 6.1). The total number of plots was 48 (4 treatments × 4 replicates × 3 stages) for each sampling area. The application rates of inhibitors to the urine treatments for all three stages were the same and based on the percentage of total urine-N applied at Stage-A. The variability in applied urine N concentration across the all 3 stages resulted in small differences in the application rate of both inhibitors (0.025% vs 0.021%) relative to the amount of N applied for equivalent treatments at different stages (Table 6.2). The timings of urine applications

with respect to inhibitor application were not included as a part of treatment in summer experiment because the experimental conditions such as amounts of N applied, soil moisture contents, and temperatures were varied. However, the difference in timings of inhibitor applications were considered as a treatment part in autumn experiment as urine with equal amount of N was applied at the same time for all treatments with urine.

Table 6.2 The application rates of inhibitors to treatments at different stages of urine application in summer experiment

Stage	Urine application time	Inhibitor rates to treatments (% of urine N applied)			
		Control	Urine	Urine + nBTPT	Urine + 2-NPT
A)	3 hrs before inhibitor application	–	–	0.025	0.025
B)	28 days after inhibitor application	–	–	0.021	0.021
C)	68 days after inhibitor application	–	–	0.021	0.021

In the autumn experiment, the six treatments included were: i) Control (water only), ii) Urine, iii) Urine immediately before nBTPT application (Urine + nBTPT immediate), iv) Urine immediately before 2-NPT application (Urine + 2-NPT immediate), v) Urine 3 hrs before nBTPT application (Urine + nBTPT 3hrs), and vi) Urine 3 hrs before 2-NPT application (Urine + 2-NPT 3 hrs). The total number of plots was 24 (6 treatments × 4 replicates) for each sampling area, NH_3 emissions measurements and soil sampling. The inhibitor application rate was 0.025% of urine N applied (Table 6.3).

Table 6.3 Treatments and inhibitor rates to treatments in autumn experiment

Treatments	Inhibitor rates (% of urine N applied)
Control (water only)	–
Urine	–
Urine immediately before nBTPT application	0.025
Urine immediately before 2-NPT application	0.025
Urine 3 hrs before nBTPT application	0.025
Urine 3 hrs before 2-NPT application	0.025

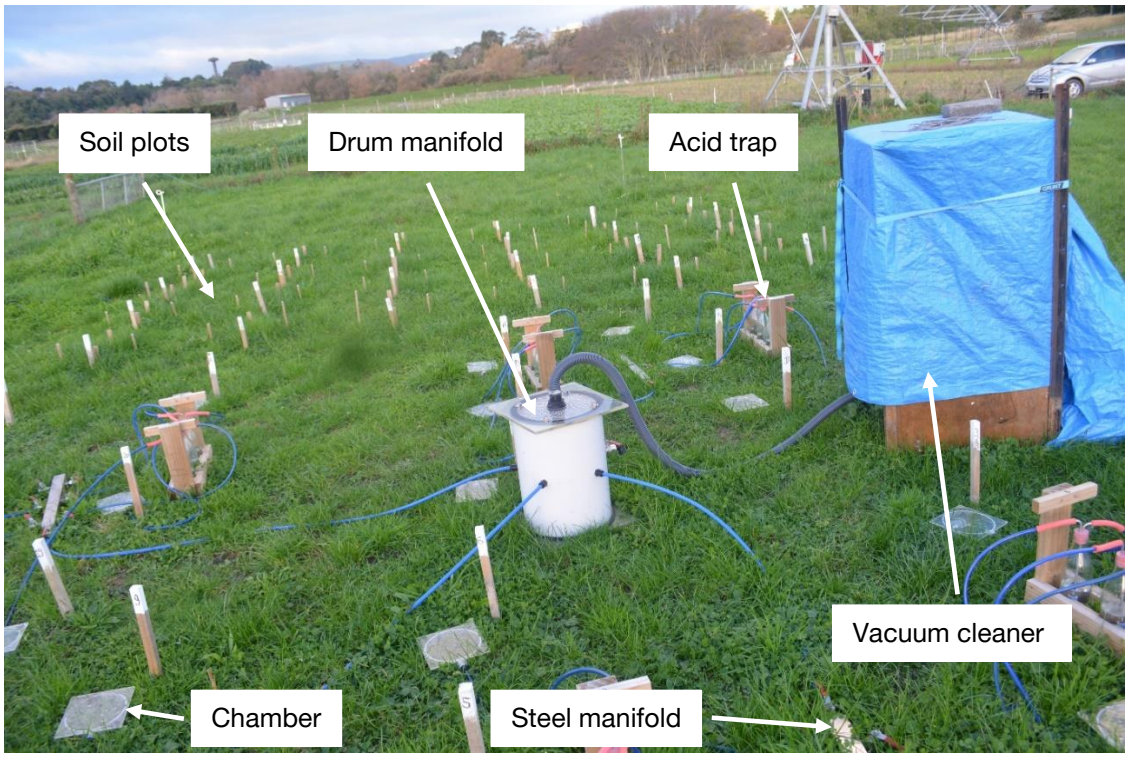

Fig. 6.1 Field experimental set up showing chamber areas for NH_3 emissions measurements and soil plots

Individual treatment plots in both sampling areas: a) a gas chamber area for NH_3 emissions measurements (circular area of diameter 15 cm) and b) an open area (circular area of diameter 50 cm) for soil sampling and harvesting pasture yield were separated by a distance of at least 50 cm. The pasture in experimental areas was mown (chamber areas for NH_3 emissions measurements - 3 cm height and soil plots - 5 cm height) before inhibitor/urine application to simulate grazing. The solutions of the nBTPT and 2-NPT treatments were prepared by dissolving Agrotain® (liquid form) and 2-NPT salt in DI water, respectively. A solution volume equivalent to 800 L ha^{-1} was sprayed on to the pasture plot areas using a syringe connected the lid of 50 mL plastic atomizer. The nBTPT oxidation to its oxygen analogue (nBTPTO) does not appear to have any significant effect on efficiency of inhibitor under partially anaerobic soil conditions of this study. Cylindrical PVC pipes, which had the same diameter as the chambers, were inserted in to the soil to a depth of 1 cm before the inhibitor/urine application. Similarly, cylindrical metal rings, which had the same diameter as the soil plot areas, were inserted in to the soil plots (1 cm depth) before the inhibitor/urine application. These cylindrical pipes/rings were used to restrict the lateral movement of urine applied (application depth of 10 mm) prior to infiltration into the soil. The urine was applied to the plot areas using a plastic container.

6.2.3 Meteorological data

Ground level air temperatures inside and outside the chambers were recorded throughout the experimental period using Temperature MicroLogger (G) (Hortplus, New Zealand). Additionally, soil temperatures at 5 cm depth was also recorded in summer experiment but not in the autumn experiment as Temperature MicroLoggers used in autumn was not working. Rainfall data were obtained from a meteorological

station (National Institute of Water and Atmospheric Research/AgResearch Ltd, Palmerston North) located at a distance of about 500 m from the experimental site.

6.2.4 Ammonia emission

Ammonia emissions were measured using the dynamic chamber method as described by Kissel et al. (1977). Emissions were measured by inserting a PVC chamber (15 cm internal diameter, 4 cm height with a tightly sealed transparent lid to allow photosynthesis) into the soil at a depth of 1 cm, providing a headspace volume of 500 cm^{-3}. The chamber used consisted of three air intake holes and one exhaust hole on its vertical surface. The exhaust hole was connected to an acid trap (glass bottle consisting of 250 mL of 0.025 M H_2SO_4) through a tube connected to a steel manifold, which regulated the air flow rate. The steel manifold was connected to the drum manifold, which in turn was connected to a vacuum cleaner. Air from each chamber was sucked at a constant flow rate of 6 L min^{-1} (monitored daily) throughout the experimental period and was then passed through the acid solution to capture NH_3 emitted (Fig. 6.1). The sampling of NH_3 was performed periodically up to day 25 in the summer experiment and up to day 16 in the autumn experiment. The emissions in the summer experiment continued for a longer period than in the autumn experiment, which was the reason for the differences in the length of the sampling periods of the two experiments. During each sampling, the volume of acid in each bottle was recorded before sub-sampling and replaced with fresh acid solution. The sub-samples were stored at 4°C until they were analysed for NH_4^+-N concentration using a Technicon AutoAnalyzer. The NH_3-N flux (kg ha^{-1} d^{-1}) was calculated by using Eqn. 6.1. A volume of water that was equivalent to the quantity of rainfall that occurred, was sprayed over the chamber area to compensate any rainfall that fell during the measurement period.

$$NH_3\text{–}N\ flux = \frac{C \times V}{a \times D \times 100} \qquad Eqn.\ 6.1$$

where, C = NH_3 concentration in the acid solution (mg L^{-1}); V = the total volume of acid solution at the time of sampling (L); a = total cross sectional area (m^2) of the chamber inserted into the soil; D = duration (days) of each sampling.

The percentage reduction in total NH_3–N flux with the application of inhibitors relative to the urine only treatment was calculated using Eqn. 6.2.

$$\%\ reduction\ in\ NH_3 - N\ flux = \frac{C - A}{C} \times 100\% \qquad Eqn.\ 6.2$$

where, C = total NH_3-N emitted from urine only treatment; A = total NH_3-N emitted from treatment with inhibitor.

6.2.5 Analyses

6.2.5.1 Urine

Urine was collected from dairy cattle during milking at the Massey University's Dairy Farm 1 and Dairy Farm 4. The fresh urine samples that were collected from individual cattle were bulked together and stored for up to five days at 4°C to avoid urea hydrolysis prior to application. Urine samples were analysed for total N, urea-N and pH before application as mentioned in Chapter 5, section 5.2.5.1. The chemical properties and application rates of urine, expressed on a total N in kg ha^{-1}, used in all of the experiments are presented in Table 6.4.

Table 6.4 Chemical properties of cattle urine and application rates used in the study

Summer Experiment		pH	Urea-N ($g\ L^{-1}$)	Total N ($g\ L^{-1}$)	Application depth (mm)	N applied ($kg\ ha^{-1}$)
Stage	Urine application time					
A)	3 hrs before inhibitor application	8.1	3.6	6.0	10	597.2
B)	28 days after inhibitor application	8.0	5.2	7.3	10	725.9
C)	68 days after inhibitor application	8.0	5.7	7.2	10	721.4
Autumn Experiment		7.5	4.9	5.9	10	589.7

6.2.5.2 Soil sampling

Soil samples were collected periodically after urine application up to day 25 during the summer experiment and up to day 13 during the autumn experiment. At each sampling, three soil cores at two depths (0-5 and 5-10 cm for the summer experiment, and 0-2.5 and 2.5-5 cm for the autumn experiment) were collected from each plot. The deeper soil samples collected in the summer experiment caused greater dilution in measured parameters (pH and NH_4^+-N), therefore, shallower depths were chosen for the autumn experiment. Similarly, soil water contents at the shallower depths were better for explaining the variations in NH_3 emissions and effectiveness of UIs. The cores were bulked together to obtain a composite sample from each replicate plot, which was sieved through a 2-mm sieve. The sieved soil was used to determine soil water content, pH and mineral N (NH_4^+ and NO_3^-). The holes left behind following each sampling were filled with soil from the area surrounding the experimental plots and 35 mL polypropylene plastic containers (P35 vial) were inserted in to the top of the hole to mark the spot. The holes were filled to reduce the potential of soil water to drain into them, which could influence N leaching from the root zone.

6.2.5.3 Soil water content

Soil water content was determined gravimetrically by weighing about 20-30 g field moist soil and then oven-dried at 105ºC for 24 hrs. The dried soils were again weighed and water content was calculated as presented in Eqn. 6.3.

$$Soil\ water\ content\ (\%) = \frac{Weight\ of\ water\ in\ moist\ soil\ (g)}{Weight\ of\ oven-dried\ soil\ (g)} \times 100\% \qquad Eqn.\ 6.3$$

6.2.5.4 Soil pH

Soil pH was determined by mixing 10 g of field moist soil with 25 mL of DI water and shaken for 30 min with the end-over-end shaker (Zaman et al. 2009). The solution was stored overnight and pH was measured using a pH meter.

6.2.5.5 Soil mineral N

Soil mineral N was determined by adding 5 g of field moist soil with 30 mL of 2 M KCl, containing 5 µg mL^{-1} of phenylmercuric acetate (as a UI), which was then shaken on an end-over-end shaker for 60 min and extracted for NH_4^+ and NO_3^-. The amount of NH_4^+-N and NO_3^--N present in the extract was determined colorimetrically using a Technicon AutoAnalyzer (Blakemore et al. 1987).

6.2.5.6 Pasture production and N uptake

Pastures from all the soil plots were harvested at a height of 4 - 5 cm using a hand shearer at various times (3 times in summer experiment and 1 time in autumn experiment) to simulate grazing. The harvested pasture samples from each plot were transferred into pre-weighed paper bags and dried at 65ºC for one week. After drying, pasture dry matter weight was recorded and the pasture samples were finely ground and

0.1 g was used for total N analysis using the Kjeldahl digestion method (as mentioned in Chapter 5, section 5.2.5.1). The recovery of applied urine N via pasture uptake was calculated using the Eqn. 6.4.

$$N\ recovery\ (\%) = \frac{Pasture\ N\ (urine\ \pm inhibitor) - Pasture\ N\ (control)}{N\ applied\ (urine)} \times 100 \quad Eqn.6.4$$

6.2.6 Statistical methods

The data for NH_3 emissions, soil pH, mineral N, pasture dry matter and pasture N uptake were analysed using an ANOVA to detect any significant difference and treatment means were compared using Tukey's Studentized Range (HSD) Test. The linear regression was used to determine the relationship between the daily NH_3 fluxes and soil pH. All of the analyses were conducted using the Statistical Analysis System software (SAS 9.4, $P < 0.05$).

6.3 Results

6.3.1 Meteorological data

The total rainfall during the summer experiment at stages A, B, and C was 5, 78, and 65 mm, respectively (Fig. 6.2) and was 28 mm during the autumn experiment (Fig. 6.3). Most of the rainfall occurred 5 days after urine application during the summer and autumn experiments.

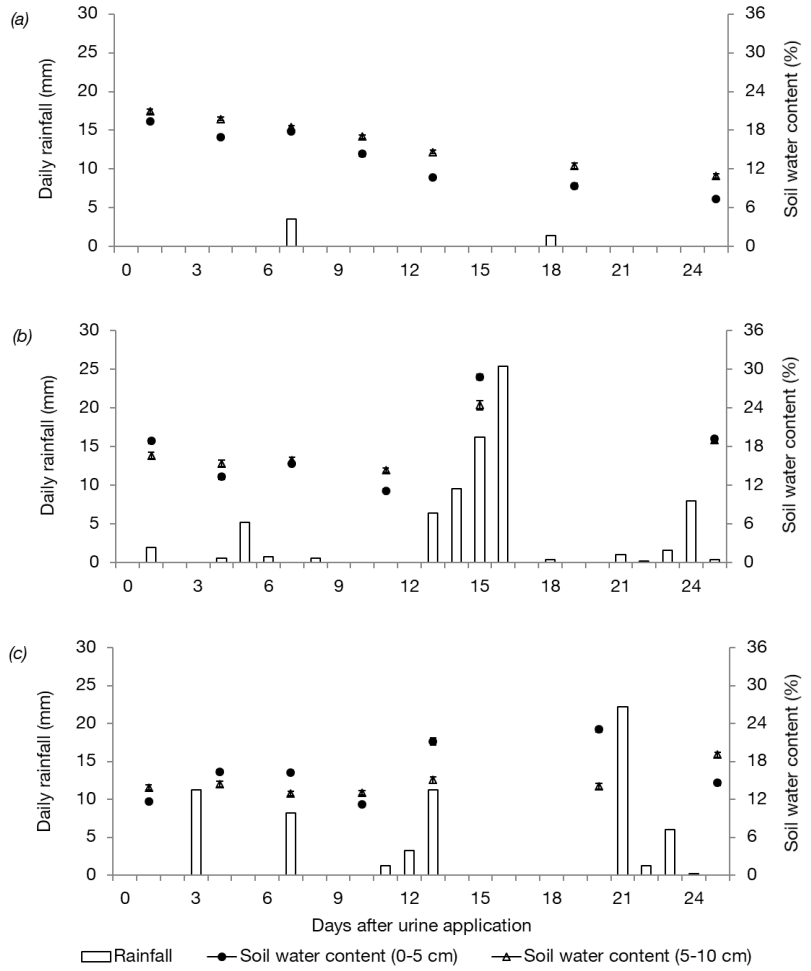

Fig. 6.2 Rainfall events, and soil water content in different soil depths during the summer experiment: *(a)* urine 3 hrs before the inhibitor *(b)* urine 28 days after the inhibitor, and *(c)* urine 68 days after the inhibitor application, vertical bars on soil water content data indicate standard error values

Soil water contents varied with rainfall events and was generally higher in autumn compared to summer (Figs. 6.2 and 6.3). The soil water contents at 0-5 cm depth during the summer experiment were <20% at most of the sampling times, with some exceptionally higher water levels when heavy rainfall occurred around the day of sampling. For example, at Stage B, soil water content was 29% on day 15 when total rainfall within 4 days of sampling was 58 mm. However, water levels at 0-2.5 cm and 2.5-5 cm depths in the autumn experiment were ranged of 27 - 33% and 23 - 28 %,

respectively. The soil water contents were similar between the treatments, therefore the mean values for all the treatments are presented.

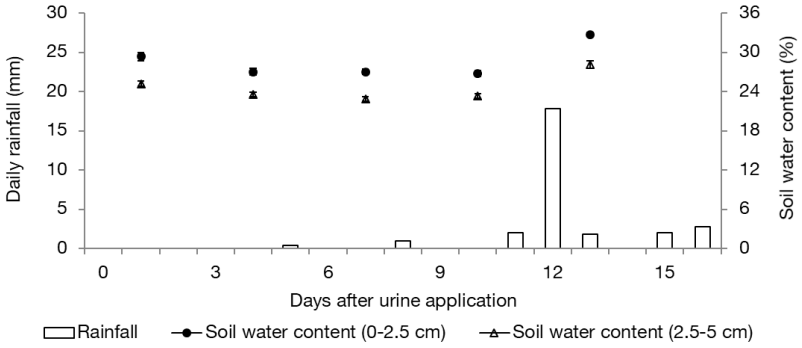

Fig. 6.3 Rainfall events, and soil water content in different soil depths during the autumn experiment, vertical bars on soil water content data indicate standard error values

During the summer experiment, the average daily ground level air temperatures inside the chambers were between 16.3 - 23.6ºC, and outside of the chambers were between 14.8 - 23.4ºC (Fig. 6.4). During the autumn experiment, the temperatures inside the chambers were between 10.5 - 15.7 ºC, and outside of the chambers were between 7 - 15ºC (Fig. 6.5). The similar temperature ranges inside and outside of the chambers are because of continuous suction of air from chambers. The soil temperatures at the depth of 5 cm were between 16.2 - 25.1ºC during the summer experiment (Fig. 6.4).

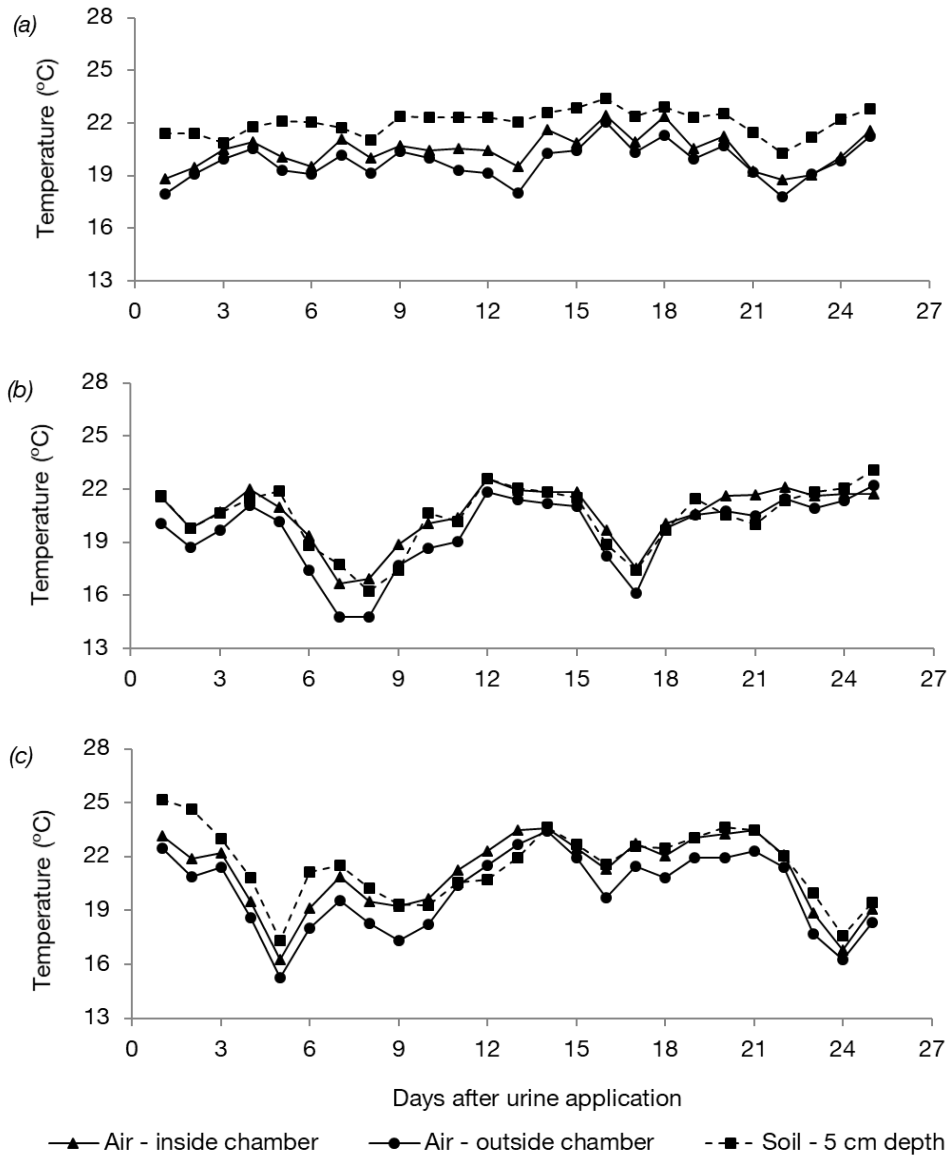

Fig. 6.4 Ground level air temperatures (inside and outside chamber) and soil temperatures during the summer experiment: *(a)* urine 3 hrs before the inhibitor *(b)* urine 28 days after the inhibitor, and *(c)* urine 68 days after the inhibitor application

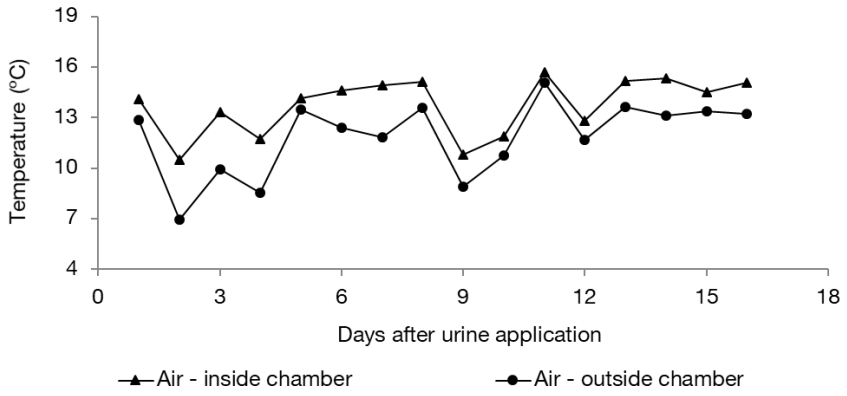

Fig. 6.5 Ground level air temperatures (inside and outside chamber) during the autumn experiment

6.3.2 Ammonia emission

Total NH_3 emitted from the urine only treatment significantly varied between the two seasons of summer (111 - 142 kg N ha^{-1}) and autumn (27 kg N ha^{-1}), being higher in summer. The NH_3-N emitted in summer was between 15.3 - 23.6 % of the total N applied (Table 6.5), however, in autumn the emissions were only 4.5 % of the total N applied (Table 6.6). Most emissions occurred within the first day of urine application. Total emissions from control were significantly lower than treatments with urine with or without inhibitors in both seasons.

In the summer, when urine was applied 3 hrs before the inhibitor application, the temporal variations in the daily NH_3 fluxes from soils treated with urine with or without inhibitors were observed. The NH_3 flux from the urine only treatment possessed the highest value on day 1 of 66 kg N ha^{-1} d^{-1}, which was 46% of total N emitted during the experimental period (Fig. 6.6 *(a)*). The NH_3 emissions decreased sharply to a level of 12 kg N ha^{-1} d^{-1} by day 2. After day 2, emissions decreased further at a slow rate, but remained slightly above those of the control (no urine) treatment. The daily NH_3 emissions from inhibitor treatments followed a similar trend to those of urine only

treatment. There was no effect of either of the inhibitors on reducing total emissions compared to the urine only treatment.

Table 6.5 Effect of inhibitors on NH_3-N emissions from urine-N added to pasture soil at different Stages; A) 3 hrs before, B) 28 days after, and C) 68 days after inhibitor application during the summer experiment [mean (standard error)]

	Stage-A	Stage-B	Stage-C
N added (kg ha^{-1})	597.2	725.9	721.4
Treatments	Cumulative NH_3-N emissions (kg ha^{-1})		
Control	0.8 (0.1) [b]	0.3 (0) [c]	0.7 (0.1) [b]
Urine	141.6 (4.2) [a]	133.2 (5.5) [a]	111.3 (3.3) [a]
Urine + nBTPT	142.3 (6.4) [a]	125.1 (1.9) [a]	113.6 (6.8) [a]
Urine + 2-NPT	144.7 (2.0) [a]	107.2 (3.9) [b]	116.8 (3.0) [a]
	% change in cumulative NH_3-N relative to urine only treatment		
Urine + nBTPT	+0.5	-6.1	+2
Urine + 2-NPT	+2.2	-19.5	+4.9
	% N emitted as NH_3 of the applied N		
Urine	23.6	18.3	15.3
Urine + nBTPT	23.7	17.2	15.6
Urine + 2-NPT	24.1	14.7	16.1

Means followed by different lower case letters in a column are significantly different ($P < 0.05$)

Following urine application at 28 days after inhibitor application in summer, the largest peak of NH_3 flux from the urine only treatment was on day 1 and was 62 kg N ha^{-1} d^{-1} (Fig. 6.6 *(b)*). Compared to the NH_3 fluxes from the urine only treatment for day 1, emissions were only lower for the treatment with 2-NPT, which were 40 kg N ha^{-1} d^{-1}. There was no significant effect of inhibitors on reducing emissions at subsequent

sampling times. Over the measurement period 2-NPT significantly reduced total NH_3 emissions by 26 kg N ha^{-1} (19.5% reduction) compared to total emissions from the urine only treatment that were equivalent to 133 kg N ha^{-1} (Fig. 6.6 *(b)* and Table 6.5).

Table 6.6 Effect of inhibitors on NH_3-N emissions from urine-N added to pasture soil during the autumn experiment [mean (standard error)]

N added (kg ha^{-1})		589.7	
Treatments	Cumulative NH_3-N emissions (kg N ha^{-1})	% change in cumulative NH_3-N relative to urine only treatment	% N emitted as NH_3 of the applied N
Control	0.3 (0.1) ^{e}	–	–
Urine	27.1 (1.5) ^{a}	–	4.5
Urine + nBTPT immediate*	12.9 (0.4) ^{c}	-52.3	2.1
Urine + 2-NPT immediate*	7.4 (0.6) ^{d}	-72.7	1.2
Urine + nBTPT 3hrs	17.6 (1) ^{b}	-35	2.9
Urine + 2-NPT 3 hrs	15.9 (1) ^{bc}	-41.2	2.7

Means followed by different lower case letters in a column are significantly different ($P < 0.05$), *after treatment indicates n = 3; (Urine + nBTPT immediate = urine immediately before nBTPT application, Urine + 2-NPT immediate = urine immediately before 2-NPT application, Urine + nBTPT 3hrs = urine 3 hrs before nBTPT application, and Urine + 2-NPT 3 hrs = urine 3 hrs before 2-NPT application)

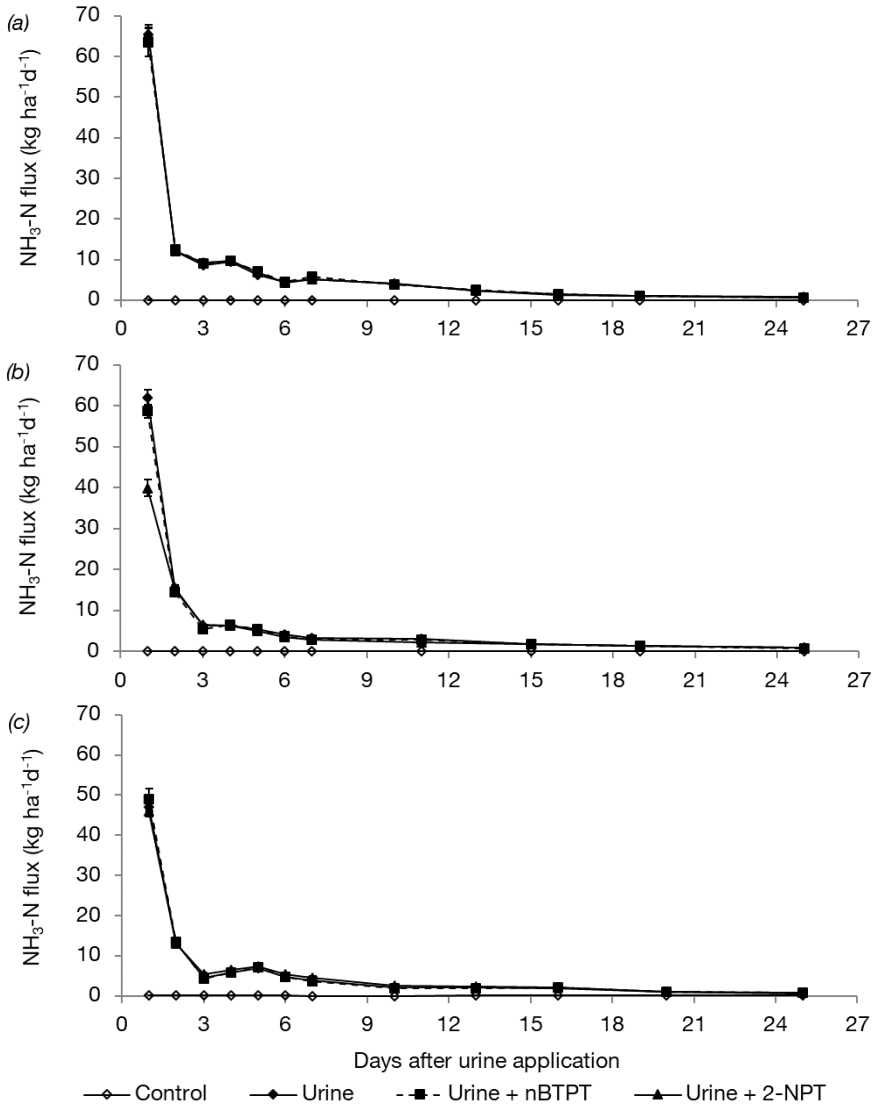

Fig. 6.6 Effect of applying cattle urine *(a)* 3 hrs before, *(b)* 28 days after, and *(c)* 68 days after the inhibitor application on mean NH_3-N emissions from pasture soils during the summer experiment, vertical bars indicate standard error values

With the cattle urine applied 68 days after inhibitor application in summer, the NH_3 flux from the urine only treatment exhibited the highest value on day 1 of 47 kg N ha^{-1} d^{-1}. The day 1 NH_3 fluxes for urine with nBTPT or 2-NPT were not significantly different from the urine only treatment. Emissions decreased sharply during the first few days after urine application, and then gradually decreased further over the subsequent three weeks, achieving values close to the control treatment by day 25. Neither inhibitor

significantly reduced total emissions over the entire sampling period, compared to the urine only treatment (Fig. 6.6 *(c)* and Table 6.5).

In autumn, all urine treatments (with and without inhibitors), except the urine applied immediately before 2-NPT treatment, had their highest emission in the first day after urine application. For urine applied immediately before 2-NPT treatment, the peak emissions occurred on day 2 (1.2 kg NH_3-N ha^{-1} d^{-1}), which was the lowest value of all of the urine treatments. The NH_3 flux from the urine only treatment on day 1 was 15 kg N ha^{-1} d^{-1} (Fig. 6.7), which was about one third to quarter the peak emissions on day 1 of the summer experiment. At day 1, the average rate of emissions for the first 3 hrs (1.1 kg NH_3-N ha^{-1} hr^{-1}) was higher than remaining 21 hrs (0.5 kg NH_3-N ha^{-1} hr^{-1}). The majority of the emissions from the treatments with urine occurred during the first 3 days, after which the values were lower and steadily decreased over the subsequent week, achieving values close to the control after day 10. The emissions from urine applied immediately before inhibitors were significantly lower than urine applied 3 hrs before inhibitors on day 1. Both inhibitors significantly reduced total NH_3 emissions compared to the urine only treatment, at both timings, but the effectiveness was greater when urine was applied immediately before the inhibitors. The amount of NH_3 emitted from nBTPT and 2-NPT treatments were 13 and 7 kg N ha^{-1}, respectively when urine was applied immediately before the inhibitors with corresponding reductions of 52.3 and 72.7 % compared to urine only treatment, being a significantly greater reduction from using 2-NPT than nBTPT. However, with the urine applied 3 hrs before the inhibitors, the reductions were 35 and 41.2% for nBTPT and 2-NPT, respectively, with corresponding emissions of 18 kg and 16 kg N ha^{-1} but the difference between the inhibitors was not significant (Fig. 6.7 and Table 6.6).

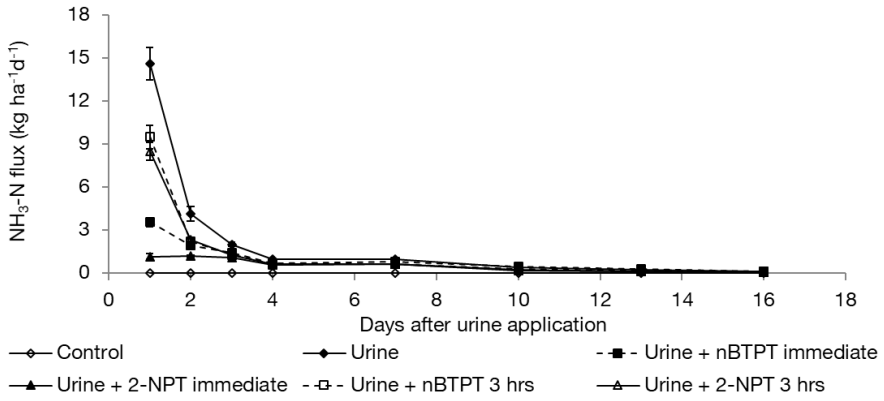

Fig. 6.7 Effect of applying cattle urine on mean NH_3-N emissions from pasture soils during the autumn experiment, vertical bars indicate standard error values (n= 3 for Urine + nBTPT immediate and Urine + 2-NPT immediate)

6.3.3 Soil pH

All urine added treatments (with or without inhibitors) significantly increased soil pH in the summer (0-5 cm depth) and autumn (0-2.5 cm) experiments for between 10-20 days, compared to the no urine control treatment. In the summer experiments, the soil pH (0-5 cm) for urine treatments ranged from 5.9 - 6.3, 1 day after urine application, compared to approximately 5.6 for the control (Fig. 6.8). In the summer, there was negligible influence of inhibitors on soil pH, compared to the urine only treatment, at most sampling times and irrespective of timing of the inhibitor application in relation to the urine application (urine applied 3 hrs before, 28 days after or 68 days after inhibitor application). The only significant effect of 2-NPT on reducing soil pH (0-5 cm) was observed on day 1 when urine was applied 28 days after inhibitor application, and the pH values were 6.3 and 6 for urine only and 2-NPT treatment, respectively (Fig. 6.8 *(b)*). An elevation in pH from urine application was not observed in the deeper soil depth (5-10 cm) in the summer experiments. However, there was evidence of urine treatments causing a decrease in soil pH, compared to the control at this soil depth, particularly at the third summer experiment (Fig. 6.8 *(c)*). By day 25 in

this experiment, soil pH (5-10 cm) was an average of approximately 5 for the urine treatments and 5.3 for the control. Overall for the summer experiments, there was no significant differences between the effects of inhibitor treatments on soil pH in the deeper soil depth (5-10 cm).

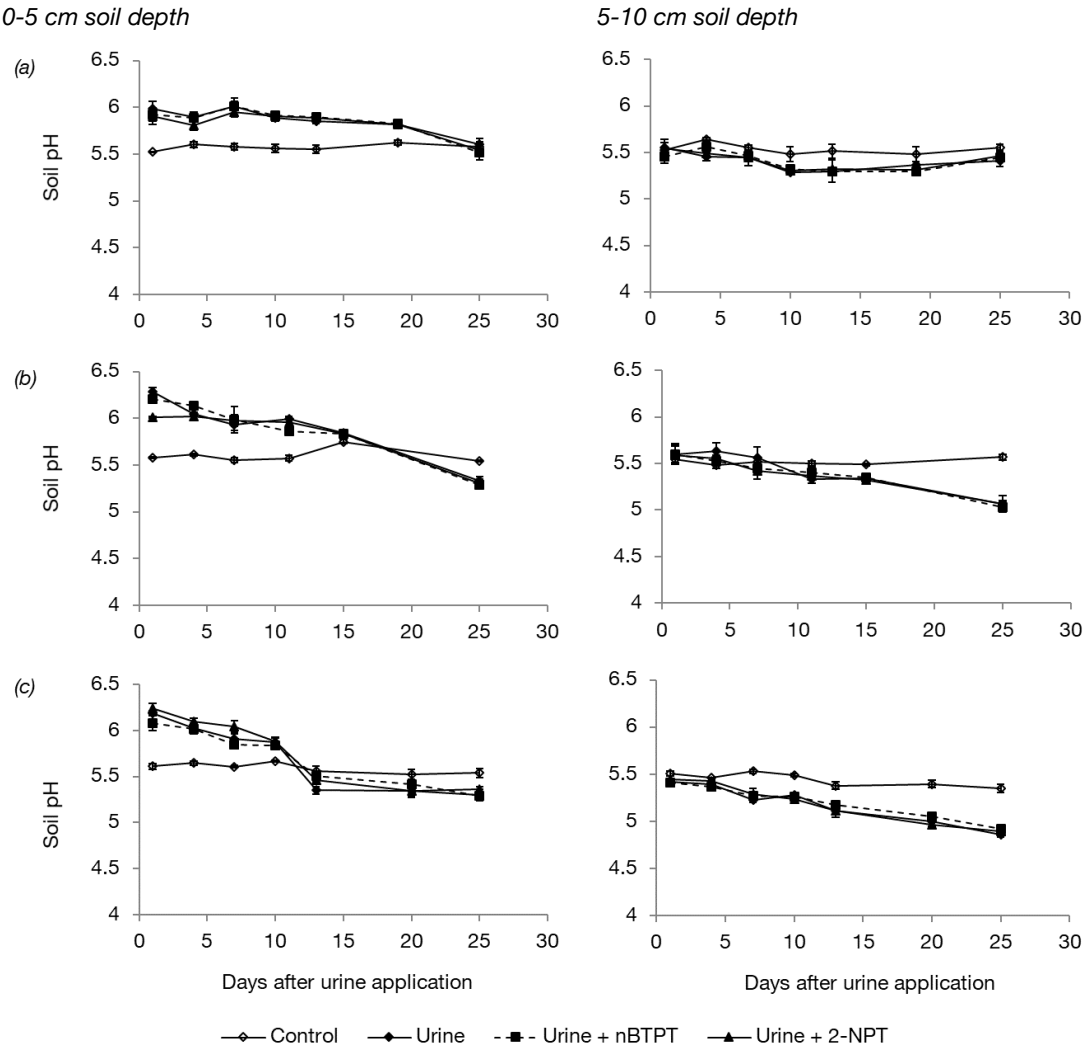

Fig. 6.8 Effect of applying cattle urine *(a)* 3 hrs before, *(b)* 28 days after, and *(c)* 68 days after inhibitor application on mean soil pH of pasture soils at different depths during the summer experiment, vertical bars indicate standard error values

In the autumn experiment, both types of inhibitors significantly reduced soil pH in the 0-2.5 cm soil depth during the first few days after urine application, at both urine

application timings, compared to the urine only treatment (Fig. 6.9). The urine only treatment achieved the maximum soil pH (0-2.5 cm) of 7 on day 1, and then steadily decreased over the subsequent two weeks to a pH closely approaching the control value of approximately 5.8. The effectiveness of inhibitors in reducing pH on day 1 was greater when urine was applied immediately before the inhibitors compared to urine applied 3 hrs before the inhibitors. The soil pH (0-2.5 cm) values achieved by the nBTPT and 2-NPT treatments on day 1 were 6.5 and 6.3, respectively, when urine was applied immediately before the inhibitors, and both had a pH of 6.7 when urine was applied 3 hrs before the inhibitors. By day 4, only the 2-NPT (urine was applied immediately before the inhibitor) treatment exhibited significantly lower pH compared to urine only treatment. Inhibitors did not significantly reduce pH in the 2.5-5 cm soil depth compared to the urine only treatment (Fig. 6.9). All urine treatments had similar soil pH levels (2.5-5 cm) to the no urine control treatment by day 4. There was a significant positive relationship between daily NH_3 fluxes and surface soil pH for the urine only treatments in both the summer (R^2 = 0.6, soil pH 0-5 cm) and the autumn (R^2 = 0.8, soil pH 0-2.5 cm) experiments (Fig. 6.10).

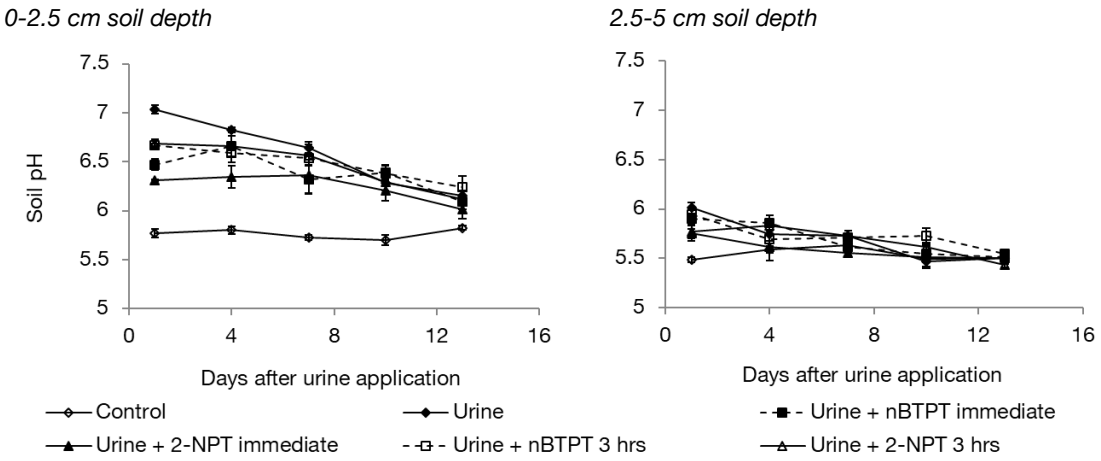

Fig. 6.9 Effect of applying cattle urine on mean soil pH of pasture soils at different depths during the autumn experiment, vertical bars indicate standard error values

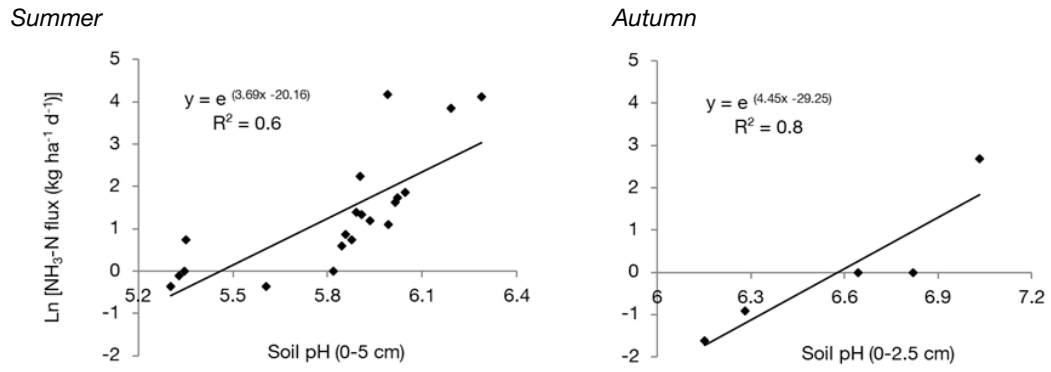

Fig. 6.10 The relationship between daily NH_3 fluxes and surface soil pH of pasture soil in urine only treatments during the two experiments ($P < 0.05$)

6.3.4 Soil NH_4^+-N and NO_3^--N

The soil NH_4^+-N concentrations in all treatments with applied urine were significantly higher compared to the control, in both the summer and autumn experiments. In the summer experiments, the NH_4^+-N concentrations for the urine treatments were higher in the 0-5 cm depth, peaking at between 244 - 360 mg N kg^{-1} at day 1, compared to the 5-10 cm depth, being between 63 - 95 mg N kg^{-1} at day 1 (Fig. 6.11). Overall the soil NH_4^+-N concentrations steadily decreased over time, but still remained above the control levels at the end of sampling period on day 25. In the summer, when urine was applied 3 hrs before inhibitor application, the effect of inhibitors on influencing soil NH_4^+-N concentrations at both soil depths (0-5 cm and 5-10 cm) was not significant. When urine was applied 28 days after inhibitor application in summer, only 2-NPT significantly reduced the NH_4^+-N concentrations, compared to urine only treatment, and only at day 1 for both soil depths (Fig. 6.11 *(b)*). By day 4, the 2-NPT treatment achieved similar level of NH_4^+-N as those of urine only treatment. With the urine applied 68 days after inhibitor application in summer, the inhibitors did not show any effect on soil NH_4^+-N concentrations at both soil depths.

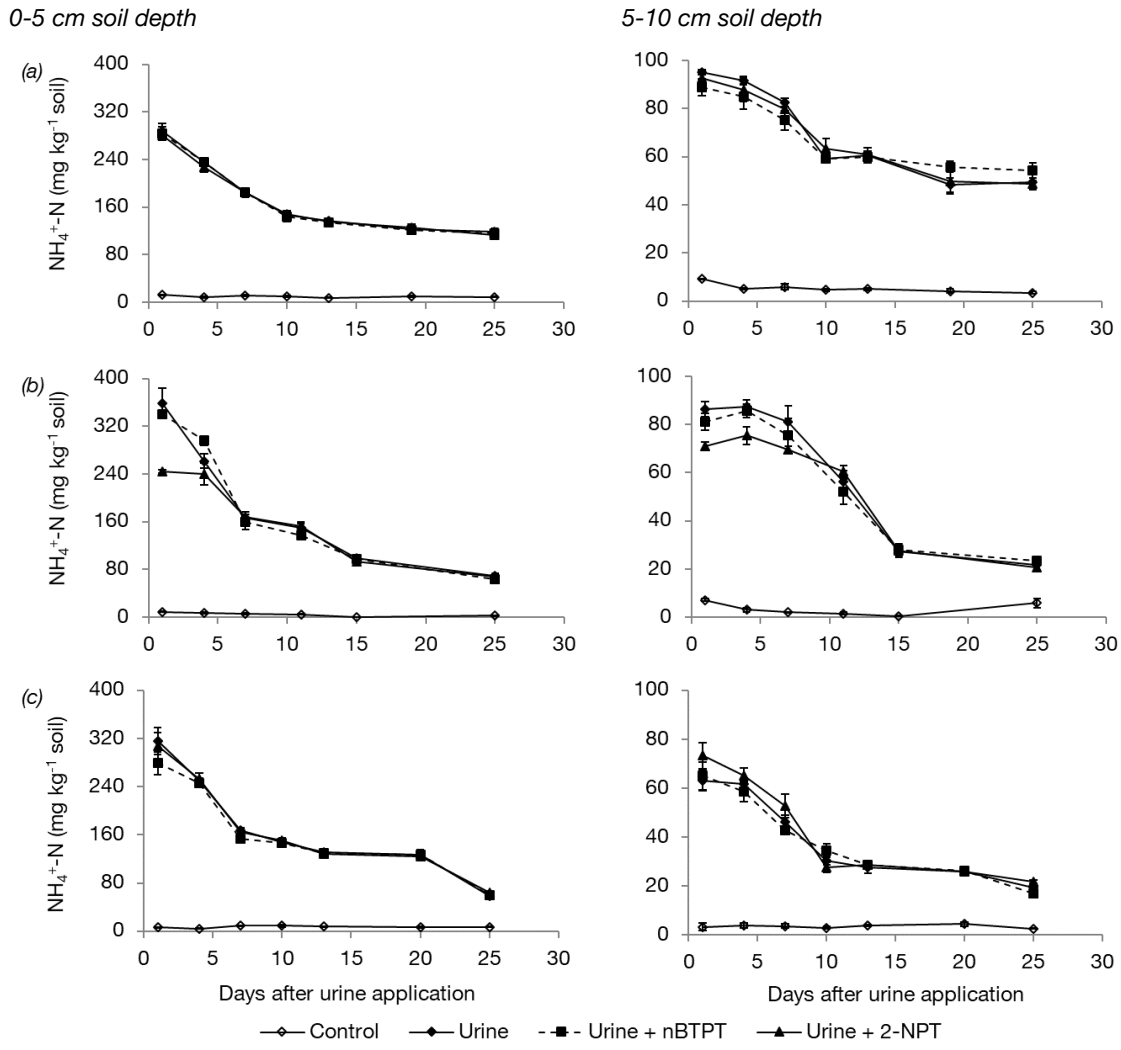

Fig. 6.11 Effect of applying cattle urine *(a)* 3 hrs before, *(b)* 28 days after, and *(c)* 68 days after inhibitor application on mean soil NH_4^+-N concentrations of pasture soils at different depths during the summer experiment, vertical bars indicate standard error values

In the autumn experiment, the urine only treatment exhibited the highest NH_4^+-N concentrations on day 1 (593 mg kg^{-1} soil at 0-2.5 cm soil depth; 384 mg kg^{-1} soil at 2.5-5 cm soil depth), which were significantly higher than the urine treatments with inhibitors (Fig. 6.12). The NH_4^+-N concentration for 2-NPT treatment (when urine was applied immediately before inhibitor application) in the 0-2.5 cm soil depth was significantly lower than other inhibitor treatments. This treatment achieved 215 mg N kg^{-1} soil on day 1 and increased to 395 mg N kg^{-1} soil by day 4, which was close to the

values of the other urine added treatments by that sampling time. The 2-NPT treatment (when urine was applied immediately before inhibitor) also achieved the lowest NH_4^+-N concentrations, of urine added treatments, in the 2.5-5 cm soil depth, being significantly lower during the first 4 days after urine application.

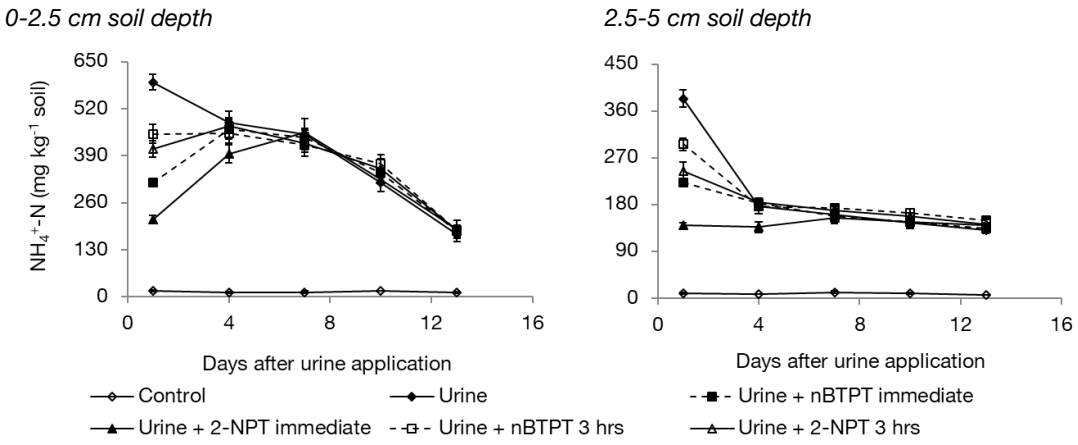

Fig. 6.12 Effect of applying cattle urine on mean soil NH_4^+-N concentrations of pasture soils at different depths during the autumn experiment, vertical bars indicate standard error values

The changes in soil NO_3^--N concentrations in the summer and autumn experiments are presented in Figs. 6.13 and 6.14, respectively. Urine addition increased soil NO_3^- concentrations, compared to the no urine control, at all sampling times from approximately day 4 in summer and autumn experiments and for all soil depths sampled. The NO_3^--N concentrations in general showed an increasing trend at most sampling times, which was the opposite trend to soil NH_4^+-N concentrations. This observation is expected due to nitrification process converting soil NH_4^+ to NO_3^-. Results showed that the rate of nitrification generally exhibited a positive correlation with soil water content. When there was a little difference between moisture levels at the different sampling times, variation in NO_3^- levels was also small, for example, average NO_3^- concentrations (0-5 cm) in treatments with urine on days 13 and 19 were 24 and 22 mg N kg^{-1} soil with corresponding moisture contents of 11 and 9%,

respectively, when urine was applied 3 hrs before the inhibitor application in summer (Figs. 6.2 *(a)* and 6.13 *(a)*). However, average NO_3^- level (0-5 cm) in urine treatments on day 11, 31 mg N kg^{-1} soil was sharply increased to 83 mg N kg^{-1} soil on day 15 with corresponding increases in moisture levels from 11 to 29%, with the urine applied 28 days after the inhibitor application in summer (Figs. 6.2 *(b)* and 6.13 *(b)*).

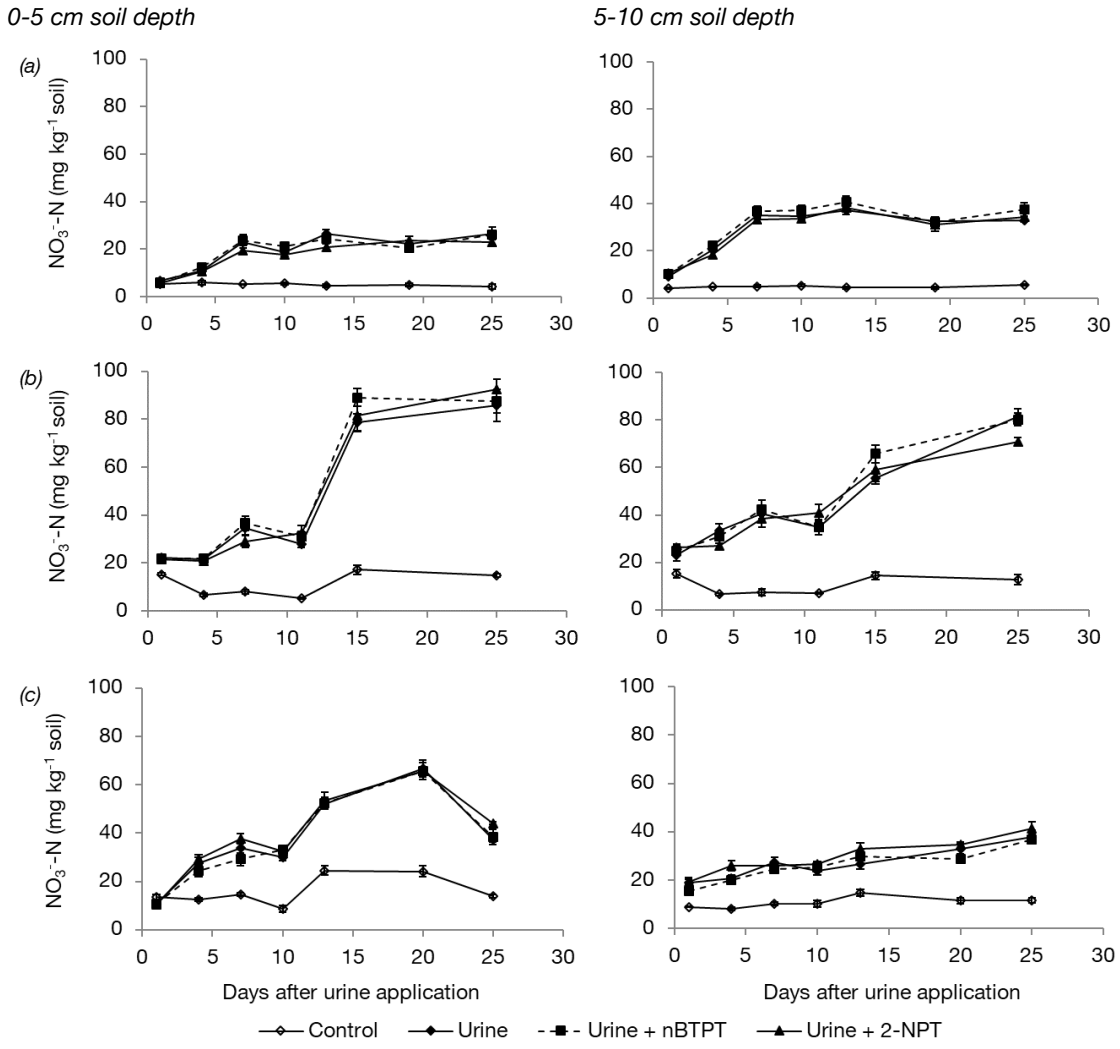

Fig. 6.13 Effect of applying cattle urine *(a)* 3 hrs before, *(b)* 28 days after, and *(c)* 68 days after inhibitor application on mean soil NO_3^--N concentrations of pasture soils at different depths during the summer experiment, vertical bars indicate standard error values

There were no significant differences in NO_3^--N concentrations among the urine treatments, either with or without inhibitors. In the summer experiment, the highest NO_3^- concentrations typically occurred 2 - 3 weeks after urine application, with up to 89 mg N kg^{-1} achieved in the 0-5 cm soil depth and 78 mg N kg^{-1} in 5-10 cm depth. The autumn experiment was only sampled up to day 13, which was when the highest NO_3^--N concentrations were measured, being 82 mg N kg^{-1} in 0-2.5 cm and 68 mg N kg^{-1} in the 2.5-5 cm soil depth. However, it is possible that the NO_3^--N concentrations may have further increased after 13 days, as was observed in the summer experiment.

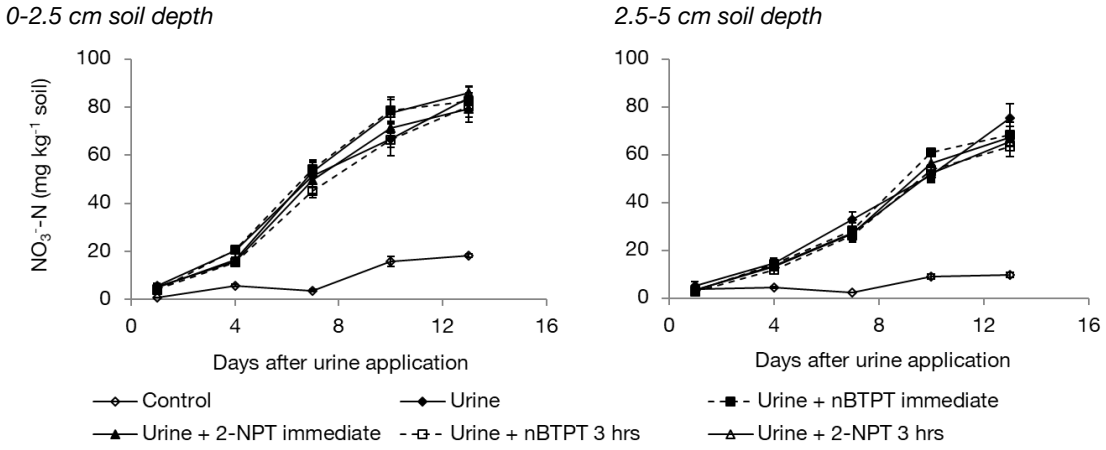

Fig. 6.14 Effect of applying cattle urine on mean soil NO_3^--N concentrations of pasture soils at different depths during the autumn experiment, vertical bars indicate standard error values

6.3.5 Pasture production and N uptake

The cumulative pasture dry matter and N uptake from all the treatments in summer are presented in Table 6.7. The cumulative pasture dry matter accumulation and N uptake in urine treatments, with and without inhibitors, were significantly higher than for the control treatment, however, differences were not significant among the urine treatments. There was no effect of inhibitors observed on pasture dry matter

accumulation and N uptake in each single harvest, therefore, cumulative values were presented.

Table 6.7 Effect of inhibitors on cumulative pasture dry matter production, N uptake, and pasture N recovery from urine-N added to pasture soil at different Stages; A) 3 hrs before, B) 28 days after, and C) 68 days after inhibitor application during the summer experiment [mean (standard error)]

Treatments	Stage-A	Stage-B	Stage-C
	Cumulative pasture dry matter accumulation (kg ha^{-1})		
Control	3684 (167) [b]	2651 (101) [b]	2260 (71) [b]
Urine	6097 (326) [a]	5534 (309) [a]	4874 (198) [a]
Urine + nBTPT	6170 (365) [a]	5761 (350) [a]	4548 (254) [a]
Urine + 2-NPT	6465 (483) [a]	5493 (280) [a]	5021 (329) [a]
	Cumulative N uptake (kg ha^{-1})		
Control	96 (4) [b]	74 (3) [b]	62 (3) [b]
Urine	183 (11) [a]	196 (4) [a]	170 (3) [a]
Urine + nBTPT	187 (7) [a]	193 (18) [a]	159 (7) [a]
Urine + 2-NPT	204 (14) [a]	194 (5) [a]	175 (9) [a]
	Cumulative pasture N recovery (%)		
Urine	14.6	16.8	15.1
Urine + nBTPT	15.3	16.5	13.5
Urine + 2-NPT	18.1	16.6	15.7

Means followed by different lower case letters in a column are significantly different ($P < 0.05$)

In the autumn experiment, all urine treatments exhibited significantly higher pasture dry matter accumulation and N uptake (single harvest) compared to the control treatment (Table 6.8). However, there were no differences in pasture dry matter

accumulation, N uptake, and N recovery among the urine treatments, with or without inhibitors.

Table 6.8 Effect of inhibitors on pasture dry matter accumulation, N uptake, and pasture N recovery (first harvest) from urine-N added to pasture soil during the autumn experiment [mean (standard error)]

Treatments	Pasture dry matter accumulation (kg ha^{-1})	N uptake (kg ha^{-1})	Pasture N recovery (%)
Control	1097 (93) [b]	34 (4) [b]	–
Urine	1986 (181) [a]	68 (5) [a]	5.9
Urine + nBTPT immediate	2168 (114) [a]	77 (3) [a]	7.4
Urine + 2-NPT immediate	2038 (73) [a]	72 (4) [a]	6.5
Urine + nBTPT 3 hrs	2118 (221) [a]	76 (7) [a]	7.2
Urine + 2-NPT 3 hrs	2014 (104) [a]	72 (4) [a]	6.5

Means followed by different lower case letters in a column are significantly different ($P < 0.05$); (Urine + nBTPT immediate = urine immediately before nBTPT application, Urine + 2-NPT immediate = urine immediately before 2-NPT application, Urine + nBTPT 3hrs = urine 3 hrs before nBTPT application, and Urine + 2-NPT 3 hrs = urine 3 hrs before 2-NPT application)

6.4 Discussion

6.4.1 Ammonia emission

The total NH_3 emissions from the urine only treatment were higher in summer than in the autumn in this field study. Similar losses as a percentage of the applied urine-N have been reported in other NZ field studies, for example, 25.7% in summer (Laubach et al. 2012) and about 5 % in autumn (Zaman and Blennerhassett 2010; Zaman et al. 2013b). The higher emissions in summer are attributed to higher temperatures (Fig. 6.4) and drier soil conditions (Fig. 6.2) than in the autumn (Figs. 6.3 and 6.5), during the measurement period. The variations in the total measurement

durations (25 and 16 days after urine application in summer and autumn, respectively) is unlikely to have contributed to the differences in NH_3 emissions between the two seasons, because measurements continued until the emissions reached near to background in both seasons.

In the present study, the influence of temperature and soil moisture content on NH_3 fluxes was greatest during the first few days after urine application when most of the emissions occurred. For example, about half of the total emissions in both seasons occurred within 24 hrs of urine applications, ranging from 47 to 66 kg N ha^{-1} d^{-1} in summer, but only 15 kg N ha^{-1} d^{-1} in autumn. The average air temperatures at ground level inside the chambers for that period were between 18.8 - 23.2ºC during the summer and 14.1ºC in autumn. Similarly, soil water contents on day 1 (24 hrs after urine application) were between 30.5 - 51.1% of field capacity in summer (0-5 cm) and 73.8% of field capacity in autumn (average of 79.5 and 68.1% of field capacity for 0-2.5 and 2.5-5 cm depth, respectively). However, by day 7 the amount of daily NH_3 emitted in both seasons were low. This variation in the effect of temperature and soil moisture content on emissions is due to the fact that emissions occur mostly during the first few days after urine application, and then rapidly decline thereafter as a result of decreasing pH and NH_4^+ concentrations in soil. The NH_3 emission model, for urine deposited by animals during grazing (Móring et al. 2016), also reported positive correlations ($R = 0.6$) between air as well as soil temperature and NH_3 fluxes, showing greater sensitivity of emissions to temperature in the first 6 hrs after urine application than at later times. The rate of emissions is regulated by the removal and dispersion of NH_3 into the atmosphere (Bolan et al. 2004). The key factors that control the extent of NH_3 emissions from soils are those that affect the rate of conversion of NH_4^+ to NH_3 gas and

influence the transfer of NH_3 gas between the soil solution and the atmosphere. These processes are regulated by changes in soil pH after urine application, soil moisture, temperature, soil texture, and wind velocity (Bolan et al. 2004). Higher temperatures reduce NH_3 solubility in soil water and increase the movement of NH_3 from the soil solution to the atmosphere (Bouwman et al. 2002). Temperature also affects emissions by changing the partial pressure of NH_3 near the soil surface (Saggar et al. 2004). Lower moisture content favours the diffusion of NH_3 in soil, while higher moisture content causes greater dilution and reduces diffusion of NH_3 in soil (Sherlock and Goh 1984). Thus, higher NH_3 emissions measured in the summer experiment, compared to the autumn experiment, are attributed to higher temperatures and lower soil moisture contents.

The significant positive relationship observed between daily NH_3 fluxes and surface soil pH in the urine only treatments in both the summer (R^2 = 0.6, soil pH 0-5 cm) and autumn (R^2 = 0.8, soil pH 0-2.5 cm) experiments is attributed to the role of pH on changing the equilibrium between NH_4^+ and NH_3 in soil solution (Saggar et al. 2004) being higher concentration of NH_3 with increasing pH (Freney et al. 1983). Rapid hydrolysis of applied urine-urea increases NH_4^+, which consumes large amounts of H^+ ions resulting in significant increases in soil pH. This high soil pH triggers the dissociation of NH_4^+ produced during urea hydrolysis to NH_3 and then emitted to the atmosphere, causing the peak emissions immediately after urine deposition (Móring et al. 2016). The subsequent fall in NH_3 emissions is due to reduced NH_4^+ concentrations in surface soil, and the decrease in soil pH levels, which are associated with NH_3 emissions and nitrification, as both processes release H^+ ions (Bolan et al. 2004).

In this study, the effect of inhibitors at reducing total NH_3 emissions, compared to the urine only treatment, were influenced by the season in which the urine was applied, with the inhibitors having an effect in autumn but not in summer, when urine was applied 3 hrs before the inhibitor application. The ineffectiveness of inhibitors in summer could be attributed to the warmer temperatures and drier soil conditions. These conditions would have limited the potential for contact between the inhibitor and the urine urea, and there by limited the inhibitor's effectiveness. The warm temperatures would have resulted in more rapid urea hydrolysis, soon after urine application, because warm temperatures increase the rate of soil UA (Cartes et al. 2009). The emissions of 11% of total N applied on day 1 also reflects the rapid hydrolysis of urea after urine application. Laubach et al. (2015) also suggested that quick hydrolysis of urea during summer temperatures, being nearly complete within a few hrs of urine application in pasture soil. This rapid urea hydrolysis reduced the amount of urea present in soil when inhibitors were applied. The higher temperatures would have also resulted in quicker drying out of the soil surface following urine application and reduced the soaking of inhibitor. Therefore, the 3 hrs duration, between urine application and inhibitor application, may be too long in these conditions to allow adequate efficacy of the inhibitors.

In contrast, the significant effect of inhibitors in reducing emissions in autumn may be due to potentially slower urea hydrolysis, at lower temperatures, which would have provided better contact time between inhibitor and urine urea. For example, the average ground level air temperature inside the chambers for the first 3 hrs after urine application in summer was 25.9ºC but lower in autumn (18.5ºC). Although urea hydrolysis following urine application was not assessed in this field study, another NZ

field study conducted in pasture soil reported that the half-life of urine urea was 3 hrs in summer but 5 hrs in autumn (Sherlock and Goh 1984). Similarly, higher soil moisture contents in autumn than in the summer would have also contributed to better soaking of inhibitor in soil and enhance the contact between the inhibitor and urine urea. The results from some previous studies also supported this assumption by showing higher effectiveness of nBTPT in wetter than drier soil conditions, when the inhibitor was applied after urine application to pasture soils. For example, the reduction in emissions by nBTPT applied immediately after urine application was only 9.6% in a dry soil conditions (approximately 55% of field capacity at the time of urine application) (Rodriguez 2014). However, the reduction was higher (23%) for another study (Hoogendoorn et al. 2017) in a wet conditions with comparable soil properties even when nBTPT was applied 4 hrs after urine application. These results suggest that the lower effectiveness of inhibitor in the first study could be mainly due to lower soil moisture content, limiting the contact between urine and inhibitor because soil temperature would have limited effect on urea hydrolysis between urine and inhibitor application and, thereby, effectiveness as inhibitor was applied immediately after urine application.

The higher effectiveness of both of the inhibitors when urine was applied immediately before the inhibitors (52.3% from nBTPT and 72.7 % from 2-NPT), compared to urine applied 3 hrs before the inhibitor (35% from nBTPT and 41.2% from 2-NPT) in autumn is probably because of the greater contact between the inhibitor and urine urea when there's a shorter duration between applications. Also, when urine was applied immediately before the inhibitors, greater reductions in emissions from using 2-NPT than nBTPT was observed. This result is different from the previous laboratory

study (Chapter 5), where no significant differences in total reductions between 2-NPT and nBTPT treatments were observed. As the amount of NH_3 emitted in this study was greatly reduced compared to laboratory study, the additional reductions achieved by 2-NPT than nBTPT for the first few days were large enough to exhibit significant reduction in total emissions. A similar result was been reported by Domínguez et al. (2008), in a laboratory study showing a 65% inhibition of UA for 10 days in a soil incubated with 2-NPT, however, inhibition in UA was only 40% with nBTPT. The effectiveness of inhibitors in summer could be improved by increasing the volume of inhibitor and/or reducing the time between urine and inhibitor application which may enhance the contact between inhibitor and urine urea. However, use of significantly higher volume of inhibitors than used in this study may increase the cost of application, therefore, the feasibility of this will depend on cost considerations. Similarly, applying inhibitors in less than 3 hrs after urine deposition is unlikely to be feasible on dairy farms in practice even if the inhibitors are applied immediately after grazing and, hence, this makes the use of inhibitors more challenging at the time of year when NH_3 emissions are the highest. Although inhibitors significantly reduced emissions in autumn, when applied 3 hrs after urine application, not all urine patches would be $\leq$ 3 hrs old even when the inhibitors are applied immediately after grazing, which would reduce the effectiveness of the inhibitors when used after grazing in autumn.

The inhibitor 2-NPT did reduce NH_3 emissions when urine was applied 28 days after inhibitor application in summer, but not nBTPT. However, the effect of 2-NPT on reducing emissions was not high, being only a reduction of 26 kg N ha^{-1} (19.5% reduction from total emissions of 133 kg N ha^{-1}). Similar results were observed in the laboratory study (Chapter 5), which showed greater effectiveness of 2-NPT in reducing

emissions compared to nBTPT, when urine was applied 29 days after inhibitor application. The effectiveness of 2-NPT is attributed to its longer stability in soil compared to nBTPT (Domínguez et al. 2008), and the presence of 2-NPT in the soil, prior to urine application, would have helped to inhibit soil UA. However, 2-NPT was not effective at reducing emissions from urine applied 68 days after inhibitor application in summer. The better performance of 2-NPT in the laboratory study (Chapter 5) may be attributed to the quicker degradation of 2-NPT in field conditions, which could be due to higher soil UA and temperatures compared to the laboratory conditions (Saggar et al. 2013; Ni et al. 2018). These results suggest that 2-NPT applied to a paddock in summer, following a grazing event, can have an effect at the subsequent grazing when the grazing cycle is short (i.e. ≤28 days). However, the total reductions in emissions from the use of this inhibitor will be lower on a whole paddock basis as urine patches cover only approximately 3% of grazed area during a typical grazing.

These results showed that the effectiveness of inhibitors at reducing NH_3 emissions is strongly influenced by soil temperatures and moisture conditions, and timing of inhibitor application after urine, showing greater effectiveness in lower temperatures and higher moisture levels, and with the inhibitor applied immediately after urine, compared to warmer and drier soil conditions, and with the inhibitor applied 3 hrs after urine. The inhibitor 2-NPT reduced emissions in the warm summer conditions when inhibitor was present in the soil prior to urine application, showing greater longevity compared to nBTPT, however, overall reductions in N loss via NH_3 emissions was low on a whole paddock basis.

6.4.2 Soil pH

The rise in soil pH following urine application, reaching a maximum level on day 1 (Figs. 6.8 and 6.9), resulted from the generation of OH^- ions from urea hydrolysis, which is supported by concentrations of NH_4^+-N also reaching a maximum on day 1 (Figs. 6.11 and 6.12). The subsequent fall in pH in urine treatments is likely the result of NH_3 emissions and nitrification, as both processes release H^+ ions, thereby decreasing soil pH (Bolan et al. 2004). A similar rise in pH after urine application and subsequent decline was also observed in a field study in pasture soil (Zaman et al. 2009), which reported a pH of about 6.5 and 5.7 on days 1 and 28, respectively at 0-5 cm soil depth in summer. In the current study, where the rate of urea hydrolysis from applied urine was decreased by inhibitors, this also coincided with lower soil pH levels.

6.4.3 Soil NH_4^+-N and NO_3^--N

The effect of inhibitors on changes in soil NH_4^+-N concentrations, resulting from urine application, varied greatly with seasons. There was significant difference in soil NH_4^+-N concentrations between inhibitor treatments and urine only treatment for day 1 in autumn but not in summer when urine was applied 3 hrs before inhibitor application. This seasonal difference is likely caused by the same factors as previously discussed in NH_3 emissions section (Section 6.4.1). Most of the NH_4^+ ions in the urine treatments were concentrated in top soil depth (0-5 cm) in this study because these ions are usually adhere to soil organic matter and clay particles (Zaman et al. 2009). After the initial rise in NH_4^+-N concentrations, following urine application, the gradual decline over time could be related to NH_3 emissions (Figs. 6.6 and 6.7), nitrification (Figs. 6.13 and 6.14), N uptake by pastures (Tables 6.7 and 6.8), and microbial immobilisation as these processes consume NH_4^+-N, which is also reported by Zaman and Nguyen (2012).

The incremental increase in NO_3^- concentrations in all urine treatments over a time in the present study is due to nitrification as explained by (Bolan et al. 2004). No effects of UIs on NO_3^- concentrations were observed in this study, which is consistent with a previous field study that also used cattle urine in dairy pasture soil (Rodriguez 2014). This is because UIs only inhibit soil UA, but not the activity of nitrifying bacteria. The less nitrification in lower soil moisture conditions is attributed to reduced activity of nitrifying bacteria as a results of dehydration of microbial cells and limited substrate supply (Stark and Firestone 1995). A similar result is reported by Zaman et al. (2009) where nitrification was lower in dry soil conditions of summer compared to autumn.

6.4.4 Pasture production and N uptake

Applying urine increased pasture accumulation and N uptake compared to the no urine control treatment due to higher soil mineral N levels. No effect of inhibitors on pasture production and N uptake were observed in summer, when urine was applied 3 hrs before and 68 days after inhibitor. This is due to the inhibitors having no influence on the availability of mineral N compared to urine only treatment at those times. Although, there was a slower rate of urea hydrolysis for the first few days with 2-NPT in the summer experiment, when urine was applied 28 days after the inhibitor, and for both inhibitors in the autumn experiment, the additional amount of N retained in inhibitor treatments was relatively small, compared to the total amount of urine N applied. In addition, the availability of inorganic N in urine patches is typically much higher than plant requirements, therefore, increasing N availability further is less likely to appreciably change pasture dry matter yield and N uptake, at least in the short-term (i.e. 1 - 3 months).

6.5 Conclusions

The effectiveness of both inhibitors, 2-NPT and nBTPT, in reducing NH_3 emissions from urine applications was greatly influenced by soil temperature and moisture content at the time of urine application. The timings of inhibitor application in relation to urine application timings also influenced emissions. When inhibitors were applied 3 hrs after urine application in summer, there was no effect in reducing total emissions, but significant reductions were observed in the autumn conditions. In autumn, the effectiveness of both types of inhibitors were higher when applied immediately after urine application, compared to 3 hrs after urine. With the inhibitors applied immediately after urine application in autumn, the effectiveness of 2-NPT was greater than nBTPT. The inhibitor 2-NPT also reduced emissions from urine applied up to 28 days after inhibitor application at a rate equivalent to 26 kg N ha^{-1} in the summer conditions. These results suggest that the UI 2-NPT applied following a grazing event may reduce emissions from urine patches at the subsequent grazing also in the warm summer conditions, especially when the grazing cycle is short (i.e. ≤28 days). This effect could be improved in low temperature seasons of the year. When the inhibitors were effective in reducing NH_3 emissions, reductions in soil NH_4^+-N concentration and pH were also observed, however, this did not translate into differences in NO_3^- concentrations because UIs generally do not inhibit the nitrification process.

Overall, the effect of inhibitors on reducing NH_3 emissions in summer could be limited by the lower volume of inhibitor and the rapid rates of urea hydrolysis at that time of year. Improving effectiveness would require applying inhibitors with higher volume and/or less than 3 hrs after urine application, which are less likely to be practical on dairy farms. This limits the potential benefits of using UIs at a time of year when

NH_3 emissions are the highest. Although inhibitors significantly reduced emissions in autumn, when applied 3 hrs after urine application, not all urine patches in a paddock would have been deposited ≤3 hrs prior to the end of the grazing. From this study it can be concluded that the effectiveness and longevity of 2-NPT in reducing NH_3 emissions is higher compared to the commonly used inhibitor nBTPT, however, overall reduction on whole paddock basis was low. Results from this study also indicated that neither inhibitors produce a positive effect on pasture dry matter production or N uptake.

Chapter 7

Overall conclusions, general discussion and recommendations for future work

7.1 Introduction

The NH_3 emissions from dairy-grazed pastures, following cattle urine deposition, are a result of the hydrolysis of urine urea, carried out by the urease enzyme present in soil. These emissions represent economic losses and have negative impacts on the human health and the environment. Urease inhibitors can be applied to reduce these emissions by inhibiting soil UA, thereby, delaying urea hydrolysis. Among the various UIs, nBTPT has been shown to reduce NH_3 emissions from NZ dairy-grazed pasture soils when it is applied with urea fertiliser or cattle urine. But the effectiveness of nBTPT persists for relatively shorts periods of up to 7 - 14 days after application. Therefore, it is potentially effective for reducing NH_3 emissions from urine deposited in a single grazing event only. However, to reduce the need to reapply the UI at each grazing event, it is necessary to identify UIs that are capable for inhibiting UA for longer periods that would reducing NH_3 emissions from multiple grazing events. The overall objective of this thesis was to assess the effectiveness and longevity of potentially longer-lasting non-specific inhibitors Cu and Zn, and the specific inhibitor 2-NPT in reducing NH_3 emissions following cattle urine applied to pasture soils. This chapter summarises the main research findings of the laboratory and field plot experiments (Chapters 3 - 6) conducted to address the objectives, and integrates discussion with practical implications of outcomes achieved. Future research that is required to provide a better understanding on the effectiveness and longevity of

inhibitors 2-NPT and nBTPT in reducing NH_3 emissions from grazed pastures is also described in this chapter.

7.2 Major findings of this doctoral research

In this section, the main research outcomes of this study are summarised.

7.2.1 Effect of inherent Cu and Zn status on soil UA of dairy-grazed pasture soils (Chapter 3)

There was no clear influence of inherent soil Cu and Zn levels on inhibiting soil UA in 23 Waikato dairy soils, although there were differences in UA levels between the soils. Soil Cu and Zn were measured using five different extractants: (i) total acid-extractable; (ii) EDTA-extractable; (iii) $SrCl_2$-citric acid-extractable; (iv) $Ca(NO_3)_2$-extractable; and (v) $CaCl_2$-extractable. The lack of effect of soil Cu and Zn levels measured using total acid-, EDTA- and $SrCl_2$-citric acid extractants on soil UA is possibly due to the inability of these methods to represent the bioavailable (ionic) forms of these metals. The Cu extracted using neutral salt extractants ($Ca(NO_3)_2$ and $CaCl_2$), which potentially approximate metal bioavailability, were below detectable levels and, thus, would help explain their ineffectiveness. Although the potentially bioavailable Zn ($Ca(NO_3)_2$-extractable) was at a detectable level, the observed levels of Zn did not exhibit an inhibitory effect on soil UA. There were, however, positive relationships between UA and the levels of total C and total N in soils studied. Soils with higher organic C may also lead to greater complexation of Cu and reduced bioavailability, suggesting that for soils with higher UA levels, Cu may be less effective as an inhibitor. Overall, these results support the view that soil inherent Cu and Zn levels are ineffective

at inhibiting UA and mitigating NH_3 emissions, especially for C rich dairy-grazed pasture soils.

7.2.2 Effect of Cu and Zn additions on soil UA of dairy-grazed pasture soils (Chapter 3)

The addition of Cu up to 20 mg kg^{-1} soil and the combination of 5 mg Cu and 5 mg Zn kg^{-1} soil did not inhibit UA of four dairy-grazed pasture soils, with contrasting organic C levels. For the soil with the lowest organic C (1.8%), of the four dairy-grazed pasture soils, $Ca(NO_3)_2$-extractable Cu and Zn, measured immediately after Cu and Zn treatment application, represented less than 5% of the Cu added. This result supports the view that low Cu bioavailability is a possible cause of Cu being ineffective at inhibiting UA. Although most of the added Zn remained extractable (bioavailable), it was also ineffective at inhibiting UA. This weaker inhibitory effect of Zn on soil UA is associated with formation of a less stable and insoluble metal sulphide by Zn ion with the SH group of urease enzyme (Hughes et al. 1969; Hughes and Poole 1989; Zaborska et al. 2004). However, the pasture soil with the lowest C content used in the current study was still high compared to the soil (organic C content 0.7%) from another study (Wyszkowska et al. 2006), which showed successful inhibition of soil UA with additions of Cu and Zn at 50 mg kg^{-1} soil. In the current study, it was decided not to exceed 20 mg Cu kg^{-1} soil considering the practicality of continued application of Cu with minimum long-term risk. Therefore, both the metals are unlikely to be practical UIs for reducing NH_3 emissions from the NZ dairy-grazed pasture soils studied.

7.2.3 Role of the soil C factor on the effectiveness of Cu and Zn inhibiting urea hydrolysis (Chapter 4)

Soil supernatants from two dairy soils (RLS and EBL soils with organic C contents 1.8 and 9.7%, respectively) used in Cu and Zn induced urease inhibition study were used to provide a medium with potentially low concentration of organic C, clay particles and cation exchangeable bases than their respective soils. When additions of Cu, at rates of 5, 10, and 20 mg Cu kg^{-1} soil, and Zn, at a rate of 20 mg kg^{-1} soil, were applied with either 120 or 600 mg urea-N kg^{-1} soil, the bioavailability of Cu was enhanced and was effective at inhibiting urea hydrolysis. Among the three Cu additions, the highest Cu rate was the most effective at inhibiting urea hydrolysis for both soil supernatants. The percentage reductions in urea hydrolysis by Cu treatments were similar for both rates of urea, indicating that Cu behaved as a non-competitive UI because the effectiveness of non-competitive UI in reducing UA is not affected by different rates of urea N additions with the same concentration of inhibitor in soil. These results suggest that Cu has urease inhibitory effect, but its ineffectiveness in organic C rich pasture soils is associated high complexation of Cu. Although bioavailability of Zn was sustained, it was not effective in reducing urea hydrolysis in the soil supernatants, suggesting it has a negligible inhibitory effect on soil UA at the Zn rate used in this experiment.

7.2.4 Summary of the laboratory evaluation on the effectiveness and longevity of UIs 2-NPT and nBTPT (Chapter 5)

In the laboratory experiment, the UIs, 2-NPT and nBTPT, equally reduced total NH_3 emissions (20.6 - 27.3%) from both soil types, when inhibitors were applied immediately after urine application. The 2-NPT continued to reduce emissions

(5.6 - 7.4%) from urine applied up to 56 days after the inhibitor application, but only for the soil with lower microbial biomass C and UA, which supports the view that the effectiveness of 2-NPT has greater longevity than nBTPT.

Wherever 2-NPT and nBTPT applications produced reductions in daily NH_3 fluxes, compared to the urine only treatment following cattle urine application in pasture soils, the effect of inhibitors on reducing soil pH (for the first few days) was also observed simultaneously. The reductions in pH were attributed to the inhibitors effect on decreasing the rate of urea hydrolysis and, thereby, reduced generation of OH^- ions.

Soil microbial biomass C, measured 31 days after inhibitors were applied immediately after urine application, showed no effect of the inhibitors on microbial biomass C. This suggests that both inhibitors exhibit a specific effect on delaying the activity of the urease enzyme rather than a broad biocidal effect on soil microbial biomass C. This specificity of inhibitors is one of the important characteristics of an ideal inhibitor, which implies that majority of soil microbes are not adversely affected with the use of it.

7.2.5 Summary of the field evaluation on the effectiveness and longevity of UIs 2-NPT and nBTPT (Chapter 6)

The field study evaluated the effectiveness of inhibitors in reducing emissions from a pasture soil following urine application in summer and autumn seasons. The summer experiment was conducted at warmer temperatures and drier soil conditions compared to the autumn experiment. These variations in conditions caused the

difference in total NH_3 emissions between two seasons, with significantly higher emissions in summer compared to autumn. A statistically significant reduction in NH_3 emissions from UIs application was achieved in autumn but not in the summer, when inhibitors were applied 3 hrs after urine application. This result suggests that the ineffectiveness of inhibitors in summer could be associated with the limited contact between the inhibitor and urine urea. This is likely to be caused by the warm temperatures and dry soil conditions immediately after urine application. Improvements in the effect of inhibitors, applied after urine application in summer, may be possible by increasing the volume of inhibitor and/or reducing the time between urine and inhibitor application. In autumn, there was higher effectiveness of both of the inhibitors when applied immediately after urine application (52.3 - 72.7% reduction in emissions) compared to when applied 3 hrs after urine application (35.0 - 41.2% reduction in emissions). The total amount of NH_3 emitted from urine only treatment in autumn was equivalent to 27 kg N ha^{-1}. The effectiveness of inhibitors in autumn is likely to be associated with greater contact between inhibitor and urine urea, as a result of slower urea hydrolysis and higher soil moisture contents at the time of urine/inhibitor application. The results from the autumn experiment also showed a greater reduction in NH_3 emissions from using 2-NPT, compared with nBTPT when applied immediately after urine application. This result is different from the laboratory study (Chapter 5), where no significant differences in total reductions between inhibitor treatments were observed when applied immediately after urine application. The amount of NH_3 emitted from urine only treatment in autumn experiment was substantially low compared to laboratory study, and therefore, the additional reductions achieved by 2-NPT than nBTPT for the first few days were large enough to show significant difference. The 2-NPT also reduced NH_3 emissions (19.5% from total emissions of 133 kg N ha^{-1}) when

urine was applied up to 28 days after inhibitor application in summer, but not nBTPT. This indicates that 2-NPT applied to a whole paddock following a grazing event may reduce emissions at the subsequent grazing when the grazing cycle is short (i.e. ≤28 days). The field study confirmed the findings of laboratory study that the effectiveness and longevity of 2-NPT, in reducing NH_3 emissions from cattle urine applied to pasture soils, is higher compared to the more commonly used inhibitor nBTPT.

As observed in the laboratory study (Chapter 5), there were reductions in soil pH also in field study when UIs reduced daily NH_3 fluxes. These inhibitor treatments also had lower soil NH_4^+ concentrations, but only during the first few days after treatment application, which also supports that urea hydrolysis was reduced in these treatments. The UIs inhibited soil UA and urea hydrolysis, which allows more time for urea molecules to move to lower soil depths. This reduces the concentrations of NH_4^+ and, thereby, lowers the pH in the soil surface, which results in lower NH_3 emissions. However, differences in soil NH_4^+ disappeared after this initial few days. The results indicated that neither 2-NPT nor nBTPT influenced the quantities of soil NO_3^- produced even when UIs reduced NH_3 fluxes, which is likely due to the difference in soil NH_4^+ (substrate for nitrification) being short-lived (i.e. a few days), and because UIs are not expected to inhibit the activity of nitrifying bacteria.

The results from the field study showed no significant effect of the UIs 2-NPT and nBTPT on pasture dry matter yield and N uptake in both the summer and autumn experiments. The lack of a pasture response to inhibitor use is likely to be associated with similar levels of total inorganic N, a few days following urine application, between the treatments even when inhibitors reduced urea hydrolysis. This could be due to

relatively small amount of additional N retained in treatments with inhibitors, compared to the total amount of urine N applied. For example, the additional amount of N available in the 2-NPT treatment in summer, when urine was applied 28 days after the inhibitor, was only 3.6% of total N applied (726 kg N ha^{-1}). Similarly, the additional amount of N available in inhibitor treatments in the autumn experiment was less than 3.3% of total N applied (590 kg N ha^{-1}), because overall NH_3 emitted was low at this time. Furthermore, N availability in urine patches is typically much higher than plant requirements, therefore, increasing N availability further is unlikely appreciably change pasture dry matter yield and N uptake, at least in the short-term (i.e. 1 - 3 months).

7.3 General discussion

This sub-chapter provides a discussion on the effectiveness and longevity of UIs evaluated in this study, for reducing NH_3 emissions from cattle urine deposited in grazed pastures, along with a consideration of the practical implications of these findings. Firstly, Cu and Zn were tested to assess their inhibitory effect on soil UA of four pasture soils. Although Cu was effective in inhibiting urea hydrolysis with the soil supernatants, it was not effective in pasture soils for inhibiting UA. This suggests that Cu has a urease inhibitory effect, but its ineffectiveness in organic C rich pasture soils is associated with high complexation of Cu and reduced bioavailability. However, Zn was not effective as an inhibitor in soils or soil supernatants, although like Cu its bioavailability was sustained in the soil supernatants. These results support the conclusion that Cu and Zn are not suitable as UIs in NZ dairy-grazed pastures.

This study also compared the effectiveness and longevity of the recently introduced UI 2-NPT with nBTPT in reducing NH_3 emissions from cattle urine applied

to pasture soils. Both the laboratory and field experiments demonstrated that 2-NPT showed greater longevity of effectiveness compared to nBTPT. Typically, summer is the season with the highest NH_3 emissions, due to the warmer and drier soil conditions. However, neither inhibitor was effective at reducing emissions in the summer experiment when they were applied 3 hrs after urine application. The effectiveness of inhibitors, applied after urine application in summer, may be improved by enhancing the contact between the inhibitor and urine urea by increasing the volume of inhibitor used and/or by implementing shorter durations between urine and inhibitor applications. The use of a significantly higher volume of inhibitor, than the 800 L ha^{-1} used in the current study, is likely to increase the cost of application, therefore, the feasibility of this will depend on cost considerations. In addition, applications of inhibitors to all urine patches deposited less than 3 hrs after deposition is not currently feasible on dairy farms, even if the inhibitor is applied immediately after the cows are removed from a paddock. This is because grazing durations are typically ~6 and ~12 hrs for day and over-night grazings, respectively, and urine depositions occur at various times spread over the whole grazing event, rather than only at the end of the grazing event. Therefore, although inhibitors showed effectiveness in autumn when applied 3 hrs after urine application, only up to about 25 to 50% of urine patches will be ≤ 3 hrs old if inhibitors are applied immediately after grazing. Furthermore, emissions are generally low in cooler and moist seasons, such as autumn, winter and spring, which would have also limited the additional benefits from using inhibitors in these periods. For example, the total amount of NH_3 emitted from urine only treatment in autumn experiment was equivalent to 27 kg N ha^{-1} (4.5 % of total 590 kg N ha^{-1} applied).

As previously discussed, in summer, when NH_3 emissions are typically the highest, both 2-NPT and nBTPT are likely to have limited benefit at reducing emissions from a grazing event when applied after the grazing event. However, because 2-NPT has a greater longevity of effectiveness than nBTPT, it has potential to reduce emissions when applied up to about a month prior to a grazing event. The 2-NPT reduced NH_3 emissions from urine patch areas on a per hectare equivalence basis by 26 kg N ha^{-1} (i.e. 19.5%, from total emissions of 133 kg N ha^{-1}), based on when urine was applied 28 days after inhibitor application in summer. This suggests that application of 2-NPT to a whole paddock after a grazing reduces emissions from urine patches at the subsequent grazing in summer when the grazing cycle is short (i.e. ≤28 days). This effect of 2-NPT could have practical benefit on dairy farms because it is not reliant on being applied immediately after (i.e. <3 hrs) urine deposition. Therefore, 2-NPT could be applied during the 1 - 2 weeks after grazing, while pasture cover is still short, to ensure the inhibitor is applied to the soil rather than mostly retained in the pasture herbage. If, for example, three applications of 2-NPT are applied following grazing in late spring, early summer and mid-summer, there is potential for reductions in emissions from subsequent grazings in early summer, mid-summer and late summer, respectively. Accordingly, a reduction in NH_3 emissions from urine patch areas is equivalent to 26 kg N ha^{-1} and for 3 grazings would reduce losses by 78 kg N ha^{-1}. This reduction in urine patch then needs to be extrapolated to whole paddock, to determine the overall benefit. Assuming the urine patches cover 3% of the grazed area per grazing, then the total reduction in N loss (26 kg N ha^{-1} x 3% x 3 grazings) is 2.3 kg N ha^{-1} yr^{-1}. Thus, overall benefit from using 2-NPT is greater than nBTPT in reducing NH_3 emissions from cattle urine deposited in dairy-grazed pastures. However, the size of the reduction from using 2-NPT on whole paddock was low, compared to the amount of N cycling in grazed

pastures annually. Therefore, decisions with using these UIs for reducing NH_3 emissions from cattle urine patches need to consider both the challenges with applying them at optimum times and the relatively small reductions in NH_3 emissions achievable, with both 2-NPT and nBTPT. However, these decisions should also consider the benefits that mitigating NH_3 emissions have on the wider environment. Reductions in NH_3 emissions minimise its atmospheric deposition and, thereby, controls the eutrophication of aquatic bodies and acidification of soil. The generation of secondary aerosols in the atmosphere and their detrimental effect to human and animal health can be minimised by lowering emissions. Decreasing emissions can potentially reduce a greenhouse gas N_2O emissions because when NH_3 emitted to the atmosphere deposited on land surfaces, it acts as a source of N_2O emissions.

It may be considered that application of about 28 % of urea fertiliser treated with nBTPT in NZ farms may have some impact in mitigating NH_3 emissions from urine patches influenced by fertiliser input. However, the concentration of inhibitors (nBTPT or 2-NPT used with fertiliser urea) from urea fertiliser applied at 25 to 45 kg N ha^{-1} is too less relative to N concentration in urine patches (typically 600 kg N ha^{-1}). These concentrations are unlikely to be effective in mitigating NH_3 emissions from urine patches.

7.4 Recommendations for future work

The results from the research presented in this thesis have highlighted several areas that require further research, which are discussed in the following sections:

i) In the current field study, 2-NPT and nBTPT applied 3 hrs after cattle urine application did not reduce NH_3 emissions in summer but were effective in autumn. Increasing the volume of inhibitor than used in this study, 800 L ha^{-1} and/or reducing the time between urine and inhibitor application to less than 3 hrs may improve the effectiveness of inhibitors in summer. Therefore, it would be useful to quantify the volume of inhibitor required in summer to reduce most of the emissions following urine deposition. Similarly, it would be also useful to determine in summer conditions how soon after urine deposition that the inhibitor will need to be applied to mitigate the majority of the emissions. This could then inform the benefits of future technology (e.g. automated devices that spray inhibitors into urine patches during grazing events).

ii) The results of laboratory incubation study conducted in this thesis suggest that longevity of 2-NPT is greatly influenced by soil type with greater longevity in pasture soil with lower microbial biomass C and UA. Therefore, it would be essential to gain a better understanding of the influence of soil microbial biomass C and UA on the longevity of 2-NPT for NH_3 mitigation, particularly during summer conditions when emissions are the highest.

iii) The long-term effect of regular applications of 2-NPT and nBTPT on soil microbial biomass C after cattle urine deposition in dairy pasture soils with varying microbial biomass C would be worthwhile studying further because this soil property is an indicator of biological activity in soil.

iv) In the present field study, there could be issue that some of the inhibitors applied may adhere to the pasture canopy and reduce the quantity of inhibitor reaching the soil and interacting with urine, however, this study did not measure concentrations of inhibitors in pasture samples. Therefore, it would be useful to quantify the effect of pasture cover on the quantity of applied inhibitor reaching the soil. The inhibitors retained on pasture cover may have effect on grazing animals and/or their products, therefore, this aspect is also recommended as a future research area.

v) It is recommended to directly measure 2-NPT and nBTPT in soil in future researches to estimate their persistency.

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Appendix

DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	KAMAL PRASAD ADHIKARI
Name/title of Primary Supervisor:	PROF. SURINDER SAGGAR
Name of Research Output and full reference:	Adhikari KP, Saggar S, Hanly JA, Guinto DF, Taylor MD (2018) Why copper and zinc are ineffective in reducing soil urease activity in new zealand dairy-grazed pasture soils. Soil Research 56, 491-502.
In which Chapter is the Manuscript /Published work:	Chapters 3 & 4
Please indicate:	
• The percentage of the manuscript/Published Work that was contributed by the candidate:	90 %
and	
• Describe the contribution that the candidate has made to the Manuscript/Published Work:	
All the experimental works and writings have performed by the candidate with guidance from supervisors.	
For manuscripts intended for publication please indicate target journal:	Adhikari KP, Saggar S, Hanly JA, Guinto DF (2019) Comparing the effectiveness and longevity of urease inhibitors 2-NPT with NBTPT in reducing ammonia emissions from cattle urine applied to dairy grazed pasture soils. Soil Research (Chapter 5)
Candidate's Signature:	
Date: 27/03/2019	
Primary Supervisor's Signature:	
Date:	27 March 2019

(This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis)

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