EFFECT OF INCREASING COW URINE PATCH AREA ON NITROGEN LOSSES FROM GRAZED PASTURES

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May Tana Hedges

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Abstract

The expansion and intensification of dairy farming in New Zealand (NZ) over the last few decades has made a major contribution to the country's greenhouse gas emissions, and to the nitrogen (N) enrichment of its surface and ground waters. The environmental concerns associated with dairy farming have led researchers to investigate potential mitigations to reduce N losses from cow urine patches, which are the main source of N losses. However, there has been little research conducted on the effect of increasing the spread area of urine patches as a mitigation for N losses. The development of a prototype urine spreading device, intended to be worn by cows during summer and autumn, has made such a mitigation possible. The primary aim of this device is to provide a method to reduce nitrate (NO_3^-) leaching, by directly reducing the N application rate in the urine patch. The impact of increasing the size of the urine patch on N emissions to the atmosphere is also an important consideration. Research was required to quantify the effects of increasing urine spread on N losses from urine patches. This research quantified the effect of increasing the urine patch spread area on ammonia (NH_3) and nitrous oxide (N_2O) emissions, and on NO_3^- leaching losses.

The first field experiment was conducted in early autumn on Dairy Farm 1, Massey University, near Palmerston North, New Zealand. The soil at the site is a Manawatu silt loam. Three urine application depth treatments of 10 mm, 5 mm and 2.5 mm were applied to an area (0.018 m^2) inside a series of 20 chambers (5 replicates). These treatments represented the depths that would result from applying 2.5 L of urine to three different patch areas: 0.25 m^2 (i.e. typical patch size), 0.5 m^2 and 1 m^2, respectively. A control treatment with no urine applied was also included. The concentration of total N in applied urine was 4.53 g N L^{-1}. Ammonia measurements were conducted over a period of 20 days using the Dynamic Chamber method. Soil samples were also collected periodically from adjacent treatment plots to measure mineral-N (nitrate and ammonium), soil moisture and pH. The results showed that increasing the urine patch area from 0.25 to 1 m^2 has increased total NH_3 emissions from 25 to 36% of the total urine-N applied and, consequently the emission factor also increased. This NH_3 increase also increases indirect N_2O emissions, which can have an influence on annual emissions. However, the loss of

NH_3 from the urine patch also reduces the amount of urinary N that is available for subsequent direct N_2O emissions and NO_3^- leaching.

The second and third field experiments were carried out on Dairy Farm 4, Massey University. The soil type at both sites is the Tokomaru silt loam soil. One of the field experiments involved urine application in early-autumn and the other in early-winter. Urine collected from lactating dairy cows was applied to small, mowed plots at application depths of 10 mm (applied to 0.25 m^2), 5 mm (applied to 0.5 m^2) and 2.5 mm (applied to 0.5 m^2 and results were extrapolated to a notional patch area of 1 m^2). A control treatment with no urine applied was also included. All treatments were replicated five times. Gas sampling was conducted in the field using the static chamber method (chamber area of 0.50 m^2). The results of these studies showed that increasing the size of the urine patches from 0.25 to 1 m^2 with the same volume of urine-N in early-winter did not signifcantly increase N_2O emissions and emission factors (EF_3). Although increasing the urine patch area increased N_2O emission by 39%, this difference was not large enough to be statistically significant ($P>0.05$). However, for the first 14 days of total N_2O emissions, the 1 m^2 urine patch treatment was statistically different ($P<0.05$) from the 0.25 m^2 urine patch treatment. In contrast, increasing the size of the urine patches from 0.25 to 1 m^2 in early-autumn decreased N_2O emissions and EF_3 by 56% ($P<0.05$). The different effect of increasing the urine patch area in these two different seasons, is likely to be attributed to the differences in soil moisture conditions at the time of urine application and the weeks that followed.

To determine the overall effect on N_2O emissions, the reduction in N_2O emissions in autumn was compared with the increase in NH_3 emissions at this time using the N_2O inventory emission value of 0.1 for indirect emissions. These indirect N_2O emissions was estimated to be about 3.6 times higher than the reduction in direct N_2O emissions. Therefore, the use of a urine spreading device to increase the spread area of cow urine in autumn is expected to result in greater overall accumulation of N_2O in the atmosphere.

The fourth experiment was conducted on Dairy Farm 4, Massey University. The experimental paddock consisted of twelve pasture plots measuring 800 m^2 per plot, with separate mole and pipe drainage systems. There were two treatments and six replicates of each treatment. The treatments were cows wearing urine-spreading devices (‘Device’ treatment) and without the device (‘Control’ treatment). The devices were used on four

grazing events over the late summer and autumn period. Drainage water from the plots was monitored and analysed for total N and NO_3^--N, and pasture accumulation measurements were also conducted. Overall N leaching losses were low, and the differences in total N and NO_3^- leaching between the two treatments were small and not statistically significant ($P>0.05$). There were also no differences in pasture accumulation over a 9-month period.

Further improvements to the device are required to consistently increase the spread area of urine patches and the uniformity of the spread. The improved device should then be evaluated over a number of years to assess its potential to reduce leaching of N and its impact on N gaseous emissions to the atmosphere.

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Table of Contents

List of Tables

List of Figures

Chapter 1

General Introduction

The agriculture industry is the major contributor to the New Zealand (NZ) economy with export revenues in the billions of dollars. Dairy products constitute 41% of total agricultural exports, representing a total revenue of $(NZ)22 billion in 2022 (Ministry for Primary Industries, 2023). This is attributed to both the expansion and intensification of dairy farming, a result of increase in the dairy cattle population from 5.2 million in 2002 to 5.9 million in 2023 (Statistics New Zealand, 2023). While there are economic benefits from increased dairy production, there are associated increased impacts on the wider environment such as enhanced nutrification of surface and ground waters with nitrogen (N) and phosphorus (P), and increased greenhouse gas (GHG) emissions.

Dairy farming is implicated as a major source of elevated nitrate (NO_3^-) in many of New Zealand's lakes and rivers in regions where dairy farming is a prominent land use (de Klein et al., 2010). The nitrate that is leached from grazed dairy pastures comes mainly from the urine-N which is deposited at high concentrations onto the pasture in small patches. Application rates of N in urine patches are typically in the range equivalent to 400-800 kg N ha^{-1} (Selbie et al., 2015a; Shepherd et al., 2011; Talbot et al., 2020). Thus, the accumulated NO_3^- within the urine patches often exceeds the pasture requirements for growth and, this surplus NO_3^- is at risk of leaching where drainage occurs following grazing events (Haynes & Williams, 1993; Shepherd et al., 2011).

Dairy farming contributes to GHG emissions in a number of ways, including cattle enteric methane emissions and the release of carbon dioxide (CO_2) associated with farm inputs like fuel and fertilisers. Another source of GHG emissions are the losses of nitrous oxide (N_2O) and ammonia (NH_3) from N fertiliser use and from cattle urine patches. Nitrous oxide is a direct GHG, with a large radiative-forcing potential and a global warming potential about 265 times greater than that of CO_2, whereas NH_3 and NO_3^- are indirect N_2O sources (IPCC, 2014). Nitrous oxide emissions from the deposition of urine and dung by grazing livestock and the use of N fertilisers increased between 1990 and 2022 (Ministry for the Environment, 2024). In NZ, agriculture contributed about 53% to the

total national GHG emissions, and 9% is N_2O (Ministry for the Environment, 2024). Ammonia emissions also cause acidification and eutrophication of natural ecosystems when returned to the earth's surface through wet or dry deposition (Cameron et al., 2013). Ammonia emissions from urine patches are regulated by soil and environmental conditions. Hence, lower NH_3 emissions are obtained in wetter and cold conditions whilst very high emissions (up to 30%) have been observed in dry summer conditions (Adhikari et al., 2020; Menneer et al., 2008; Singh et al., 2013; Zaman et al., 2013b).

These environmental concerns have led researchers to investigate potential mitigation options to reduce N losses from urine patches. Although there are a number of mitigation options available to reduce the N load to the environment, such as Duration-controlled (DC) grazing and restricted grazing (Christensen et al., 2019b; de Klein et al., 2006), the majority of them are costly and often have limitations. For example, nitrification inhibitors (NIs) are expensive to apply in winter and their effectiveness is short lived. Spreading the urine stream as it leaves the cow may be a more direct, effective and cheaper method to reduce the rate of N deposition in urine patches. Ramirez (2017) found that increasing the urine patch surface area from 0.2 m^2 to 1 m^2 (i.e., spreading it widely) reduced inorganic N movement below the 45 cm soil depth by 64%. This is likely to reduce the risk of N leaching. A modelling exercise by Cichota et al. (2018) showed a reduction in N leaching with greater lateral spread of urinary N. These studies support the potential benefits of increasing the urine patch area as a means of mitigating NO_3^- leaching. However, there is little data on the effect of increasing the spread of the urine patch on N_2O and NH_3 emissions. Therefore, quantitative information is required to assess the benefits associated with the greater lateral spread of urine-N and, in turn, the potential effectiveness of any new technologies to increase the lateral spread of cattle urine patches.

A NZ based company, Novataro Ltd, previously commissioned the Fertiliser and Lime Research Centre (FLRC), Massey University, to assess the urine spread achieved by a device that they had developed to spread cow urine. In preliminary evaluations, these devices were attached to a small number of cows. These initial observations suggested that the devices have the potential to spread urine in a manner that is likely to be significant in terms of NO_3^- leaching mitigation. More research is required to obtain

quantitative information on the effect of spreading urine patches on NO_3^- leaching and on NH_3 and N_2O emissions.

Therefore, the overall objectives of this PhD research are:

- to quantify the effect of increasing the urine patch spread area on NH_3 emissions.
- to quantify the effect of increasing the urine patch spread area on N_2O emissions.
- to determine the ability of a proto-type device to spread urine and its effects on NO_3^- leaching and pasture production.
- to identify the practical limitations associated with the use of such a device in dairy herds.

Thesis structure

This thesis is comprised of six chapters, including this chapter (Chapter 1 – General Introduction) and a Literature Review Chapter (Chapter 2). Chapters 3 to 5 describe four field experiments conducted over three years. Each of these chapters is written in a manuscript format. Chapter 3 describes an experiment where NH_3 emissions were measured from three urine patch sizes (0.25, 0.5 and 1 m^2) in autumn. The aim of this experiment was to investigate the influence of increasing the area of the urine patch (i.e., decreasing urine application depth) on NH_3 emissions from dairy cow urine applied to a pasture soil. Chapter 4 describes two experiments conducted in early-winter and early-autumn, where N_2O emissions were measured from three urine patch sizes (0.25, 0.5 and 1 m^2). The main objective of this experiment was to quantify the effect of increasing cow urine patch area on N_2O emissions. Chapter 5 describes an experiment where a novel urine spreading device was attached to cows during late-summer and autumn grazings. The main aim of this experiment was to quantify the effect of the urine spreading device on nitrate leaching and pasture accumulation. Chapter 6 summarises all the findings from this research work and makes some recommendations for future research.

Chapter 2

Literature Review

2.1 Introduction

The expansion of intensive dairy farming in NZ has resulted in an increase in the quantity of urine deposited by grazing dairy cows onto small areas of pasture (urine patches). Urine-N is the main contributor to NH_3 volatilisation, N_2O emissions and NO_3^- leaching (Davidson, 2009; Laubach et al., 2013; López-Aizpún et al., 2020). Ammonia volatilisation causes acidification and eutrophication of natural ecosystems (Saggar et al., 2005; Sutton et al., 2008); N_2O is a potent GHG that contributes to climate change and depletion of the ozone layer (Ravishankara et al., 2009). Nitrate (NO_3^-); and leaching poses health and environmental concerns such as increased cancer risk and declining water quality (Schullehner et al., 2018; Ward et al., 2018). Therefore, the main aim of this chapter is to review the published NZ and international research information on the N transformations in the urine patch and transfers from grazed pastures.

Over the years, several mitigation strategies have been conducted to reduce the N load in urine patches. However, widespread adoption of many of these mitigation strategies is either costly or has practical constraints which make them difficult to implement. Some of these strategies and limitations will be discussed in more detail in this chapter, which will conclude with the identification of the key gaps in our knowledge. A better understanding of N transformations in the urine patch and mitigation strategies to reduce N loss from grazed pastures will help explain how a novel urine-spreading device worn by cows could potentially reduce NH_3 volatilisation, N_2O emissions and NO_3^- leaching.

2.2 Nitrogen transformations and transfers from grazed pastures

Under grazed pastures, the transformations of N in soils are dependent on inputs of N (atmospheric deposition, legume fixation, plant residues, N fertilisers and animal manure (urine and dung)), and outputs (pasture uptake, immobilisation, gaseous and leaching losses) (Cameron, 1992; Haynes & Williams, 1993; Jarvis, 1993) (Fig 2.1).

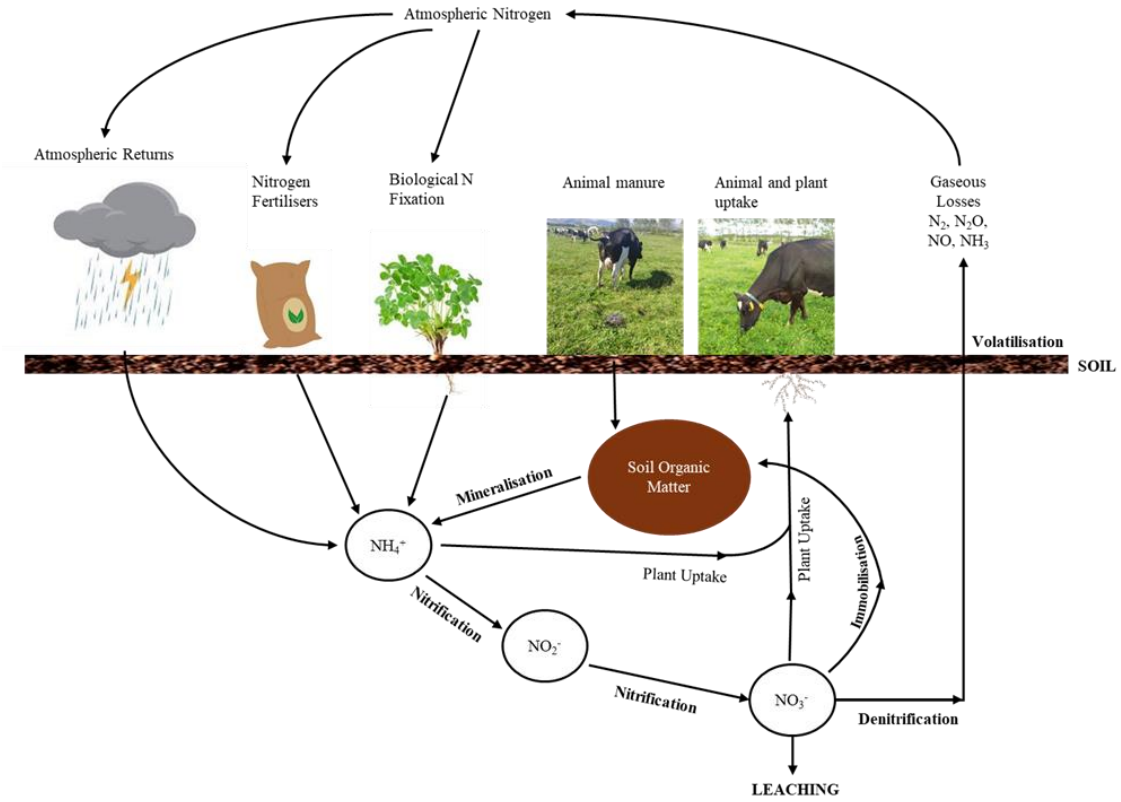


Figure 2.1: The soil-plant-animal nitrogen cycle (Adapted from Cameron, 1992)**.**

In grazed pastures, dietary N use is inefficient as the grazing animals only convert 5–30% of N intake into animal products (milk and meat), and 70-95% is excreted in urine and dung (Cameron et al., 2013; López-Aizpún et al., 2020; Oenema et al., 2005). Under a dairy cattle urine patch, the high N loading rates can typically range from 400–800 kg N ha^{-1} (Selbie et al., 2015a; Shepherd et al., 2011; Talbot et al., 2020), which exceeds the pasture's requirements for growth, especially in winter months (Haynes & Williams, 1993). Therefore, the urinary-N deposited by the grazing animals is the largest source of N which is at risk to loss to the environment (Ball & Ryden, 1984; Selbie et al., 2015a). The three main losses to the environment are; ammonia (NH_3) volatilisation which

contributes to indirect N_2O (indirect GHG), N_2O emissions (direct GHG) and N leaching, which degrades water quality. These three losses are discussed in more detail below.

2.2.1 Ammonia volatilisation losses

Ammonia volatilisation is a result of a chemical process catalysed by the ubiquitous urease enzyme, whereby urea ($(NH_2)_2CO$) in urine undergoes hydrolysis immediately after being deposited on the soil (Bolan et al., 2004; Zaman et al., 2009; Zaman et al., 2013b). Urea hydrolysis results in the formation of ammonium carbonate ($(NH_4)_2CO_3$) (Equation (Eqn.) 2.1), which then dissociates to produce ammonium, ammonia and hydroxide ions (Cameron et al., 2013; Saggar et al., 2004b). The hydrolysis process increases soil pH, which favours NH_3 volatilisation (disassociation of NH_3 from NH_4^+) (Cameron et al., 2013; Saggar et al., 2004b; Selbie et al., 2015a).

$$(NH_2)_2CO + 2H_2O \rightarrow (NH_4)_2CO_3 \rightarrow NH_4^+ + NH_3\uparrow + CO_2 + OH^- \qquad \textbf{Eqn. 2.1}$$

Most of the NH_3 volatilisation occurring in dairy grazed pastures is emitted from dairy cow urine patches. In a study conducted by Laubach et al. (2013), 89% of the NH_3 volatilisation came from urine patches and only 11% was from dung. This is attributed to the high concentrations of urea in urine-N (80-91%) deposited by grazing cattle in urine patches which are relatively small in areas (Jolly et al., 2021).

Several factors regulate both the rate and degree to which NH_3 volatilisation occurs. These include soil conditions, such as soil pH, cation exchange capacity (CEC), temperature and moisture. Climate also influences NH_3 volatilisation via its effect on both soil temperature and moisture. Another important factor is the concentration of urea in the urine patch.

After urine deposition, the soil pH increases (pH > 7.5) in the urine patch as a result of hydrolysis, which favours NH_3 volatilisation (Bolan et al., 2004; Cameron et al., 2013; Saggar et al., 2004b; Selbie et al., 2015a). Laubach et al. (2013) found that a day after urine application, the soil pH peaked between 8.5 and 9.0 and decreased steadily thereafter. Other studies also reported that NH_3 volatilisation peaked 1-2 days after urine application (Rodriguez et al., 2019; Saarijarvi et al., 2006; Zaman et al., 2009). After this peak, the soil pH decreases, resulting in a decrease in the NH_3 volatilisation rate, and the nitrification processes becomes more significant (Cameron et al., 2013). Therefore, a

soil's initial pH and its pH buffering capacity can influence the amount of NH_3 volatilisation. For example, on calcareous soils, which have a high (alkaline) pH, NH_3 volatilisation is expected to be high (Saggar et al., 2004b). Likewise, sandy soils with low organic matter contents typically have low pH buffering capacity and are also expected to have higher NH_3 volatilisation, compared to a soil with a high pH buffering capacity (Cameron et al., 2013). The pH in the urine patch after urine deposition is the key driver of NH_3 emissions (van der Weerden et al., 2023).

Soil CEC also influences NH_3 volatilisation from urine as NH_3 concentration is affected by the reaction of NH_4^+ ions with OH^- ions (the negatively charged cation exchange sites) to produce NH_3 gas and water (H_2O). Consequently, soils with low CEC levels are more prone to NH_3 volatilisation than soils with high CECs (Li et al., 2015; Saggar et al., 2004b). For example, clay soils (high CEC) generally have a lower ammonia volatilisation potential compared to sandy soils (low CEC) (Whitehead & Raistrick, 1993). In addition, soils with lower CEC, typically have lower pH buffering capacities.

The rate of urea hydrolysis and NH_3 volatilisation is also affected by soil and atmospheric temperature (Black et al., 1985a; Black et al., 1985b). Lockyer and Whitehead (1990) found that there was a positive correlation between soil temperature and NH_3 volatilisation from late March to October. This resulted in 3.7 to 26.9% of urinary N being volatilised. Saarijarvi et al. (2006) also reported that NH_3 volatilisation was highest when the soil temperature was high. Zaman et al. (2009), reported that NH_3 volatilisation was higher in summer than in spring. This was due to elevated temperature (hot and dry) during summer and lower temperature (cool and moist) in spring. Soil moisture content can influence NH_3 emissions since it affects the concentration of ammonia/ammonium in soil solution. High solution concentrations are promoted when there is low soil moisture contents resulting in high NH_3 emissions (Cameron et al., 2013). A study carried out by Sherlock and Goh (1984) found that when urine was deposited in a soil with high moisture content (33.9%), ammonia loss was low as a result of the dissolution effect of ammonia into the soil solution.

In addition to their impact on soil moisture, significant amounts of rainfall or irrigation applied to the soil soon after urea or urine deposition can reduce ammonia volatilisation. This results from urea being washed below the soil surface, therefore, keeping the surface solution concentration low (Black et al., 1987). Studies carried out by Whitehead and

Raistrick (1991), Bussink (1996) and Saarijarvi et al. (2006) reported that heavy rainfall reduced ammonia volatilisation. Zaman et al. (2013b) also found that when a grazing event or fertiliser urea application is followed by rainfall or irrigation, ammonia volatilisation can be significantly smaller.

Wind affects NH_3 volatilisation due to its effect on partial pressure close to the soil surface. Therefore, NH_3 volatilisation increases with increasing wind speed (Bolan et al., 2004).

The amount of NH_3 volatilisation from a soil is also related to the quantity of N that is in the form of urea (approximately 80% of urine N is in the form of urea) (Bolan et al., 2004; Zaman et al., 2007). The concentration of urea in the soil solution determines the rate of ammonia volatilisation. In general, the higher the concentration of urea in the soil solution, the higher the ammonia volatilisation rate. Therefore, applying N fertiliser (especially urea) and animal urine can significantly increase the ammonia volatilisation rate. The loss of N as NH_3 from urine patches ranges from 5-30% of the total N applied as urine (Adhikari et al., 2020; Laubach et al., 2013; Menneer et al., 2008; Rodriguez et al., 2019; Zaman et al., 2013b).

2.2.2 Nitrous oxide emissions

From the latest NZ inventory data, agriculture soils contributed the most (91.9%) to total N_2O emissions in 2022, and 51.5% of emissions came from urine and dung deposited by grazing animals (Ministry for the Environment, 2024). Most of the animal excreta N deposited onto grazed pasture is in the form of urea present in the urine, while mostly organic forms of N are deposited in the dung (Chadwick et al., 2018; Selbie et al., 2015a). Ruminant urine patches are hotspots for the production and emission of N_2O, which results from the soil microbial processes of nitrification and denitrification (Selbie et al., 2015a). Nitrification involves the biological oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) by ammonia oxidising bacteria (AOB), such as Nitrosospira and Nitrosomonas (Equation 2.2); and NO_2^- is further oxidised to nitrate (NO_3^-) by Nitrobacter (Bolan et al., 2004; Cameron et al., 2013; de Klein et al., 2008b) (Equation 2.3). The latter conversion takes place very rapidly and, therefore, NO_2^- rarely accumulates in soil (Cameron et al., 2013).

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ + \text{energy} \qquad \textbf{Eqn. 2.2}$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- + \text{energy} \qquad \textbf{Eqn. 2.3}$$

Denitrification is a stepwise process that is carried out by denitrifying bacteria and each step is catalysed by reductase enzymes namely; nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase (Cameron et al., 2013; de Klein et al., 2008b; Di & Cameron, 2016; Saggar et al., 2013) (Equation 2.4). Biological denitrification and complete reduction of NO_3^- to N_2 has the following key requirements: 1) the presence of soil bacteria harbouring the genetic ability to perform the steps in denitrification, and 2) suitable environmental conditions for expression of the genetic potential (Samad et al., 2016).

$$\underset{\text{Nitrate}}{NO_3^-} \xrightarrow[\text{reductase}]{\text{Nitrate}} \underset{\text{Nitrite}}{NO_2^-} \xrightarrow[\text{reductase}]{\text{Nitrite}} \underset{\text{Nitric oxide}}{[NO]} \xrightarrow[\text{reductase}]{NO^-} \underset{\text{Nitrous oxide}}{N_2O} \xrightarrow[\text{reductase}]{N_2O^-} \underset{\text{Dinitrogen}}{N_2} \qquad \textbf{Eqn. 2.4}$$

Nitrification and denitrification are affected by a number of soil factors, which in turn are affected by various other distal factors (de Klein et al., 2001c). Many soils and climatic factors such as temperature, soil moisture, soil texture, soil pH and soil mineral N/urinary N affect N_2O emissions (Charteris et al., 2020; de Klein & Eckard, 2008a). A meta-analysis by López-Aizpún et al. (2020) reported that mean air temperature, soil pH and urine type are the main drivers of N_2O emissions from urine patches. However, they recommended that future studies should also include the following factors: soil texture; experimental set up; soil moisture and temperature; amount and composition of urine applied, animal type and diet; N_2O emissions with a measure of uncertainty; data from a control (no N application) and meteorological data.

Denitrification rates and N_2O emissions increase with increasing temperatures (Dobbie & Smith, 2001; Ryden, 1986). de Klein and van Logtestijn (1996) found that denitrification rates increased 10-fold in a grassland soil when temperature increased from 10°C to 20°C. Studies carried out by Dobbie and Smith (2001) and de Klein and van Logtestijn (1996) showed that the effect of temperature on denitrification rates was greater in non-irrigated dry soil compared to irrigated soil. Another study showed that in sandy and loess soils, N_2O production increased with the soil temperature until 15-20°C and above this temperature range, lower emissions were detected (Horváth et al., 2010).

Soil moisture can influence N_2O emissions since it can directly regulate oxygen availability in soil pores, which determines the activity of nitrification and denitrification organisms within the soil profile (van der Weerden et al., 2023; Zheng et al., 2000). As soils become wetter, they become more anaerobic, which usually increases N_2O emissions (Dobbie & Smith, 2001). In grazed pastures, N_2O emissions are often greater when the water-filled pore space (WFPS) is above field capacity, as this is a conducive environment for denitrification (Saggar et al., 2004b). Di et al. (2014) also showed that soil moisture content was a major driver of N_2O emissions from soils treated with animal urine. Furthermore, this study showed that the growth of ammonia oxidiser and denitrifier communities were significantly affected by the soil moisture content and the activity of functional genes increased with increased soil moisture content. As the soil moisture content increased, the soil became increasingly anaerobic, leading to higher denitrification rates. However, when the soil is completely saturated or at high soil moisture content level (> 90% WFPS), N_2O is converted to N_2 (Saggar et al., 2013; K. A. Smith et al., 1998). Heavy rainfall and irrigation can also cause denitrification (Di & Cameron, 2003).

Soil texture influences N_2O emissions (Jamali et al., 2016). For example, N_2O emissions are higher in clay soils compared with sandy soils, due to higher denitrification activity because clay soils have slower drainage rates that cause longer periods of anaerobic soil conditions (Cameron et al., 2013; Jamali et al., 2016; Luo et al., 2010).

Soil pH affects both the nitrification rate and the denitrification rate because it affects the abundance and activity of soil microbial communities (Mørkved et al., 2007; van der Weerden et al., 2023). For example, AOB and ammonia oxidising archaea (AOA) prefer to grow in different soil pH environments: the growth of AOB is favoured in neutral to alkaline pH soils and AOA may out-compete AOB in more acidic soils (Robinson et al., 2014). Furthermore, this study found that N_2O emissions increased when soil pH decreased. Contrary to this study, van der Weerden et al. (2022) found that when the soil pH increased (6.6-7.1), there was no significant effect on N_2O emissions after urine deposition.

Nitrogen (N) is available in the soil as NH_4^+ and NO_3^- and this has a major influence on the denitrification process (Cameron et al., 2013; Saggar et al., 2013). During outdoor grazing, animal excreta (urine or dung) is deposited on the soil, and this results in

substantial amounts of N in the soil. The resulting increase in soil inorganic N induces large increases in denitrification (de Klein et al., 2001c; Di & Cameron, 2003; Di et al., 2007b), which results in the production of N_2O. The proportion of N emitted as N_2O-N from urine-N is called the 'emission factor' (EF_3) (IPCC, 2007). The IPCC's default EF_3 for N_2O emissions from urine deposited on grazed pasture soil is 2% (IPCC, 2006) and the New Zealand specific default emission factor is 1% (van der Weerden et al., 2020). However, a meta-analysis by van der Weerden et al. (2020) reported that the EF_3 values for urine ranged from 0.08% to 0.98%, based on livestock type and topography. This agrees with the IPCC default EF_3 values (0.77% for wet climates and 0.32% for dry climates), which they have updated recently (IPCC, 2019).

2.2.3 Nitrogen leaching and the urine patch

Nitrogen leaching is the downward movement of nitrogen through the soil profile, and beyond the plant root depth, in drainage water. In grazed pastures, urine patches are the major contributor to NO_3^- leaching. The amount of NO_3^- that typically leaches under dairy pasture ranges from 15 to 60 kg N ha^{-1} (Christensen et al., 2019b; Decau et al., 2004; L. C. Smith & Monaghan, 2020) and it depends on plant uptake, soil type, climate, drainage properties, and management practices such as grazing and tillage (Cameron et al., 2013; L. C. Smith & Monaghan, 2020). The characteristics of the urine patch that specifically affect leaching are; urine N deposition rate, deposition time, and patch size (Selbie et al., 2015a; Vogeler et al., 2013).

In a meta-analysis, Selbie et al. (2015a) found that the N loading rate of cow urine patches range from 200–2000 kg N ha^{-1}. Several studies showed that NO_3^- leaching increased with increasing N loading rate. For example, Di and Cameron (2007a) reported leaching losses increased with increasing N loading rates; they measured the total NO_3^- leached was 4.3 times greater at a loading rate of 1000 kg N ha^{-1} compared to a loading rate of 300 kg N ha^{-1}. Shepherd et al. (2011) also reported that there was an increase in NO_3^- leaching at a loading rate of 800 kg N ha^{-1} compared to 400 kg N ha^{-1}. A modelling study by Shorten and Pleasants (2007) reported that urine patch overlap is an important factor in increasing nitrogen leaching. For example, they predicted that 38, 61 and 71% of N in single, double, and triple urine patches is leached into ground water.

Urine N deposited on pastures during grazings in the late summer to early-winter period was identified as the main contributor to nitrate (NO_3^-) leaching in drainage (in late autumn, winter and early spring) (Buckthought et al., 2015; Christensen, 2013; Howes, 2019; Shepherd et al., 2011). Typically when grazing events occur from about mid-summer and onwards, then there is limited time for the pasture to remove much of the inorganic N generated under urine patches, therefore, surplus NO_3^- will be susceptible to leaching when drainage occurs (Cameron et al., 2013; Haynes & Williams, 1993). This poses environmental risks, such as the N enrichment of ground and surface waters (Cameron et al., 2013; Di & Cameron, 2002a; Selbie et al., 2015a).

2.3 Methods for reducing N losses from grazed pastures

2.3.1 Grazing duration

Reducing grazing duration is one of the methods for reducing N losses from urine patches because it decreases the number of urine patches deposited during grazing. Christensen et al. (2019a); (2019b) studied the effect of Duration-controlled (DC) grazing over three years. In these studies, the cows were allowed to graze for four hours before they were moved to stand-off facilities to ruminate. The excreta collected from the stand-off facility was stored and then returned to pastures by an irrigator which spread the effluent evenly over a larger area. This resulted in a much smaller N application rate than typical urine patches. Furthermore, during the stand-off period, total GHG emissions were increased by 2-8% (Monaghan et al., 2008). Additionally, if there is sufficient capacity, the effluent can be stored for longer periods and only applied when the soil water deficit is larger, thereby increasing the uptake of applied N by pasture and reducing the risk of N leaching (Christensen et al., 2019b). Nitrate leaching under year-round DC grazing was 52% less than leaching under standard grazing management (SG) where cows were left at pasture between milking (Christensen et al., 2019b).

A field study carried out by de Klein et al. (2006) over three years found restricted autumn grazing reduced both N_2O emissions and NO_3^- leaching losses from grazed pasture by 57% and 41%, respectively. In this study, cows were allowed 3 hours grazing per grazing and then moved to a feed pad. The NO_3^- leaching results from this study are close to the

average reductions reported by Christensen et al. (2019b). Romera et al. (2017) also found 23 to 32% reduction in NO_3^- leaching losses when modelling restricted grazing. In this modelling, restricted grazing for the treatments that they used was defined as keeping the cows on the standoff pad for 8 h d^{-1} with no feed between morning and afternoon milking. This did not stop the cows urinating and defecating on the standoff pad, which potentially could increase NH_3 and N_2O losses.

Despite the benefits of reduced NO_3^- leaching losses in restricted or DC grazing, it also has some disadvantages, such as increases in gaseous N losses (NH_3 and N_2O) from captured effluent and higher capital and/or operating costs (de Klein, 2001a; de Klein & Ledgard, 2001b).

2.3.2 Animal diet

Cows grazing dairy pastures have a diet which is rich in N diet, and this results in high N concentrations in their urine and large application rates in the patches. This leads to significant N losses (NO_3^- leaching and gaseous losses; NH_3 and N_2O) (Selbie et al., 2015a). Therefore, manipulating animal diet is a potential method for reducing N losses from urine patches. Dairy cows that are on a low protein and high carbohydrate diet are likely to have smaller urinary-N concentration and lower N losses from the soil (Dalley et al., 2017; Lee et al., 2014). Diets high in soluble sugars and starch (SSS) content and 16-20% crude protein (CP) are required by lactating cows for optimal milk production so it is important that farmers select feeds that meet animal metabolic requirements (Dalley et al., 2017; de Klein & Eckard, 2008a). In winter and spring the CP in pastures can vary between 20-28% (Burke, 2020) which is above the 16-20% CP range suggested by Dalley et al. (2017). Therefore, lower protein supplements can be added to the diet at these times to reduce the CP intake, which will help to reduce the N concentration in urine patches. N leaching can also be reduced by substituting the grass that would be grown with the use of N fertiliser in winter or early spring with supplements with a low CP content (e.g. maize).

Talbot et al. (2020) found that cattle on a fodder beet diet and grazing on a winter active crop reduces N leaching losses but has no significant effect on N_2O emissions. Forage herbs such as chicory and plantain have the potential to reduce N load onto pasture by increasing the urination frequency and lowering urinary N concentrations i.e. a diuretic

effect (Box et al., 2017; Box et al., 2023; Mangwe et al., 2019; Nguyen, 2023; Podolyan et al., 2020). Rodriguez et al. (2020) found that plantain may also reduce N_2O emissions. Vi et al. (2023) found that plantain in ryegrass and white clover pastures reduced urine-N content and N_2O emissions from cow urine patches in summer/late autumn.

Cows taking in salt as a supplement with their feed will increase their water intake. For example, Ledgard et al. (2015) found that salt supplement given to cows (200 g sodium chloride per cow per day) increased their water intake and urination frequency, resulting in a 59% decrease in urine N deposition rate which, in turn, was likely to reduce N leaching. However, high rates of salt and prolonged use of salt supplement could result in soil degradation and affect animal health.

2.3.3 Inhibitors

Urease inhibitors (UIs) and nitrification inhibitors (NIs) have been used as mitigation tools to reduce gaseous losses (NH_3 and N_2O) and N leaching from grazed pastures. Urease inhibitors (UIs) are chemical compounds that regulate the transformation of urea in urine into NH_4^+, resulting in less available NH_4^+ to be converted into NH_3 (Volatilisation) (Bolan et al., 2004; Singh et al., 2013). Nitrification inhibitors inhibit the first-step of the nitrification process (NH_3 oxidation) (Di & Cameron, 2018), which subsequently reduces NO_3^- leaching and N_2O emissions. A study by Di and Cameron (2012) suggested that the liquid formulations of nitrification inhibitors (NIs) dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP) have the potential to reduce N_2O emissions and NO_3^- leaching from grazed pastures in winter.

A study by Singh et al. (2013) found that UI N-(n-butyl) thiophosphoric triamide (nBTPT), commercially named Agrotain, reduced NH_3 and N_2O emissions when applied with urine. Kim et al. (2012) suggested the potential increase in NH_3 emission with the use of NIs. This was attributed to higher ammonium levels in NI treated soils as a result of slower transformation of NH_4^+ to NO_3^-. A global meta-analysis by Wu et al. (2021) reported NIs increased NH_3 volatilisation by 36% and that contributed to increased indirect N_2O emissions by 3-15%.

When urine was applied 3 hours before or immediately before inhibitor application, nBTPT was not very effective at reducing NH_3 emissions compared to UI *N*-(2-

Nitrophenyl) phosphoric triamide (2-NPT) (Adhikari et al., 2020). Despite the effectiveness of 2-NPT in reducing NH_3 emissions, it will be impractical on dairy farms to apply the UIs in less than 3 hours after urine deposition. Contrary to this study, Rodriguez et al. (2019) found a greater reduction in NH_3 emissions when nBTPT was applied before urine deposition than when it was applied after urine deposition in autumn. However, the application of UIs before grazing is not recommended because the residues can remain on the pasture canopy, which can be subsequently grazed by the animals (Adhikari et al., 2020; Rodriguez et al., 2019).

Although NIs have been shown in some conditions to be effective at reducing NO_3^- leaching (59% reduction annually), N_2O (62-66% reduction in total emissions) and NH_3 losses (52-73% reduction) (Adhikari et al., 2020; Di & Cameron, 2002b, 2012), there are disadvantages such as the expense to apply in winter, short-term effectiveness (UIs) and they have to be applied at the right time (e.g. not effective in summer). Recent studies have found that increasing the volume of NIs is a potential option for effectively mitigating N_2O emissions from urine patches (Adhikari et al., 2024a; Adhikari et al., 2024b; Giltrap et al., 2023). This reduces the NI residues on the pasture canopy, increases the movement of NIs into the soil profile, thus increasing effective mixing with urine-N. However, further research should be carried out to optimise NI application rates and volumes. Nitrification Inhibitors are not available for commercial use on farms in NZ because a maximum permissible level in foods has not yet been established. Currently, all the NIs in NZ must be registered under the MPI (Ministry for Primary Industries) ACVM (Agriculture Compounds and Veterinary Medicine) regulations and provide the scientific data on their effectiveness and residues.

Furthermore, there were several studies of Biological Nitrification Inhibitors (BNIs) which could potentially reduce N_2O emissions from grazed pastures. It was found that aucubin which is a secondary metabolite in plantain could potentially inhibit nitrification (Gardiner et al., 2018; Luo et al., 2018; Rodriguez et al., 2021; Simon et al., 2019). Although aucubin reduced N_2O emissions, it did not have any effect on nitrate leaching and there was no significant differences in the soil inorganic nitrogen concentrations (Gardiner et al., 2018; Rodriguez et al., 2021). Recently, Vi et al. (2023) found that 30% and 50% plantain content in ryegrass white clover pastures reduced urine N content and N_2O emissions from cow urine patches in summer/late autumn.

2.3.4 Urine patch characteristics

The key characteristics of the urine patch are; urine N composition, urine volume and frequency, urine patch area and conditions in the urine patch (Selbie et al., 2015a). Understanding these characteristics is important to the identification of methods to reduce N losses from grazed pastures. A meta-analysis by Selbie et al. (2015a) produced an average urine N concentration of 6.9 g N L^{-1} for dairy cattle with a range of N concentration from 1 to 20 g N L^{-1}. The main form of urine N is urea, and its concentration can be variable depending on the cow's crude protein and water intake, as previously discussed. Apart from urea, the other constituents of cow urine containing N are allantoin, hippuric acid, creatine, creatinine, ammonia, amino acids, uric acid and (hypo)xanthine (Selbie et al., 2015a).

A cow's water intake influences daily urination volume and frequency. The average urination volume for dairy cattle is 2.1 L (Selbie et al., 2015a) and the frequency of urination for a cow may be around 9 - 14 times per day (Cameron et al., 2013; Lantinga et al., 1987; Selbie et al., 2015a). A study carried out by Shepherd et al. (2017) measured an average urine volume of 1.98 L which is within the range 1.6 - 2.2 L given by Haynes and Williams (1993). They also measured a urination frequency of 14 times per day which was at the upper end of the range summarised by Selbie et al. (2015a). They also observed that diurnal patterns affected the urination volume, which peaked in the early morning, after the cows had rested. Minnee et al. (2020) found that cows that were on a diet of 30% or more plantain had increased urination volume and more frequent urination throughout the day. The increase in urine volume was associated with the high water content in the plantain diet.

The urine patch area consists of the wetted area (where urine is directly deposited) and the area immediately outside the wetted area (Selbie et al., 2015a). However, the area outside the wetted area or the 'edge effect' introduced further complexity because plants beyond the wetted area can take up to 50% additional urinary N (Buckthought et al., 2015; Buckthought et al., 2016; Cichota et al., 2018). Therefore, the urine patch (wetted area) can vary in shape and size. When a dairy cow urinates, it covers the wetted area and it ranges from 0.14 to 0.49 m^2 with an average area of 0.24 m^2 (Haynes & Williams, 1993; Selbie et al., 2015a). Under the patch, urine can penetrate through the soil to a depth of 400 mm (Williams & Haynes, 1994). Recently, Jolly et al. (2021) compared

measurements of wetted areas using three sensor technologies: namely, thermal imagery, Spikey-R and a Drone. The wetted areas measured were 0.2-0.8 m^2 depending on the urine volume (1 L to 3 L). The sensor technologies were effective in measuring the urine patch configurations; however, they still have some disadvantages which require more research.

The urine patch area is determined by the volumes of deposited urine, environmental factors (e.g. wind and oxygen availability), soil physical conditions (e.g. moisture and temperature), and these factors can affect the plant uptake of urinary N, thereby, affecting the potential for N losses (Marsden et al., 2016; Selbie et al., 2015a). Ramirez (2017) demonstrated that increasing urine deposition area five-fold (from 0.2 to 1 m^2) reduced the quantity of net inorganic N in the 45-120 cm soil depth by 63%. This measurement of soil inorganic N occurred 53 days after urine deposition in autumn and 24 days after the commencement of the drainage season. A modelling study carried out by Cichota et al. (2018) found that there was a reduction in N leaching when the N deposited in urine patches was spread over a larger area. This demonstrates an effective reduction in the N load and a greater potential N uptake by plants.. Orwin et al. (2009) and Moir et al. (2016) found that large urine patches (e.g. 2 L) deposited from dairy cows have greater pasture yield and N uptake; which potentially reduces the N in soil at risk to loss.

2.4 Cow urine spreading device developed by Novataro Ltd

Novataro Ltd has developed a prototype urine-spreading device that is designed to be placed on cows to increase the size of urine patches and, thereby, reduce nitrate leaching. The intention is to use the device on dairy cows for up to about five and a half months each year (mid-January to late-June), after mating, to increase the spread area of urine patches.

Novataro Ltd previously commissioned the Fertiliser and Lime Research Centre (FLRC), Massey University, to assess the urine spread achieved by the device using a small number of cows in initial evaluation studies (Hanly et al., 2018). One study, which used catch containers, demonstrated that the urine-spreading device could achieve low average

application depths of ~2 mm compared to more typical urine application depths (i.e., ~10 mm or greater) (Hanly et al., 2017b). Field measurements, using a conductivity meter supported the earlier observation that, compared to standard urine patches, the device can increase the actual spread in the soil (Dewhurst, 2021). This preliminary study showed that the device has the potential to spread urine in a manner that is likely to be significant in terms of nitrate leaching mitigation. However, further research is required to evaluate the effect of the device on NO_3^- leaching and NH_3 and N_2O emissions from urine patches.

2.5 Summary

The main findings of this literature review are:

- In grazed pasture systems, urine patches are the major source of the N that is lost to the environment. Considerable research has been conducted on urine patch dynamics. However, few studies have measured NH_3 emissions, N_2O emissions and NO_3^- leaching when the urine stream is spread, and the patch size is increased.
- Nitrogen losses from urine patches contribute to three environmental problems: NH_3 volatilisation (indirect GHG), N_2O emissions (direct GHG – contributes to global warming) and NO^-_3 leaching (causes degradation of water quality). These forms of N loss from urine patches have been studied in dairy grazed pastures, especially from a typical urine patch.
- A common result in these studies is that N loss from urine patches can be mitigated by lowering the rate of N application in patches. However, to date, there is no published data on the effect of increasing the size of the urine patch on the above-mentioned pathways. Therefore, further research is required in which direct measurements of NH_3, N_2O and NO_3^- leaching from a spread urine patch are made.
- Earlier studies have suggested mitigation options to reduce N losses from urine patches. Although there are a number of mitigation options available to reduce the N load in urine patches, they vary in their costs and the ability to implement them. Therefore, a simple and cost-effective device for increasing the size of the urine patch could prove to be a very useful way to mitigate nitrate leaching from grazed pastures.

- Novataro Ltd has developed a prototype urine-spreading device which was used on a small number of cows in some preliminary evaluation studies conducted by FLRC at Dairy 4 Farm, Massey University. The device has the potential to spread the urine stream as it leaves the cow, and it may be a more direct, effective and cheaper method to reduce the rate of N deposition in urine patches. Therefore, further research is required to directly quantify the effect of this urine-spreading device on N leaching and N losses to the atmosphere.

Chapter 3

Effect of increasing cow urine patch area on ammonia emissions from a pasture soil

3.1 Introduction

The urine patches of grazing animals are well recognised as concentrated areas of gaseous [ammonia (NH_3), nitrogen oxides (NO_x), nitrous oxide (N_2O) and nitrogen gas (N_2)] losses to the atmosphere and nitrate (NO_3^-) leaching to waterways (López-Aizpún et al., 2020; Uwizeye et al., 2020). This poses environmental concerns, such as acidification and eutrophication of natural ecosystems (Saggar et al., 2005; Sommer et al., 2019), increases in greenhouse gas concentrations (Tian et al., 2020) and the indirect catalysis of stratospheric ozone depletion (Ravishankara et al., 2009).

There are a number of approaches to reducing the N losses from pastoral farms. Examples of mitigation measures include: restricted or duration controlled grazing (DC) (Christensen et al., 2019a, 2019b; de Klein et al., 2006); changing animal diet (Dalley et al., 2017; Ledgard et al., 2015; Talbot et al., 2020); and the use of urease or nitrification inhibitors (Adhikari et al., 2020; Di & Cameron, 2012; Rodriguez et al., 2019; Singh et al., 2013). The objective of most of these mitigation options is to decrease the N load in urine patches or slow down its transformation in the soil. However, the widespread adoption of many of these mitigations have been limited by either the cost or other practical constraints to implementation (Adhikari et al., 2020; Dalley et al., 2017; de Klein, 2001a; de Klein & Ledgard, 2001b; Ledgard et al., 2015). Another challenge is that a mitigation used to target one source of N loss can inadvertently increase other sources of N loss. For example, the use of urease inhibitors to decrease the emissions of NH_3 from urine patches may increase the amount of inorganic N retained in soil, which has potential to contribute to subsequent N_2O emissions and NO_3^- leaching (Singh et al., 2013). Therefore, the evaluation of N loss mitigations should consider interactions between the various sources of N loss that have potential to be environmentally damaging.

In 2017, a New Zealand based company, Novataro Ltd, commissioned the Fertiliser and Lime Research Centre (FLRC), Massey University, to assess the urine spread achieved by a spreading device worn by cows in initial evaluation studies (Dewhurst, 2021; Hanly et al., 2017b, 2018). The primary aim of this device is to provide a method to reduce NO_3^- leaching from dairy farms by directly reducing the concentration of N in urine patches by increasing the urine patch area and decreasing the urine application depth. These studies showed that the device can spread urine in a manner that has potential to mitigate NO_3^- leaching. Previous studies have shown that increasing the urine patch area and the lateral spread of urinary N reduces the risk of N leaching (Cichota et al., 2018; Ramirez, 2017). Therefore, these studies support the potential benefits of increasing the urine patch area for mitigating NO_3^- leaching, but further research is required to quantify these benefits and also the effect of increasing the urine patch area on other N losses, particularly NH_3 and N_2O emissions.

Ammonia emissions are one of the sources of indirect greenhouse gases (GHG) (Cameron et al., 2013). Emissions of NH_3 that result from urea hydrolysis, where ammonium (NH_4^+) dissociates to NH_3 (Selbie et al., 2015b), contribute to indirect emissions of N_2O. Ammonia emissions within the range of 5-26% of the N applied as urine in cow urine patches have been measured (Adhikari et al., 2020; Laubach et al., 2013; Rodriguez et al., 2019; Zaman et al., 2013b). In New Zealand (NZ), the estimated NH_3 volatilisation from agricultural soils has increased by 19.4% (139.0 kt CO_2-e) between 1990 and 2021 (Ministry for the Environment, 2023).

Urea in livestock urine begins hydrolysis immediately after being deposited on the soil, catalysed by the urease enzyme, which can increase soil pH to levels (>7.5) that favour NH_3 emissions (Bolan et al., 2004; Rodriguez et al., 2019; Saggar et al., 2004b; Selbie et al., 2015a; Zaman et al., 2013b). The majority of NH_3 emissions from livestock urine patches occur within about 2 days of urine deposition. The rate and degree to which NH_3 emissions occur are influenced by a soil's cation exchange capacity (CEC) (Li et al., 2015; Whitehead & Raistrick, 1993), soil temperature (Black et al., 1985a; Black et al., 1985b; Sherlock & Goh, 1984; Zaman et al., 2009), soil moisture, the amount of rainfall or irrigation (Black et al., 1987; Bussink, 1996; Saarijarvi et al., 2006; Zaman et al., 2013b) and soil urinary N urea concentration (Selbie et al., 2015a). In general, warm soils with low CEC and low soil moisture levels, result in higher NH_3 emissions. There is a

lack of comprehensive and quantitative information on how urine patch size affects NH_3 emissions and, thus, indirect N_2O emissions. This chapter is focussed on assessing the effect of increasing the surface area of urine patch on NH_3 emissions.

This research comprised a field experiment conducted on a pasture site with a Manawatu silt loam soil in the Manawatu, during early autumn. This experiment quantified NH_3 emissions and emission factors (EF_3) from three urine patch areas: 0.25, 0.5 and 1 m^2. The objectives of this study were to (i) quantify the effect of increasing the urine patch area (i.e., decreasing urine application depth) on NH_3 emissions from dairy cow urine applied to a pasture soil in early autumn, (ii) quantify the effect of urine patch sizes on how much NH_4^+ and NO_3^- has moved further down into the soil.

3.2 Materials and Methods

3.2.1 Field trial location and site description

A field experiment was carried out in the early autumn of 2019 on Dairy Farm 1, one of Massey University's research farms, near Palmerston North, New Zealand. The soil type is the Manawatu Silt Loam soil, a Weathered Fluvial Recent soil (NZ Classification) or Dystric Fluventic Eutroichrept (USDA Classification). The experimental site had a standard ryegrass (*Lolium perenne)* and white clover (*Trifolium repens*) mixed pasture, with some weed species. Surface soil characteristics are as defined in Adhikari et al. (2019) (Table 3.1). The trial area was fenced to exclude livestock and had not been grazed for more than a year when the trial commenced.

Table 3.1: Physical and chemical characteristics of soil at 0-5cm. Adapted from Adhikari et al. (2019)**.**

Depth (cm)	Soil pH (water)	Total C (%)	Total N (%)	CEC (meq 100 g^{-1})	Soil Urease Activity (mg kg^{-1} hr^{-1})	Field capacity (%)	Bulk density (Mg m^{-3})
0-5	5.5	2.8	0.3	18.5	37	37	1.2

3.2.2 Experimental design

The experimental design consisted of four treatments and five replicates of each treatment, which were randomised (Table 3.2). The treatments were no dairy cow urine (control treatment), and dairy cow urine applied to pasture (flat topography) at depths of 10, 5 and 2.5 mm, which represent the depths that would result from the deposition of 2.5 L of urine to patch areas of 0.25, 0.5 and 1.0 m^2, respectively (Table 3.2). The application depth of 10 mm was used to represent the urine application depth to a typical urine patch area (Selbie et al., 2015a; Shepherd et al., 2011), whereas the other two smaller application depths represent urine depths applied to patches with greater levels of urine spread. The experimental design was repeated in two separate areas: one for ammonia capture chambers (each 0.018 m^2) and one for soil sampling plots (each 0.20 m^2).

Table 3.2: Urine application depth treatments used in the ammonia capture chambers.

Treatment #	Urine patch area (m^2)	Application depth* (mm)	Volume of urine applied (ml $chamber^{-1}$)**	Amount of urine N applied (mg N $chamber^{-1}$)
1	0	0	0	0
2	0.25	10	177	802
3	0.5	5	89	401
4	1	2.5	44	201

*****The application depths represent the depths achieved when a urine volume of 2.5 L is applied to each of the urine patch areas.
******Chamber areas are 0.018 m^2.

3.2.3 Urine collection, analyses, and application to ammonia capture chambers and soil sampling plots

Urine was collected from dairy cows during milking times at Massey University Dairy Farm 4. The urine was refrigerated at 4˚C after collection (7 days) until the start of the experiment. Before application to the chambers on 7 March 2019, all of the urine was mixed together. A sub-sample of the urine was analysed for urea-N using the 'Diacetyl Monoxime method', which forms a red colour when the extract is heated with diacetyl monoxine (DMA), thiosemicarbazide (TSC), sulphuric and orthophosphoric acid and ferric chloride hexahydrate (Douglas & Bremner, 1970; Mulvaney & Bremner, 1979). Total N in the urine samples was determined using the Kjeldahl digestion method

(McKenzie & Wallace, 1954) and analysed using a spectrophotometer (Bibby Scientific, Stone, UK). The concentrations of total N and urea-N in the applied urine were 4.53 g N L^{-1} and 3.30 g N L^{-1} (73%), respectively. The percentage of urine-N in this experiment was 27% which falls within the range of 20–30% of the urine-N reported in previous studies (Rodriguez et al., 2019; Zaman et al., 2013b).

Urine was applied evenly to the soil in the chambers and to the soil sampling plots, using a small watering bottle and a watering can, respectively, on the same day. The volume of urine applied to the soil sampling plots to achieve 2.5 mm, 5 and 10 mm application depths were 0.5, 1 and 2 L, respectively.

3.2.4 Ammonia measurement

Gas sampling was conducted in the field using the Dynamic Chamber method similar to that described by Kissel et al. (1977). It is comprised of a volatilisation chamber, an acid trap for trapping NH_3 and a manifold that consists of four air valves that regulate the flow rate inside four PVC pipe chambers (Fig 3.1). The chambers, with a transparent top (clear Perspex), have an internal diameter of 150 mm and a height of 40 mm. The chambers were pushed into the soil to a depth of approximately 10 mm, leaving a headspace volume of 0.5 L. Each chamber had a vent on the side that connected, via tubing, to an acid trap (containing 250 mL of 0.025 M H_2SO_4), which in turn is connected to a vacuum cleaner via a drum manifold. A vacuum cleaner, running continuously for approximately 3 weeks, was used to draw air from the chambers and through the acid traps at a constant flow rate of 6 L min^{-1} (monitored daily), which provided an air exchange rate of 12 air changes per minute. The acid solution was sampled and replaced with fresh acid solution every day for the first 5 days, then at intervals between 1 and 6 days, thereafter. At each sampling, the volume of the acid solution in each bottle was recorded before sub-sampling. Sub-samples of the acid solution (0.025 M H_2SO_4) were stored at 4 °C until they were analysed for NH_3-N concentration using a Technicon II Autoanalyzer (Blakemore et al., 1987). The NH_3-N (mg) was calculated using Eqn. 3.1. Daily NH_3-N emitted (mg $chamber^{-1}$ d^{-1}) was calculated using Eqn. 3.2. The net NH_3-N flux (mg $patch^{-1}$ day^{-1}) was calculated using Eqn. 3.3, which involved subtracting the control treatment (without cow urine) concentration from the urine treatment concentrations. Ammonia volatilisation was continuously measured until the NH_3-N concentration in the acid trap reached background levels. The chambers were covered with shade cloth to avoid direct sunlight

and this, along with the continuous airflow, also helped to avoid elevating the temperature inside the chambers above ambient temperature levels (Li et al., 2014). Following rainfall events, each chamber area was sprayed with a volume of water that was equivalent to the quantity of rainfall that occurred. This was done to compensate for any rainfall that fell while the chambers were covered during the period of NH_3 measurement.

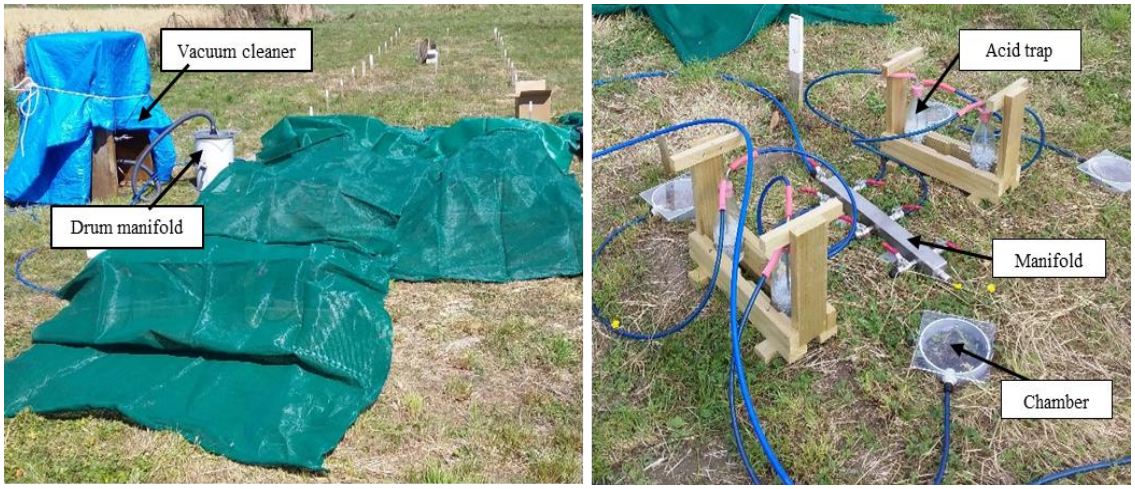


Figure 3.1: Ammonia sampling site and set up of the Dynamic Chamber method.

$$NH_3\text{-}N \text{ quantity} = C \times V \qquad \textit{Eqn. 3. 1}$$

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).

The daily NH_3 emitted per chamber was calculated using Eqn. 3.2.

$$NH_3\text{-}N \text{ emitted} = NH_3\text{-}N \div D \qquad \textit{Eqn. 3.2}$$

where NH_3-N = NH_3-N emitted per chamber (mg $chamber^{-1}$); D = duration (days) of each sampling.

The daily net NH_3 emitted per patch was calculated using Eqn. 3.3.

$$NH_3\text{-}N \text{ flux} = (((NH_3\text{-}N - Control) \div a) \times A) \div D \qquad \textit{Eqn. 3.3}$$

where NH_3-N = NH_3-N emitted per chamber (mg $chamber^{-1)}$; Control = control treatment (without cow urine) (mg $chamber^{-1}$); a = total cross-sectional area (m^2) of the chamber inserted into the soil; A = area of urine patch (m^2); D = duration (days) of each sampling.

The NH_3 emission factor (EF_3) was also calculated using Eqn. 3.4.

$$EF\ (\%) = \frac{CE\ excreta\ (urine) - CE\ control}{Urine\ N\ load} \times 100 \qquad Eqn.\ 3.4$$

where CE is the cumulative emission of NH_3 (kg N ha^{-1}), and Urine N load is the total N applied as urine (kg N ha^{-1}).

3.2.5 Soil sampling and measurements

Soil sampling plots consisted of five replicate blocks of the four treatments, each plot measuring 1.20 m x 1.25 m (Fig 3.2). Within the 1.5 m^2 plot, urine was applied to an area of 0.18 m^2, represented by the red circle, and soil cores were sampled from this area. Soil sampling was conducted in the afternoon of Days 1 (a day after urine application), 4, 7, 14, and 20. Soil cores (three cores per plot) were collected from each of the 20 plots using a soil corer at depths of 0-25 mm and 25–50 mm. The three cores from each plot were placed in a plastic bag and taken back to the laboratory and sieved immediately using a 2 mm sieve without drying. These soil samples were analysed for extractable NO_3^- and NH_4^+, moisture content and pH.

Figure 3.2: Soil sampling area showing the individual soil plots. The red circle indicates where one of the urine application areas is within a plot.

3.2.6 Extractable soil nitrate and ammonium

Soil NO_3^- and NH_4^+ analyses involved weighing 5 g of moist field soil into a 50 mL centrifuge tube and adding 30 mL of 2 M KCl (Blakemore et al., 1987). The samples were shaken on an end-over-end shaker for 1 hour. They were removed from the shaker, centrifuged for 5 minutes at 5,000 rpm, and then filtered into 30 mL containers using Whatman 41 filter papers. The collected filtrates were stored at 4˚C for up to a week until they were analysed using the Technicon II Auto Analyzer (Blakemore et al., 1987).

After analysis, the NO_3^- and NH_4^+ concentrations were used to calculate the net NH_4^+-N and NO_3^--N concentrations expressed as mg N kg soil^{-1}, using Eqn. 3.5, and mg N patch^{-1}, using Eqn. 3.6. Equation 3.7 was used to calculate the soil mass per patch (SMP).

Net Mineral N concentration (NH_4^+-N and NO_3^--N; mg N kg soil^{-1}) = $U_{conc.} - C_{conc.}$ *Eqn. 3.5*

Net Mineral N quantity (NH_4^+-N and NO_3^{-1}- N; mg N patch^{-1}) = ($U_{conc.} - C_{conc.}$) x SMP *Eqn. 3.6*

Where $U_{conc.}$ = concentration of NH_4^+-N or NO_3^-–N (mg N kg soil^{-1}) for treatments with cow urine; $C_{conc.}$ = concentration of Control treatment (mg N kg soil^{-1}); SMP = soil mass patch^{-1} (kg patch^{-1}).

SMP = DS x UPA x BD *Eqn. 3.7*

Where DS = sampling depth (m); UPA = urine patch area (m^2 patch^{-1}); BD = bulk density of soil (kg m^{-3}).

3.2.7 Soil moisture and pH

Gravimetric soil moisture content and soil pH were determined for the 0-25 and 25-50 mm soil depths for all collected soil samples. To measure the soil moisture content gravimetrically, 12 g (approximately) of fresh soil was weighed into an aluminium foil cup, oven dried at 105˚C for 24 hours and reweighed. The soil pH was measured by weighing 10 g of fresh soil in a pH beaker with 25 ml of deionized water and then was stirred vigorously with a laboratory spatula. The solution was left to stand overnight and the pH was measured using a pH metre (pHM83, Autocal pH metre) (Blakemore et al., 1987)

3.2.8 Climate data

Daily air temperature, daily soil temperature and daily rainfall for the trial period were collected from the NIWA/AgResearch meteorological station located about 500 m from the experimental site.

3.2.9 Data and statistical analysis

The mean values and standard error of the means for NH_3 emissions, soil pH and the soil NO_3^- and NH_4^+ concentrations were calculated, using Microsoft Excel for Microsoft 365. Least significant differences and p-values were calculated using one-way analysis of variance (ANOVA) to detect any significant difference and different treatment means were compared using Tukey Pairwise Comparisons in Minitab 19.

The combined apparent recovery of urinary-N (Table 3.4) was calculated by adding the total amount of N measured in NH_3-N emissions to net soil NO_3^- and NH_4^+ and then comparing this total with the amount of N added to each treatment in urine.

3.3 Results

3.3.1 Meteorological data

During the experimental period (Day 1 to Day 20), the daily maximum air temperature ranged from 21.4 to 26.1°C and the daily minimum air temperature ranged from 10.5 to 18°C (Fig 3.3). The daily average soil temperature ranged from 18.9 to 22.1°C.

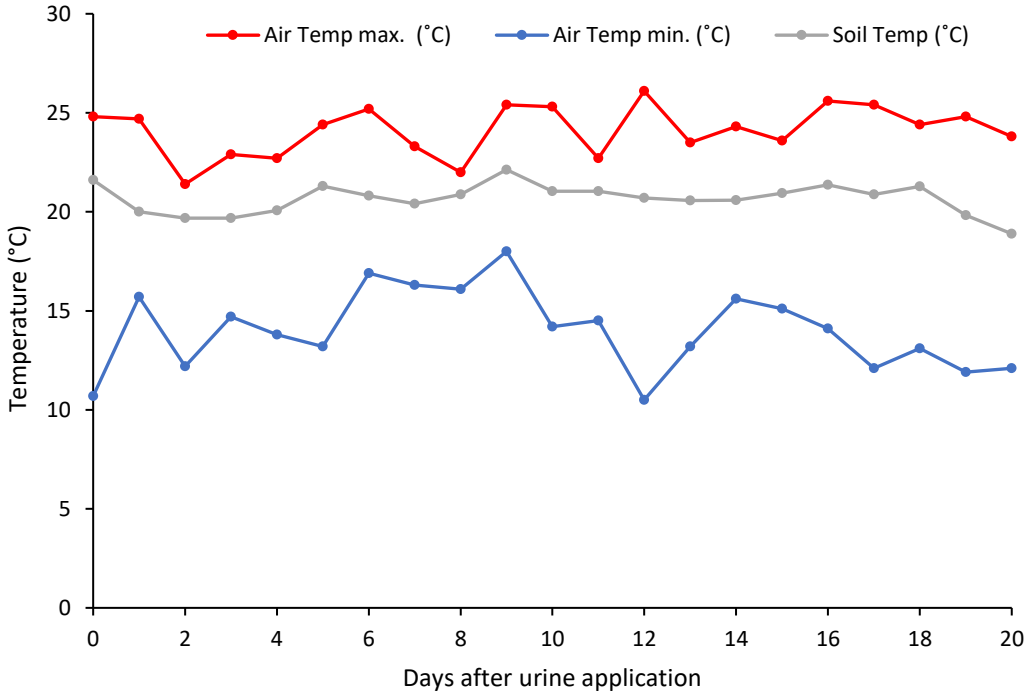


Figure 3.3: Daily air and soil temperature at a depth of 100 mm (Day 0 was 7th March 2019).

The total rainfall over the experimental period was 43 mm (Fig 3.4). Rainfall occurred on Days 1, 2, 6 and 7 after urine application. There was 5 mm of rainfall on Day 1, which started approximately 24 hours after urine application on Day 0. The highest daily rainfall of 15 mm occurred on both Day 2 and 7. After Day 7 there was no rainfall up until Day 20 when the experiment ended.

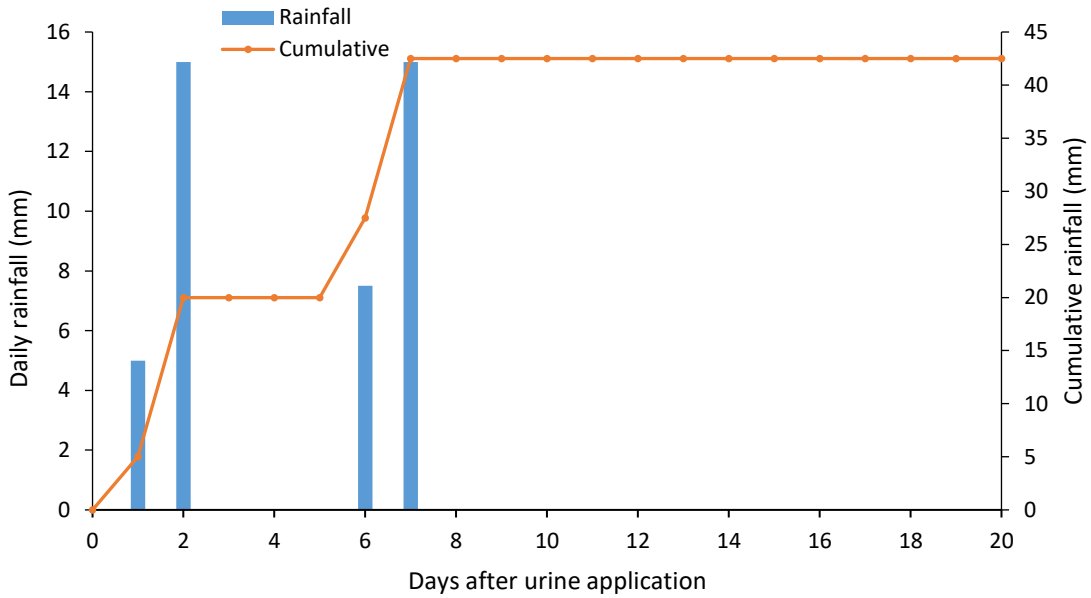


Figure 3.4: Daily and cumulative rainfall (Day 0 was 7th March 2019).

During the trial period, differences in soil moisture showed a general trend of increasing with urine application depth (Fig 3.5), particularly in the surface soil depth. On Day 1 in the 0-25 mm soil depth, the Control (0 mm urine) treatment had the lowest gravimetric soil moisture content of 20.5% and the Urine (10 mm) treatment had the highest soil moisture content of 26.1%. These soil moisture contents would encourage greater ammonia volatilisation from each of the urine treatments since these levels of soil moisture content are less than the field capacity (approximately 37%) for the Manawatu silt loam soil (Table 3.1). The soil moisture content in both soil depths responded to the rainfall, with the highest moisture content for all treatments occurring at Day 7 (Fig 3.5 a). The soil moisture content in the 0-25 mm depth peaked at 33.3, 34.9, 33.9 and 35.6% for the Control (0 mm), Urine (2.5 mm), Urine (5 mm) and Urine (10 mm) treatments, respectively, and decreased thereafter (Fig 3.5 a). In the 25–50 mm soil depth on Day 7, the soil moisture content peaked at 24.5, 25.4, 23.5 and 25.8%, for the Control (0 mm), Urine (2.5 mm), Urine (5 mm) and Urine (10 mm) treatments, respectively (Fig 3.5 b). In the 0-25 mm soil depth there was a decline in soil moisture for all treatments over the first four days on average. Over the same period, the soil moisture content increased in the 25-50 mm soil depth.

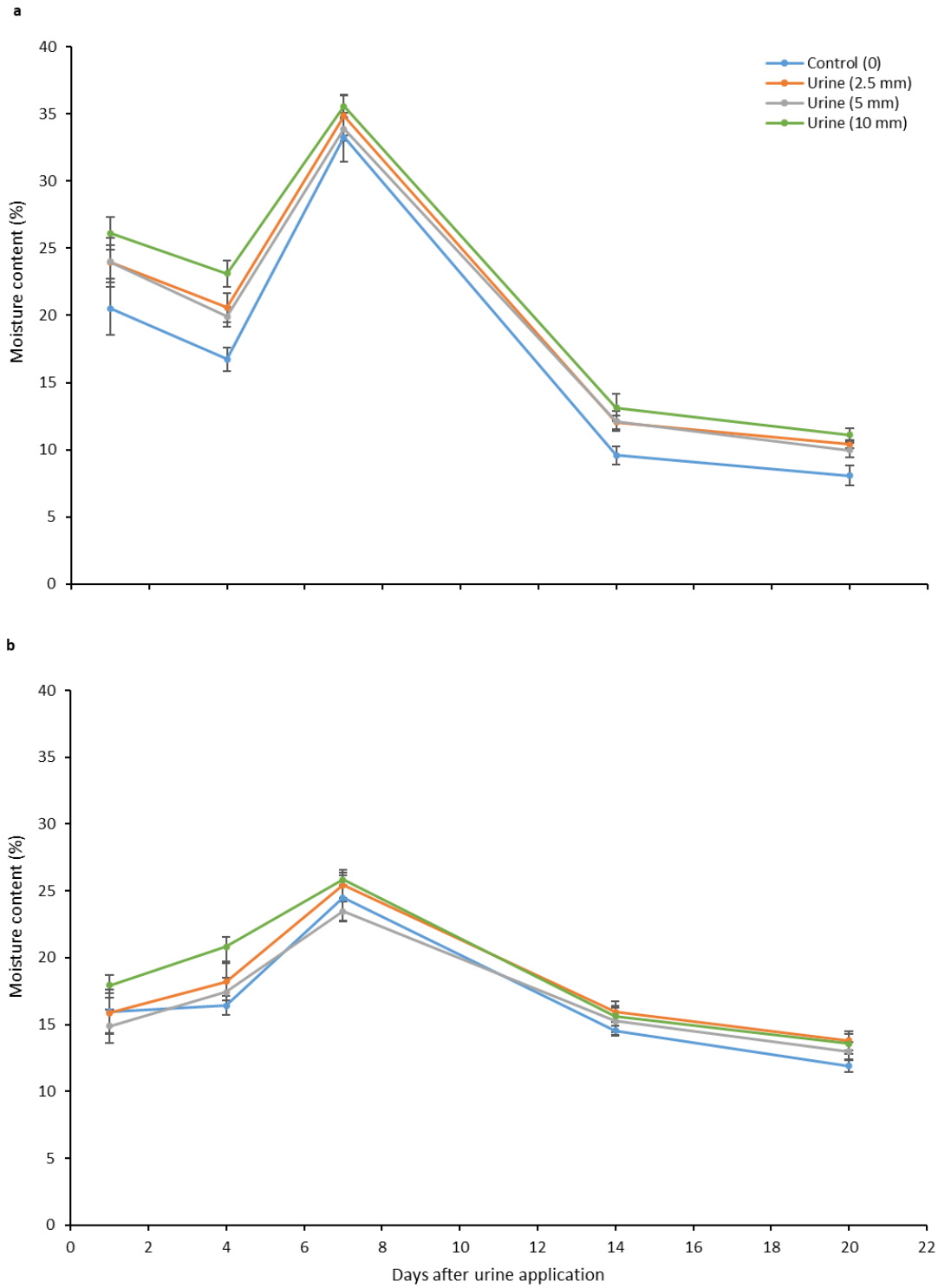


Figure 3.5: Gravimetric moisture content in the (a) 0–25 mm and (b) 25–50 mm soil depths for the control (no urine) and urine application depth treatments. Error bars are standard error of the mean.

3.3.2 Ammonia emissions

One day after urine application, the NH_3 emissions peaked at 39 (± 2.1), 60 (± 4.8) and 115 (± 2.6) mg NH_3-N chamber^{-1} day^{-1}, for the 2.5, 5 and 10 mm urine application depth treatments, respectively (Fig 3.6 a). This compares to negligible NH_3 emissions from the Control (no urine) treatment. After the peak, there was a steep decline over the following two days and then a gradual decrease to background levels approximately two weeks after urine application.

When the net NH_3 losses per chamber for each urine application depth treatment were extrapolated to the urine patch areas that they represented (i.e., 10 mm = 0.25 m^2, 5 mm = 0.5 m^2, 2.5 mm = 1 m^2), the NH_3 emissions from the 0.25, 0.5 and 1 m^2 urine patch areas peaked, one day after urine application, at 1,620 (± 36), 1,702 (± 135) and 2,207 (± 117) mg NH_3-N patch-1 day^{-1}, respectively (Fig 3.6 b). Comparing the NH_3 emissions on Day 1, the NH_3 emissions from the 1 m^2 urine patch area was significantly different ($P<0.05$) from the 0.25 and 0.5 m^2 urine patch areas, whilst the emissions from the latter urine patch areas were not significantly different from each other. The emission from the 1 m^2 urine patch remained significantly higher than the two smaller patches on Day 2, 3 and, 4. On Day 5 till the end of the experiment (Day 20), the NH_3 emissions from the 0.25, 0.5 and 1 m^2 urine patch areas declined to background levels and were not significantly different from each other. The total cumulative net NH_3 emissions over the 20-day period of the experiment was equivalent to an average of 2,867 (± 162), 3,087 (± 122) and 4,019 (± 222) mg NH_3-N patch^{-1} for the 0.25, 0.5 and 1 m^2 urine patch areas, respectively (Fig 3.6 c). The total cumulative net value for the 1 m^2 urine patch area was significantly different ($P < 0.05$) from the 0.25 and 0.5 m^2 urine patch areas, whereas the emissions from the two smallest urine patch areas were not significantly different from each other. These losses represent 25, 27 and 36% of the total urine N applied (Table 3.4), respectively, which are also the NH_3 emission factors.

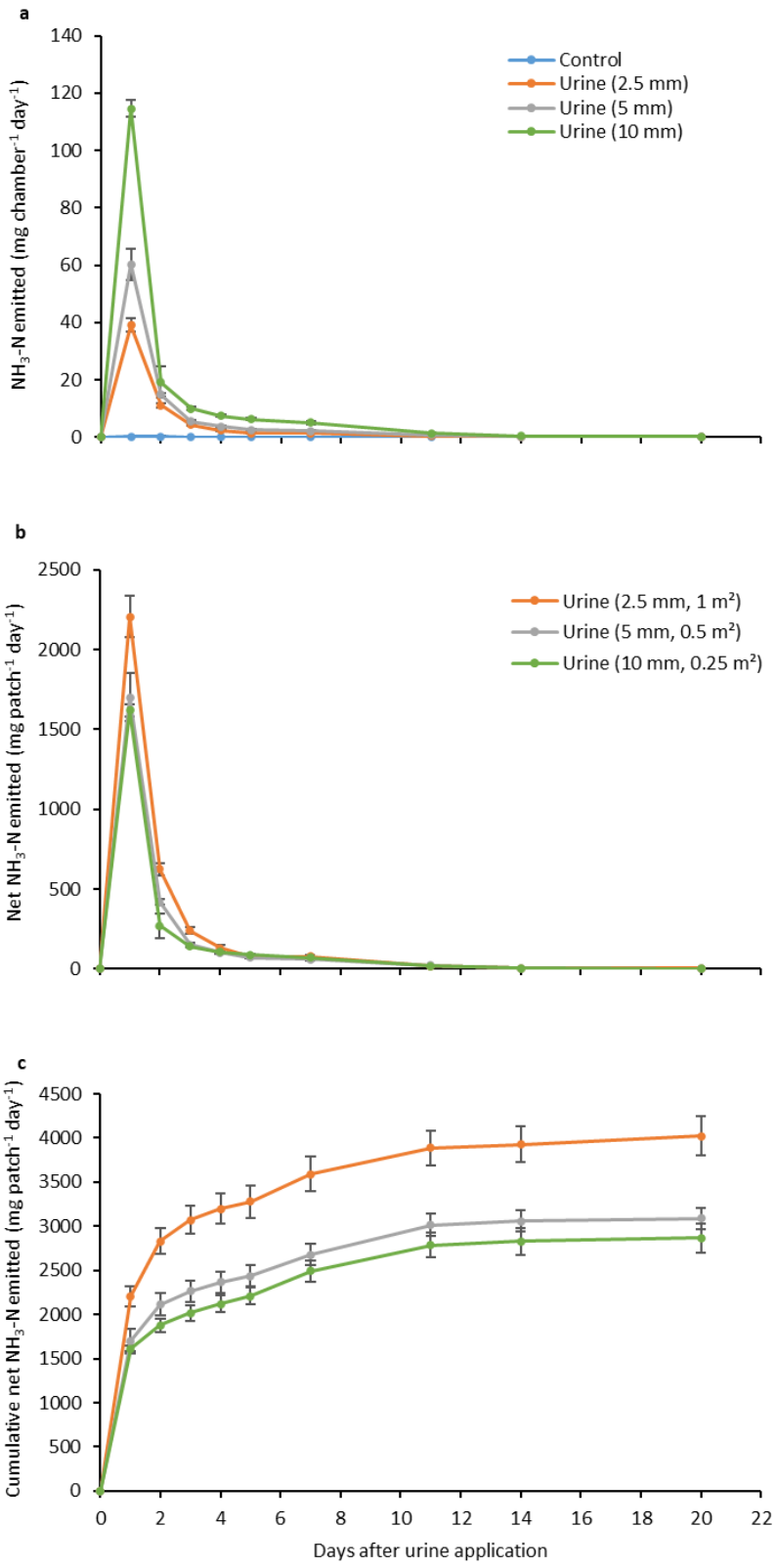


Figure 3.6: Ammonia losses following dairy cow urine applications to pasture, presented as daily NH_3-N emitted per chamber (a), daily net NH_3-N emitted per urine patch (b) and cumulative net NH_3-N emitted per urine patch (c). Error bars are standard error of the mean.

3.3.3 Soil pH

The effect of urine treatments on soil pH was more pronounced in the 0-25 mm soil depth than the 25-50 mm soil depth. During the experiment the pH for the Control (no urine) treatment remained between 5.6 and 5.8. One day after urine application, the soil pH in all the urine patch treatments increased to values in the range of 6.6 to 6.7 (Fig 3.7 a). At most of the sampling times, the highest urine application depth (10 mm) treatment had the highest surface soil pH, peaking at a pH of 6.8 four days after urine treatment. The highest soil pH for the other two treatments occurred at one day after urine application. The soil pH levels in the 0-25 mm soil depth for the three urine treatments gradually decreased and returned to levels close to the Control treatment value (pH 5.8) by Day 20 after urine application.

In the 25-50 mm soil depth, the 10 mm urine application depth treatment also had the highest soil pH of 5.9 at one day after urine application, compared to the other three treatments (Control, 2.5 and 5 mm), which had pH values of 5.6, 5.8 and 5.6, respectively (Fig 3.7 b). At four days after urine application, the pH decreased to pH 5.3, 5.3, 5.4 and 5.7, for the Control, 2.5, 5 and 10 mm urine treatments, respectively, and continued to decrease to pH values of between 5.1 and 5.2 by Day 7 and showed little change until Day 14. Between Day 14 and the end of the experiment at Day 20, the pH values increased again to 5.6, 5.3, 5.4 and 5.2 for the Control, 2.5, 5, and 10 mm urine treatments, respectively.

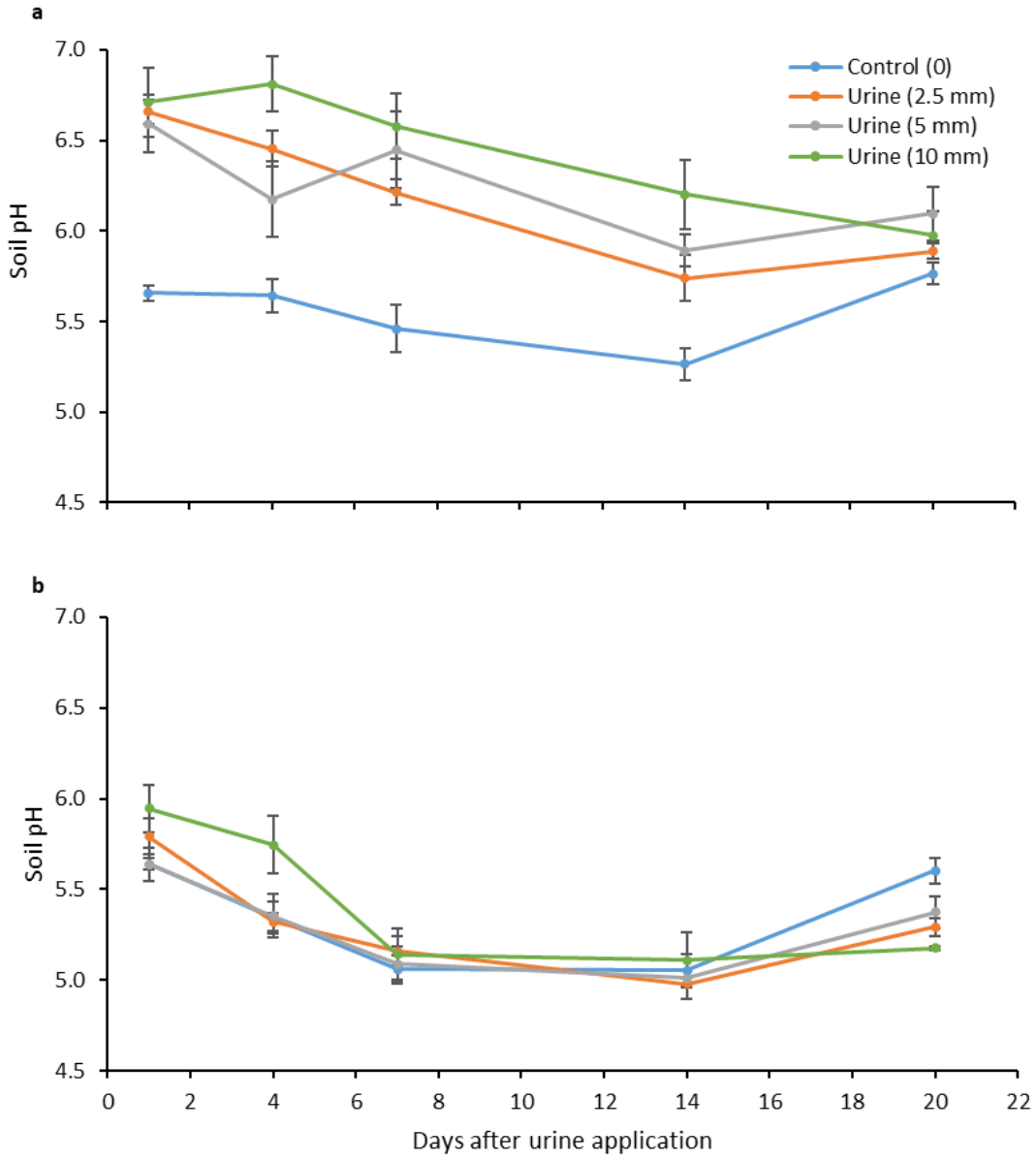


Figure 3.7: Average pH in (a) 0–25 mm and (b) 25–50 mm soil depths during the study period. The error bars indicate standard errors of the mean (SEM).

3.3.4 Soil inorganic N

3.3.4.1 Soil ammonium

In the 0-25 mm soil depth, net soil NH_4^+ concentrations peaked at one day after urine application for all three urine treatments (Fig 3.8 a). The net concentrations for each urine patch areas were similar, at 367 (±21), 362 (±61) and 347 (±18) mg NH_4^+-N kg $soil^{-1}$, for the 2.5, 5 and 10 mm urine application depths, respectively. In addition, all three

treatments showed a general trend of net NH_4^+ concentrations decreasing over the remainder of the experiment up to 20 days after urine application to be between 8-34% of their Day 1 values. When the net NH_4^+ concentrations were used to extrapolate the amounts of net NH_4^+ patch^{-1}, based on the different urine patch areas that they each represent, then there were clear differences between the urine application treatments. The peak values, at one day after urine application, were 2,609 (±136), 5,437 (±920) and 11,016 (±634) mg NH_4^+-N patch^{-1} for the 0.25, 0.5 and 1 m^2 urine patch areas, respectively (Fig 3.8 b). At the end of the experiment (Day 20), the net NH_4^+ quantities in the soil were 884 (±189), 1,064 (±62) and 920 (±315) mg NH_4^+-N patch^{-1}, respectively.

In the 25-50 mm soil depth, the net NH_4^+ concentrations were lower (Fig 3.8 c) compared to the surface soil depth (Fig 3.8 a). Over the duration of the experiment, the 10 mm urine application depth consistently had the highest net soil NH_4^+ concentrations, peaking one day after urine application at 221 (±18) mg NH_4^+-N kg soil^{-1}. In comparison, the net NH_4^+ concentrations for the 5 and 2.5 mm urine application depth treatments were 47 (±6) and 113 (±72) mg NH_4^+-N kg soil^{-1}, respectively. When extrapolated to 0.25, 0.5 and 1 m^2 urine patch areas, the estimated net NH_4^+ quantity was 1,661 (±138), 704 (±92) and 3,389 (±2,173) mg NH_4^+-N patch^{-1}, respectively (Fig 3.8 d). By Day 4 there were little differences between the three treatments, with the treatment values remaining similar until the end of the experiment. At Day 20, the estimated net NH_4^+ quantities had decreased to 207 (±39), 209 (±64) and 150 (±19) mg NH_4^+-N patch^{-1}, for the 0.25, 0.5 and 1 m^2 extrapolated urine patch areas, respectively (Fig 3.8 d).

At Day 20, the percentage of urine N applied (Total N) which was lost as NH_4^+-N in the 0-25 mm soil depth was 8, 9 and 8% for the 0.25, 0.5 and 1m^2 urine patch areas, respectively. In the 25-50 mm soil depth, the percentage of urine N applied which was lost as NH_4^+-N was 2, 2 and 1% for the 0.25, 0.5 and 1 m^2 urine patch areas, respectively (Table 3.3).

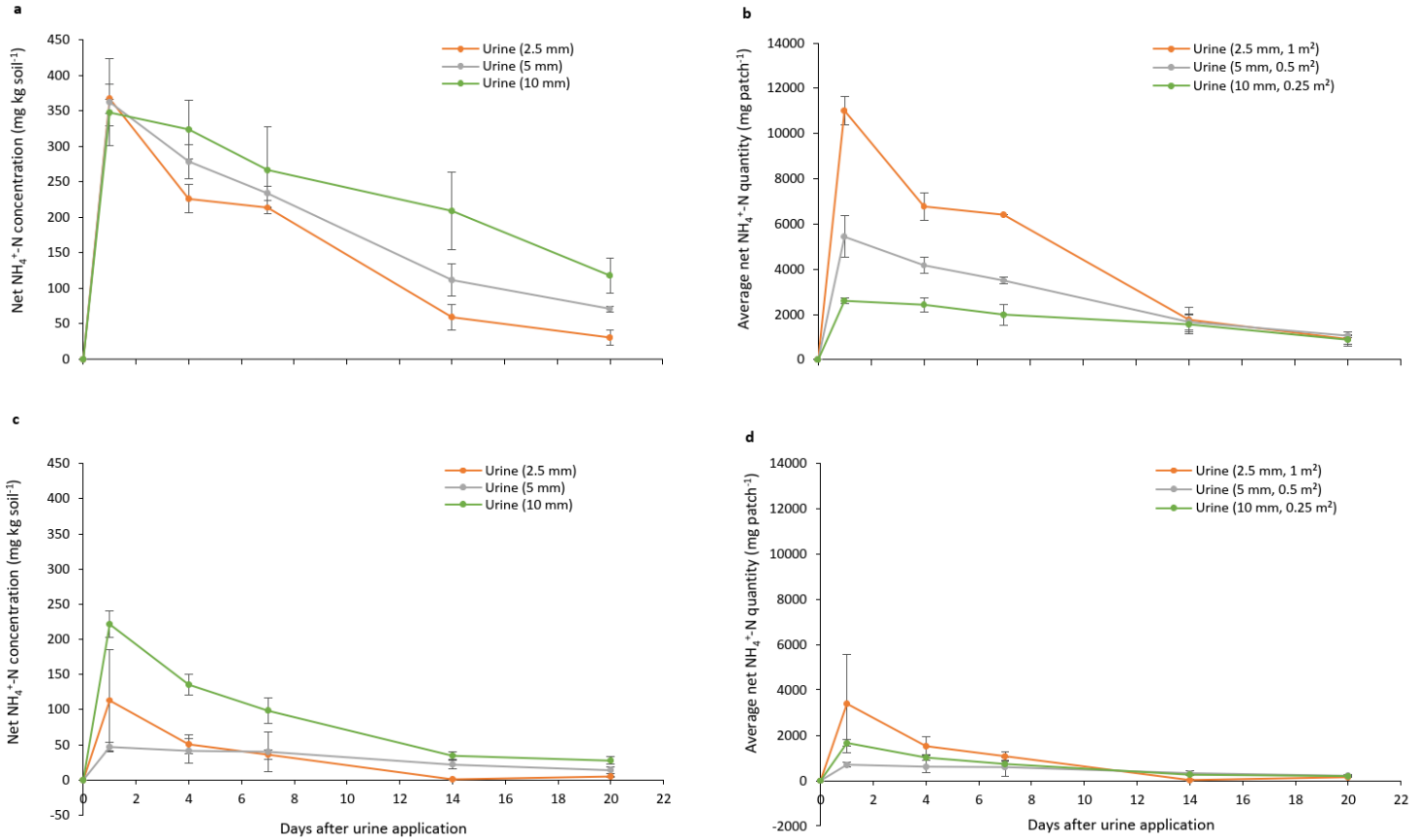


Figure 3.8: Soil net NH_4^+-N concentration and the equivalent average net NH_4^+-N quantity per urine patch for each urine application treatment at two soil depth (0-25 mm a & b; 25-50 mm c & d). The error bars indicate standard errors of the mean (SEM).

3.3.4.2 Soil nitrate

In the surface soil depth (0-25 mm), there was initially only a small increase in net soil NO_3^- concentrations in all the urine patch treatments during the first week after urine application (Fig 3.9 a). From day 8 after urine application there was a steady increase in soil net NO_3^- concentrations, peaking at Day 14 at 98 (± 37), 34 (± 6) and 78 (± 6) mg NO_3^--N kg $soil^{-1}$ for the 2.5, 5 and 10 mm urine application depths, respectively. After the peak, the net NO_3^- concentrations for all urine treatments decreased over the subsequent week but remained high. While all three urine application treatments had somewhat similar net NO_3^- concentrations in the 0-25 mm soil depth, there were clear differences in the amount of NO_3^- in the soil when extrapolated to their respective patch sizes (Fig 3.9 b). At 14 days after urine application, the 1 m^2 urine patch peaked at 2,937 (± 1118) mg NO_3^--N $patch^{-1}$, compared to only 515 (± 85) mg NO_3^--N $patch^{-1}$ in the 0.5 m^2 urine patch and 582 (± 47) mg NO_3^--N $patch^{-1}$ in the 0.25 m^2 urine patch. By Day 20, the amount of NO_3^- in the soil for the 1 m^2, 0.5 m^2 and 0.25 m^2 urine patches were 1,058 (± 43) mg NO_3^--N $patch^{-1}$, 455 (± 82) mg NO_3^--N $patch^{-1}$ and 274 (± 32) mg NO_3^--N $patch^{-1}$ (Fig 3.9 b), respectively.

In the 25-50 mm soil depth, there was an increase in the net NO_3^- concentrations until Day 14 days after urine application where concentrations peaked at 24 (± 3), 40 (± 3) and 93 (± 13) mg NO_3^--N kg $soil^{-1}$ for the 2.5, 5 and 10 mm urine treatments, respectively (Fig 3.9 c). After the peak, the net NO_3^- concentrations for all urine treatments decreased over the subsequent week. When the net NO_3^- concentrations at Day 14 were extrapolated to the urine patch areas they represented, then the quantities of net NO_3^- per patch were similar for the three treatments, being 697 (± 96), 599 (± 51) and 721 (± 86) mg NO_3^--N $patch^{-1}$ for the 0.25, 0.5 and 1 m^2 urine patches, respectively. Between Days 14 and 20, there was a decrease in NO_3^--N $patch^{-1}$ for all the urine patches but concentrations remained high. At Day 20, the net NO_3^- concentrations per patch were 214 (± 13), 137 (± 9) and 504 (± 88) mg NO_3^--N $patch^{-1}$ for the 0.25, 0.5 and 1 m^2 urine patches, respectively (Fig 3.9 d).

At Day 20, the percentage of urine N applied (Total N) which was lost as NO_3^--N in the 0-25 mm soil depth was 2, 4 and 9% for the 0.25, 0.5 and 1m^2 urine patch areas, respectively. In the 25-50 mm soil depth, the percentage of urine N applied which was

lost as NO_3^--N was 2, 1, and 5% for the 0.25, 0.5 and 1 m^2 urine patch areas, respectively (Table 3.3).

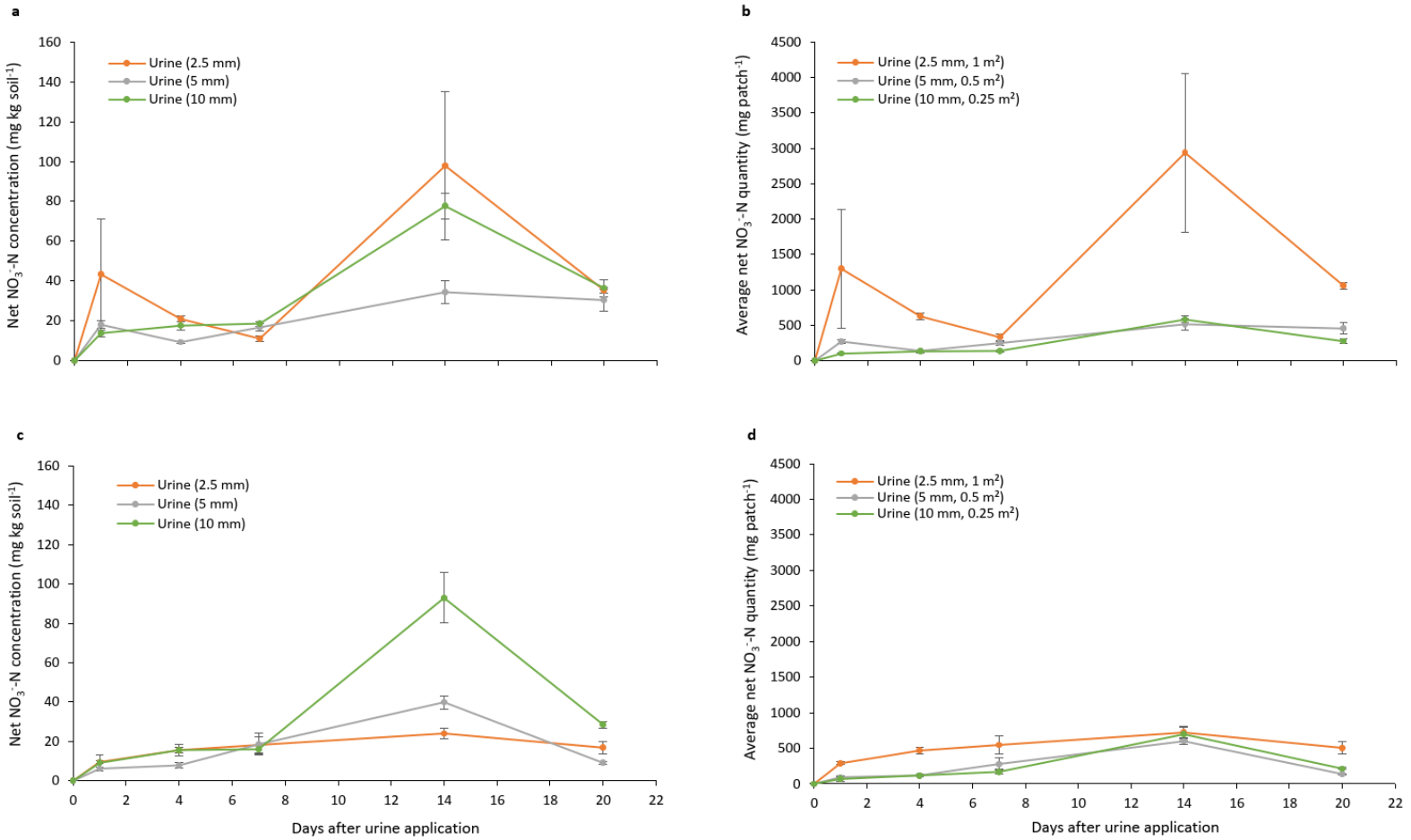


Figure 3.9: Soil net NO_3-N concentrations and the equivalent average net NO_3-N quantity per urine patch for each urine application treatment at two soil depth (0-25 mm a & b; 25-50 mm c & d). The error bars indicate standard errors of the mean (SEM).

Table 3.3: The effect of increasing the size of urine patches on extrapolated soil net NH_4^+ and NO_3^- present in the soil, as a percentage (%) of total urinary N applied.

Treatments (size of urine patches)	Day 1	Day 7	Day 14	Day 20
	Net NH_4^+-N present in the soil as a percentage (%) of total urine N applied			
		Soil depth 0-25 mm		
0.25 m²	23	18	14	8
0.5 m²	48	31	15	9
1 m²	97	57	16	8
		Soil depth 25-50 mm		
0.25 m²	15	7	2	2
0.5 m²	6	5	3	2
1 m²	30	10	0	1
	Net NO_3^--N present in the soil as a percentage (%) of total urine N applied			
		Soil depth 0-25 mm		
0.25 m²	1	1	5	2
0.5 m²	2	2	5	4
1 m²	12	3	26	9
		Soil depth 25-50 mm		
0.25 m²	1	2	6	2
0.5 m²	1	3	5	1
1 m²	3	5	6	5

3.3.5 Total nitrogen recovered from the urine patch areas

The amount of total urine N applied to each urine patch treatment was at an equivalent rate to 11330 mg N patch^{-1} (2.5 L of urine per patch with an N content of 4532 mg N L^{-1}) being applied to the patch areas that they represent. On Day 1, the apparent recovery total percentage of urine-N together as NH_3 emissions and soil (0-50 mm soil depth) net NH_4^+ and NO_3^- were 54, 72 and 161% for the 0.25, 0.5 and 1 m^2 extrapolated urine patches, respectively (Table 3.4). The 1 m^2 urine patch, which had a 2.5 mm depth of application of urine, had the highest total percentage of urine N recovery, which suggests that most of the urinary N is likely to have been retained in the surface 0-50 mm of the soil. An apparent recovery greater than 100% may be due to the addition of urine N stimulating greater N mineralisation from soil organic matter in the surface 0-25 mm of the loose surface area of these patches. This is supported by 97% of the amount of urinary

N applied as being recovered as net NH_4^+ on Day 1 (Table 3.3). In contrast, for the 0.25 m^2 urine patch (10 mm application depth of urine), it is likely that close to half of the urinary-N applied may have moved below the 0-50 mm soil depth soon after application. The percentage recoveries of the urine patch areas decreased by Day 7 but did not show further large changes between Day 7 and Day 20. By Day 20 , the total percentage recovery of urine N decreased to 39, 44 and 59% for the 0.25, 0.5 and 1 m^2 urine patches, respectively (Table 3.4).

Table 3.4: Apparent recovery of total urine-N applied per patch as NH_3 emissions and soil net NH_4^+ and NO_3^-.

Treatments (size of urine patches)	Day 1	Day 7	Day 14	Day 20
	Cumulative NH_3-N emissions as a percentage of total urine N applied per patch			
0.25 m^2	14	22	25	25
0.5 m^2	15	24	27	27
1 m^2	20	32	35	36
	Net NH_4^+-N present in the soil (0-50 mm) as a percentage (%) of total urine N applied per patch			
0.25 m^2	38	24	16	10
0.5 m^2	54	36	18	11
1 m^2	127	66	16	9
	Net NO_3^--N present in the soil (0-50 mm) as a percentage (%) of total urine N applied per patch			
0.25 m^2	2	3	11	4
0.5 m^2	3	5	10	5
1 m^2	14	8	32	14
	Combined apparent recovery* (NH_3-N emissions, net NH_4^+-N and NO_3^--N) as a percentage (%) of total urine N applied per patch			
0.25 m^2	54	49	52	39
0.5 m^2	72	65	55	44
1 m^2	161	106	83	59

*****Recovery does not include plant uptake, which is expected to be only a minor contribution for periods less than 20 days.

3.4 Discussion

The findings from this study showed that NH_3 emissions peaked 24 hours after cow urine application and exhibited a trend of increasing with increasing urine application depth.

Previous studies have also shown that NH_3 emissions from cow urine patches mostly occurred during the first couple of days after urine deposition, due to the rapid rate of the urea hydrolysis which causes an increase in soil NH_4^+ concentration coupled with an increase in soil pH (Rodriguez et al., 2019; Saggar et al., 2004b; Zaman et al., 2013b). In the current study, soil moisture conditions were relatively dry, and the soil temperature was high at the time of urine application. These soil conditions would have aided greater NH_3 emissions due to dilution of urinary N in soil water. This finding is consistent with that of Sherlock and Goh (1984) and Cameron et al. (2013). Although 5 mm of rainfall occurred within a day of the urine application, this was excluded from the NH_3 monitoring chambers, and instead simulated rainfall was added to the chambers a day after the actual rainfall event. Therefore, this rainfall event would have had minimal influence on decreasing the NH_3 emissions measured from the chambers during the first day after urine application. Zaman et al. (2013a) found that if 5 to 10 mm of rainfall was to suppress NH_3 volatilisation from a urea fertiliser application, the rainfall would need to occur very soon (<8 hr) after urea application. The influence of the rainfall on reducing NH_3 volatilisation also depends on initial soil moisture contents.

When the NH_3 emissions for each application depth treatment were extrapolated to the urine patch areas that they represented, the 2.5 mm urine application depth treatment (i.e., extrapolated to 1 m^2 urine patch) had significantly ($P<0.05$) higher NH_3 losses, compared to the other two urine treatments. The extrapolated total cumulative NH_3 emitted from applied cow urine for each of the urine patch areas (0.25 m^2, 0.5 m^2 and 1 m^2) in this study was 25, 27 and 36% of the total urine-N applied (equivalent to 11,330 mg N $patch^{-1}$), respectively. In previous studies (Adhikari et al., 2020; Laubach et al., 2013; Rodriguez et al., 2019; Zaman et al., 2013b), measured NH_3 losses from typical urine patches were in the range of 5 to 26% of the total amount of N applied in urine. These studies were carried out in autumn with mostly similar soil moisture contents to the current study. Therefore, the NH_3 emitted from the typical urine patch (0.25 m^2) in our study (25%) was at the top range of the previous studies' ranges as previously mentioned. The larger two urine patch areas (0.5 and 1 m^2) resulted in NH_3 emissions higher than this range.

The NH_3 emission factors for this autumn applied urine was higher than the New Zealand NH_3 emission factor of 10%. This emission factor represents the annual average losses,

which accounts for the emissions that will be lower at other times of the year when conditions are less conducive to NH_3 volatilisations, such as Winter and early-Spring.

The higher extrapolated NH_3 volatilisation for the largest urine patch area (1 m^2), demonstrates the influence that increasing surface area has on retaining a higher proportion of urinary N in the surface soil (0-25 mm soil depth) and, in turn, on volatilisation. The increase in surface soil (0-25 mm soil depth) pH and net NH_4^+ concentrations at 1 day after urine application were similar for the three urine patch treatments. However, because these increases occur over a larger surface area for the extrapolated 1 m^2 urine patch, then this favours greater NH_3 volatilisation (Bolan et al., 2004; Cameron et al., 2013; Selbie et al., 2015a).

The results from this study showed that the soil NH_4^+ concentrations, which peaked on Day 1, were the result of rapid hydrolysis of urea to NH_4^+ soon after urine application and declined thereafter over the first 14 days. This coincided with an increase in soil NO_3^- concentration, which will be due to the majority of nitrification occurring over this period. Soil NO_3^- increased and reached a peak at 14 days after urine application. This agrees with the established understanding that the majority of nitrification in a urine patch occurs during the first 10-14 days after urine deposition (Adhikari et al., 2020; Bolan et al., 2004; Haynes & Williams, 1993; Selbie et al., 2015a).

High NH_4^+ volatilisation from urine patches represents a potential loss of N from the soil/plant system and contributes to indirect GHG emissions. However, there are also some potential benefits from reducing the quantity of inorganic N in autumn urine patches, which includes the potential of lower risk of N being subsequently lost via leaching or N_2O emissions. In addition, increasing the spread area of a urine patch (i.e., lowering the application depth) results in a greater proportion of the urinary N being retained in the surface soil over a larger area. This can enhance the potential for greater plant uptake of urinary N and reduce the N that moves to lower soil depths. For example, by Day 7 after urine application the net inorganic N, in the 0-50 mm soil depth, for the extrapolated 1 m^2 urine patch treatment was equivalent to 74% of urinary N applied, compared to only 27% for the 0.25 m^2 treatment. This was despite the 1 m^2 urine patch treatment also having higher NH_4^+ volatilisation losses. This suggests that a higher proportion of urinary N is likely to have moved below the 0-50 mm soil depth for the 0.25 m^2 urine patch treatment, which has potential to increase the leaching risk. Ramirez

(2017) observed that increasing the urine patch area from 0.2 m^2 to 1 m^2 decreased inorganic N movement below the 0-450 mm soil depth by 64% (53 days after urine application) following a period of drainage.

3.5 Conclusions

This study has shown that increasing the urine patch area of a specific volume of urine has the potential to increase total NH_3 emissions and, consequently emission factors, in early autumn. The largest extrapolated urine patch area of 1 m^2 (represented using the smallest urine application depth) had the highest total NH_3 emissions and emission factor, compared to the smaller two extrapolated urine patch areas. This difference was attributed to the influence that urine patch area has on volatilisation. A greater proportion of urinary N is retained in the surface soil, when the same volume of urine is applied at a smaller application depth over a larger area. While this may result in higher NH_3 volatilisation, an indirect greenhouse gas emission, it also further reduces the movement of urinary N below the 0–50 mm soil depth, which potentially improves plant uptake or immobilisation by soil microbes, which has potential to reduce the risk of NO_3^- leaching and N_2O emissions during Winter and early-Spring. Thus, further research is required to assess the full impacts of increasing urine spread area on total N losses to the wider environment.

Chapter 4

Effect of increasing cow urine patch area on nitrous oxide emissions in a pasture soil

4.1 Introduction

In grazed pastures, urine patches are the main source of N_2O emissions and NO_3^- leaching due to their high concentrations of N (Ball & Ryden, 1984; Selbie et al., 2015a). Nitrous oxide is a potent GHG that can stay in the atmosphere for more than a century, making it a long-lived GHG that contributes to global warming and depletion of the stratospheric ozone layer (Ravishankara et al., 2009). In NZ, N_2O accounts for ~10.3% of NZ's total GHG emissions and 21% of the country's agricultural emissions, with approximately two-thirds of the total N_2O emissions obtained either directly or indirectly from livestock excreta (mainly urine) deposited onto the soil (Ministry for the Environment, 2023).

Grazing animals only convert 5–30% of ingested N into products (e.g., meat, milk, fibre) and the remaining 70-95% is excreted in urine and dung (Cameron et al., 2013; Oenema et al., 2005). Under a dairy cattle urine patch, the equivalent rate of N can typically range from 400–800 kg N ha^{-1} (Selbie et al., 2015a; Shepherd et al., 2011; Talbot et al., 2020), which often exceeds pasture requirements for growth, especially in winter months (Haynes & Williams, 1993). The surplus N in urine patches undergoes soil microbial transformations, leading to the environmental issues described in Chapter 3 (Cameron et al., 2013).

Although there are mitigations that have the potential to reduce N_2O emissions (Kim et al., 2012; Wu et al., 2021), there are disadvantages to them as mentioned in Chapter 3. Therefore, a device was developed by Novataro Ltd to increase the size of cow urine patches with the intention of decreasing the rate of N application in patches, increasing plant uptake of N and reducing the accumulation of soil NO_3^-. As demonstrated in Chapter 3, increasing the area of a urine patch from 0.25 m^2 to 1 m^2 in autumn increased NH_3 emissions from 25 to 36% of the total urine N applied. While the increase in NH_3

emissions has some disadvantages, including potential environmental impacts, it also results in less urinary N being left in the urine patch to convert to NO_3^-. Hence, this potentially reduces the risk of subsequent N_2O emissions and N leaching. Therefore, this chapter is focused on assessing the effect of spreading the surface area of urine patches on N_2O emissions.

The research described in this chapter was comprised of two field experiments carried out in a dairy pasture in the Manawatu Region. The main purpose of this study was to quantify the effect of increasing the urine patch area on N_2O emissions from dairy cow urine applied to a pasture soil in early winter and early autumn. The specific objectives of this study were to: (i) quantify the effect of increasing the urine patch area on N_2O emissions and EF_3 from cow urine applied in early winter and early autumn, (ii) quantify the effect of urine patch area on the movement of NH_4^+ and NO_3^- down the soil profile in early winter and early autumn, and (iii) quantify the effect of spreading cow urine on pasture dry matter (DM) accumulation and N uptake in early winter and early autumn.

4.2 Materials and Methods

4.2.1 Experimental sites

The experimental sites were in two paddocks (40°23'38.5"S 175°37'05.1"E and 40°23'45.8"S 175°36'36.4"E) on Massey University's Dairy Farm 4 near Palmerston North, Manawatu Region. Average annual (2002-2022) rainfall at these sites is 980 mm, which, on average, has a relatively even monthly distribution throughout the year. The month with the lowest average rainfall is January (56 mm) and the month with the highest average rainfall is June (104 mm). The average annual sunshine hours was 1,764. The average annual daily air temperature is 14°C, with the coldest month being July (7°C) and the warmest month being February (19°C).

Soil temperature, soil moisture, soil water filled pore space (WFPS), and rainfall data was collected during the two study periods. The first experiment (referred to as the 'Early-winter experiment') started on the 9 June 2020 and the second experiment (referred to as the 'Early-autumn') started on the 30 March 2022. The soil type at both sites is the Tokomaru silt loam soil or Argillic-fragic Perch-gley Pallic soil , 2010), which is formed

from windblown loess. The soil has been artificially drained with a mole and pipe drainage system. Soil characteristics are described in Table 4.1.

Table 4.1: Physical and chemical characteristics of the Tokomaru silt loam for the 0-100 mm depth. Adapted from Pereira et al. (2019), Palmada (2020) and author's own results.

Early-winter experiment (2020)							
Depth (mm)	Soil pH (water)	Total C (%)	Total N (%)	CEC (meq 100 g^{-1})	Total porosity (%)	Field capacity (%)	Bulk density (Mg m^{-3})
0-100	5.8	3.8	0.4	13.9	58.9	45	1.1
Early-autumn experiment (2022)							
0-100	6.0	3.8	0.4	12.1	54.7	45	1.2

The pasture consisted of predominantly a mixture of ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) with some weed species. The experimental sites were fenced off six months prior to the Early-winter experiment, and three months prior to the Early-autumn experiment. The sites remained fenced off for the duration of N_2O flux measurement, which was over a period of 94 days for the Early-winter experiment, and over a period of 132 days for the Early-autumn experiment. This was to exclude grazing cows depositing excreta on the sites.

4.2.2 The experimental design, treatments, and application

The experimental design consisted of four treatments and five replicates of each treatment, which were randomised (Table 4.2). The experiments consisted of twenty static gas chambers, where N_2O emissions were measured, and a series of rings adjacent to these chambers, which were used to collect soil samples. All treatments except for the control treatment received cow urine in the area within these rings (Fig 4.1). Soil samples collected from the plots were for NO_3^- and NH_4^+ analysis.

The treatments were no dairy cow urine (control treatment), and dairy cow urine applied to pasture at application depths of 10, 5 and 2.5 mm, which represent the depths that would result from the deposition of 2.5 L of urine to patch areas of 0.25, 0.5 and 1 m^2, respectively (Table 4.2). These treatments were allocated to plots randomly (Fig 4.1). The application depth of 10 mm was used to represent the typical urine application depth in a naturally deposited urine patch, whereas the lower application depths represent soil urine concentrations for urine patches with higher levels of urine spread.

The area inside the gas chambers is 0.5 m^2. Therefore, to achieve the 10 and 5 mm urine application depth treatments, urine volumes of 2.5 L were applied to cover areas of 0.25 and 0.5 m^2, respectively, in the gas chamber areas. For the 2.5 mm urine application depth treatment, 1.25 L of urine was applied to the area of 0.5 m^2 in the chamber (Fig 4.2, Table 4.2). The 2.5 mm application depth represented the same application depth that would be achieved by applying 2.5 L of urine to a 1 m^2 urine patch. For the soil plot areas, 2.5 L of urine was applied to 0.25 and 0.5 m^2 urine patch areas and 1.6 L of urine was applied to a confined area of 0.64 m^2 (simulating the urine application depth of a 1 m^2 urine patch area). Urine was measured using a measuring jug and uniformly applied to the chambers and soil plots using a watering can. The concentration of total N and urea in the applied urine is presented in Table 4.3.

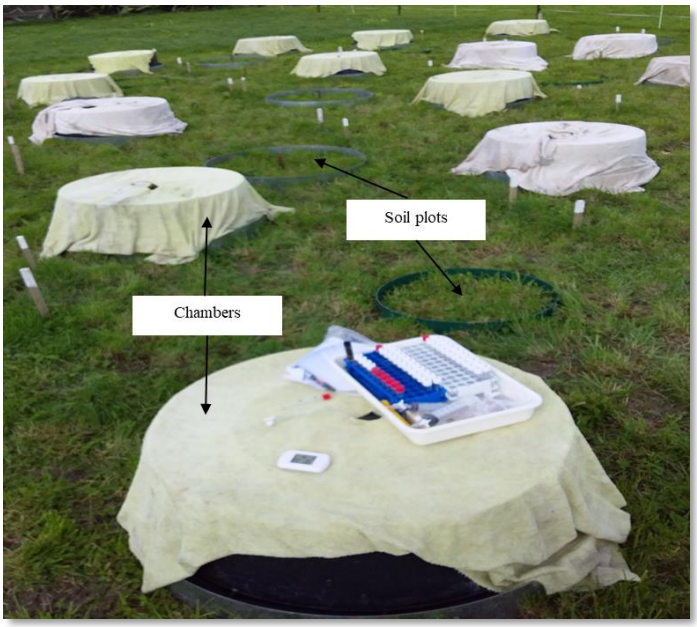


Figure 4.1: Layout of static gas chambers and soil sampling plots.

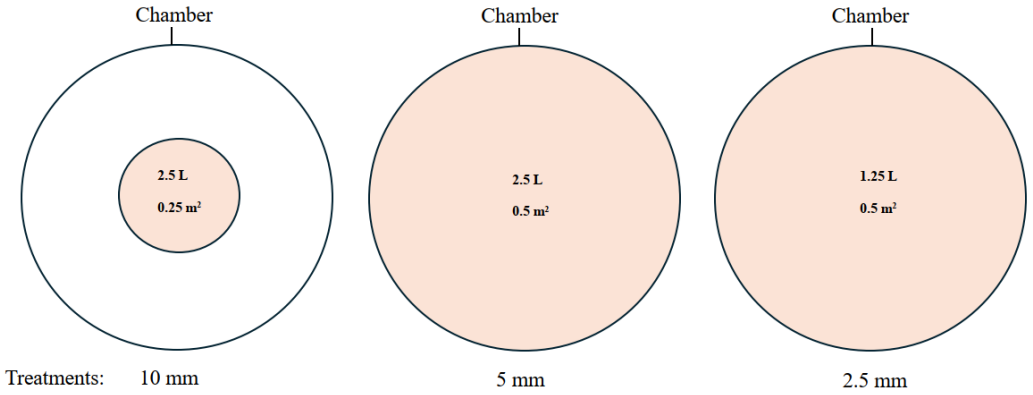


Figure 4.2: Diagram showing the spread of urine volumes onto the urine patch areas in the chambers, to achieve the urine application depth treatments (not to scale).

Table 4.2: Urine application treatments for the static gas chambers.

Urine application depth (mm) treatments	Area of chamber treated with urine (m^2)	Volume of urine applied (L area of the chamber^{-1})	Early-winter experiment: Amount of urine N applied (g N chamber^{-1})	Early-autumn experiment: Amount of urine N applied (g N chamber^{-1})
0	0.5	0	0	0
10	0.25	2.5	14.5	10.0
5	0.5	2.5	14.5	10.0
2.5	0.5*	1.25	7.25	5.0

*This area was used to simulate an application depth of 2.5 mm, resulting from applying 2.5 L of urine to an area of 1 m^2.

4.2.3 Urine collection and analysis

Urine was collected from dairy cows during milking times at Massey University Dairy Farm 4 over a period of 3 to 5 days. The urine was stored at 4°C after collection until the start of the experiment (12 days for the Early-winter experiment and 19 days for the Early-autumn experiment). Before application to the chambers on 8 June 2020 (Early-winter experiment) and on 29 March 2022 (Early-autumn experiment), all the urine was mixed. Sub-samples of the urine were collected and stored frozen at -20°C until analysed for urea using the Enzymatic Kinetic Method (Urease Kinetic UV assay). Total N in the urine samples was analysed using the Dumas method (AOAC 968.06, 2000) involving three processes: combustion, reduction and separation, and detection. These analyses were conducted in the Nutrition Laboratory, Massey University.

Table 4.3: Total N and urea-N of the cow urine used in the two experiments (Early-winter and Early-autumn).

Seasons	Urine Total N ($g\ L^{-1}$)	Urine Urea-N ($g\ L^{-1}$)	Urea as a percentage of Total N (%)	Urine Total N applied per patch (g N/patch)
Early-winter 2020	5.80	4.69	78	14.5
Early-autumn 2022	4.00	2.65	66	10.0

4.2.4 Nitrous oxide measurement

Gas sampling was conducted in the field using the static chamber method (Fig 4.4) to measure N_2O emissions. The methodology used was based on previous published studies that also measured N_2O emissions from livestock excreta (de Klein et al., 2003; Luo et al., 2019; Rodríguez Gelós, 2020; Saggar et al., 2004a; van der Weerden et al., 2017b). In this method, a chamber is placed inside a base, which is a galvanised metal ring (900 mm diameter). The ring was inserted 50-100 mm into the soil, one week before the experiments began and remained in the field until the end of each experiment. Each chamber had a 800 mm internal diameter (0.5 m^2), a height of 300 mm and a volume of 151 L. Bicycle tyre inner tubes were fitted around the perimeter of the base of each chamber to establish a seal between the chamber and metal ring (Fig 4.3).

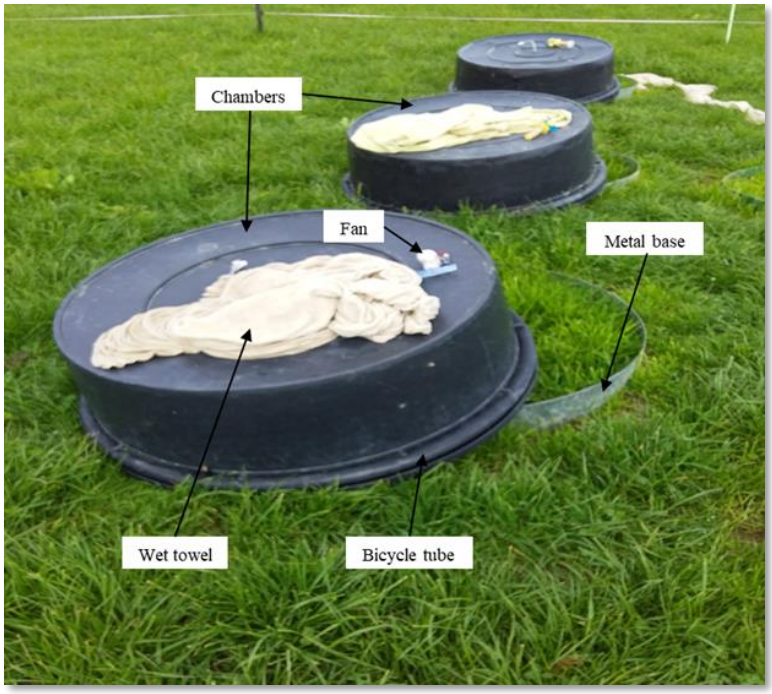


Figure 4.3: The chambers lined up beside the metal bases.

On each sampling day, before samples were collected, a small fan was placed inside the top of each chamber (to circulate air in the chamber) and the chambers were placed into the galvanised metal rings and the bicycle tyre inner tubes were inflated to provide a gas-tight seal (Fig 4.3).

Figure 4.4: Trial treatment plots showing the chambers used for N_2O collection and the set up used for N_2O gas sampling.

The chambers were covered with wet towels to help minmise temperature increases inside the chambers during sampling (Fig 4.4). The schedule for gas sampling conformed to those recommended in the guidelines for N_2O chamber methodology (Harvey et al., 2020). Gas samples were taken from the chamber headspace immediately (t_0), 60 minutes (t_{60}) and 120 minutes (t_{120}) after covering. On each sampling day, three background air samples were also taken at t_0, t_{60} and t_{120}.

A plastic syringe (60 mL volume) was used to collect gas samples from each chamber. This involved inserting the syringe into the sampling port on the chamber, drawing chamber air, closing the chamber and syringe taps and then removing the syringe (Fig 4.5). Another syringe (35 mL volume) with a needle (plunger fully pushed in) was connected to the 60 mL syringe then their taps were opened and the chamber air was exchanged between the two syringes in order to mix the chamber air. Then 25 mL of chamber air was transferred from the 60 mL syringe into the 35 mL syringe. The syringe contents were compressed into evacuated septum-sealed screw-capped glass vial: the vial was then removed (with 12 mL of compressed chamber air) and the remaining chamber air in the syringe was discarded. Gas sampling was carried out between 10:00 am and 12:00 noon on each sampling day, a time when N_2O daily flux can be extrapolated without bias (van der Weerden et al., 2013).

Figure 4.5: Collecting gas samples from the chamber and mixing the chamber air in the syringes.

For the Early-winter experiment, gas sampling was carried out twice a week for the first six weeks and then once a week. For the Early-autumn experiment sampling was carried out twice a week for the first four weeks, once to twice weekly for the next ten weeks and once every two weeks until the N_2O flux reached background levels. The differences in the sampling times for the Early-autumn experiment was due to longer periods of no rainfall, which meant that there was likely to be less N_2O emissions and so longer durations between sampling times. The N_2O concentrations were determined using gas chromatography (Shimadzu Nexis GC-2030 Gas Chromatograph). These gas samples were analysed by the Landcare Research Laboratory, Palmerston North.

Nitrous oxide emission rates were calculated from the increase in N_2O concentration (Hutchinson & Mosier, 1981). Daily N_2O-N fluxes were calculated using Eqn. 4.1. It is assumed that the calculated hourly flux represented the average hourly flux for that day (de Klein et al., 2003; Luo et al., 2019; van der Weerden et al., 2020) using the following equation:

$$N_2O\text{-N flux (mg N m}^{-2}\text{ h}^{-1}) = \frac{\delta N_2O}{\delta T} \times \frac{M}{Vm} \times H \qquad \text{(Eqn. 4.1)}$$

where δN_2O is the increase in head space N_2O during the enclosure period ($\mu L\ L^{-1}$); δT is the enclosure period (hours); M is the molar weight of N in N_2O ($g\ mol^{-1}$); Vm is the molar volume of gas at the sampling temperature ($L\ mol^{-1}$); H is the height of headspace (m).The total N_2O emissions were calculated by integrating the daily emission fluxes for each chamber over the measurement period (de Klein et al., 2003). The emission factor (EF_3), or the proportion of N emitted as N_2O-N from urine applied, was calculated using Equation 4.2 (Luo et al., 2019):

$$EF_3\ (\%) = \frac{N_2O\text{-N total (urine)} - N_2O\text{-N total (control)}}{\text{Urine-N applied}} \times 100 \qquad \text{(Eqn. 4.2)}$$

where EF_3 (%) is the emission factor, 'N_2O-N total (urine)' is the cumulative total N_2O-N emitted (mg N_2O-N $chamber^{-1}$) from a urine treatment, 'N_2O-N total (control)' is the cumulative total N_2O emitted (mg N_2O-N $chamber^{-1}$) from the comparative no-urine treatment, and 'Urine-N applied' is the amount of N added as urine (mg N $chamber^{-1}$).

In this study, the daily N_2O fluxes were presented as g N_2O-N day^{-1} kg N $applied^{-1}$ and the total N_2O emissions were presented as g N_2O-N kg N $applied^{-1}$ due to the different urine patch sizes.

4.2.5 Soil sampling

For the Early-winter experiment, an initial soil sampling was conducted before the application of urine treatments on 8 June 2020 (Day 0) followed by sampling on Day 1 (a day after urine application), Day 7, Day 14, Day 31, Day 66, and Day 94. For the Early-autumn experiment, an initial soil sampling was conducted on 28 March 2022 (a day before urine application), and then on Day 1 (a day after urine application), Day 7, Day 14, Day 43, Day 78, Day 118 and Day 135. The soil samples were collected within rings which were placed adjacent to the N_2O collection chambers. These rings received the same urine application depth (treatment) applied to the adjacent chambers. For both experiments, the soil sampling area consisted of a total of twenty rings, which provided five replicates of each of the four treatments located near the gas sampling chambers (Fig 4.6). Soil cores (3 cores per soil sampling ring) were collected from the twenty rings, using a soil corer, at depths of 0–50 and 50–100 mm for the early winter experiment, and at depths of 0–50, 50–100, 100–200 and 200–300 mm for the early autumn experiment. The deeper cores (100–200 and 200–300 mm) were only collected on three sampling days (Day 14, 78 and 135). The samples were placed in a plastic bag and taken back to the lab and refrigerated at 4°C until they were analysed. The soil cores were used to measure extractable NO_3^- and NH_4^+, soil moisture and pH.

Figure 4.6: The chamber and the soil plot.

4.2.6 Extractable soil nitrate and ammonium

Soil samples were thoroughly mixed prior to analysis for NO_3^- and NH_4^+. Soil NO_3^- and NH_4^+ analyses involved weighing a 5 g sample of moist field soil into a 50 mL centrifuge tube and adding 30 mL of 2 M KCl. The samples were shaken in an end-over-end shaker for 1 hour. They were removed from the shaker, centrifuged for 5 minutes at 5,000 rpm, and then filtered into 30 mL containers using Whatman 41 filter papers. The collected filtrates were stored at 4˚C until they were analysed using the Technicon II Auto Analyzer (Blakemore et al., 1987).

After analysis, the NO_3^- and NH_4^+ concentrations were used to calculate the net NO_3^--N and NH_4^+-N (mg kg soil^{-1}) concentration using equation 4.3, and the quantities of NO_3^--N and NH_4^+-N per urine patch using equation 4.4. Equation 4.5 was used to calculate the soil mass (SM). This estimated the soil mass after correcting the actual area receiving urine for the representative urine patch area (UPA) for each application depth.

Net Mineral N (NO_3^--N and NH_4^+-N mg kg soil^{-1}) = C – c (Eqn. 4.3)

Mineral N (NO_3^--N and NH_4^+-N mg patch^{-1}) = (C – c) x SM (Eqn. 4.4)

where C = concentration of NO_3^-–N or NH_4^+-N (mg kg soil^{-1}) for treatments with cow urine; c = concentration of NO_3^-–N or NH_4^+-N in the control treatment (mg kg soil^{-1}); SM = soil mass (kg).

SM = AAS x DS x BD x (UPA/AAS) (Eqn. 4.5)

where AAS = actual area of sampling site where urine was applied (m^2); DS = depth of sampling (m); BD = bulk density of soil (kg m^{-3}); UPA = urine patch area (m^2).

4.2.7 Soil moisture

Volumetric soil moisture content was measured daily for the monitoring period at a soil depth of 0–200 mm for the Early-winter experiment and the Early-autumn experiment. The daily average moisture content values were then calculated and recorded for the duration of the experiments. These data were later used to determine a relationship between soil moisture content and N_2O emissions as described in section 4.3.2.1.

Water-filled pore space (WFPS) was calculated as a ratio of the volumetric soil water content (SWC) to the total pore space (Saggar et al., 2004b). The equation below (Eqn. 4.6) was used to calculate the total pore space:

$$\text{Total pore space (\%)} = 100[1 - (bulk\ density/particle\ density)] \quad \text{(Eqn. 4. 6)}$$

The soil bulk density was determined from undisturbed soil cores taken from the experimental sites with pasture growing on them. The particle density was assumed to be 2.65 Mg m^{-3}. Total porosity (TP) was calculated for each soil depth using Eqn. 4.6, which is shown in Table 4.4.

Table 4.4: Soil bulk density and total porosity.

Experiments	Soil Depth (mm)	Bulk density (Mg m^{-3})	Total pore space (%)
Early-winter 2020	0-100	1.09	58.9
Early-autumn 2022	0-200	1.20	54.7

4.2.8 Pasture yield and plant N uptake

Pasture samples were collected from each gas chamber at a height of approximately 45 mm above the soil surface, using Ryobi One+ 18V cordless grass shears. For the Early-winter experiment, there were three harvests over a period of up to 14 weeks after urine application, whereas for the Early-autumn experiment three harvests were made over a period of up to 19 weeks after urine application. All pasture harvested from each chamber was transferred into pre-weighed paper bags and the fresh weight of the pasture samples recorded. These pasture samples were then oven-dried at 65˚C, and dry matter (DM) accumulation was determined. The pasture samples were finely ground, and 0.1 g was used for total N (TN) analysis using the Kjeldahl digestion method (McKenzie & Wallace, 1954). The recovery of applied urine N via pasture uptake was calculated using Eqn. 4.7. The effects of spreading cow urine on pasture DM accumulation and N uptake were assessed at each single harvest and for the total of all 3 harvests combined.

$$\text{N recovery (\%)} = \frac{\text{Pasture N (urine)} - \text{Pasture N (control)}}{\text{N applied (urine)}} \times 100 \quad \text{(Eqn. 4. 7)}$$

4.2.9 Climate data

For the early-winter and early-autumn experiments, daily soil temperature, rainfall and volumetric soil moisture content data for the trial period were obtained from the NIWA/AgResearch weather station, which is located approximately 3 km from the field trial sites.

4.2.10 Data and statistical analysis

The mean values and standard error of the means for daily and total N_2O emissions, NO_3^- and NH_4^+ concentrations, pasture DM and N uptake were calculated based on the five replicates for each treatment using Microsoft Excel for Microsoft 365. Least significant differences and P values were calculated using one-way analysis of variance (ANOVA) to detect any significant difference and different treatment means were compared using Tukey Pairwise Comparisons in Minitab 19.

4.3 Results

4.3.1 Climate

In 2020 and 2022, when the two N_2O experiments were conducted, the annual rainfall at the study sites were 826 and 1,136 mm, respectively. In comparison, the long-term (2002-2022) average annual rainfall for the study sites was 980 mm (Appendix A). The two study years especially had contrasting winter (June-August) rainfall, being 169 and 460 mm in 2020 and 2022, respectively. Compared to the long-term (2002-2022) average winter rainfall of 281mm, the winter rainfall was 40% lower in 2020, making it a relatively dry winter. Whereas the winter rainfall in 2022 was 64% higher than the long-term average, therefore was a comparatively wet winter.

During the 14-week monitoring period of the Early-winter grazing experiment (9th June to 10th September 2020), the total rainfall was 165 mm. The highest weekly rainfall was recorded in the fifth week of the trial (29 mm) and the lowest weekly rainfall was in the eighth week (0 mm). From the eleventh week to the fourteenth week, the weekly rainfall ranged from 7 to 19 mm (Fig 4.7). As expected, the soil moisture content (SMC) showed

peaks after rainfall events. Throughout the 14-week period after urine application, the soil moisture content was relatively uniform, ranging from 38 to 44% (WFPS of 64-75%) in the 0-200 mm soil depth. This indicates that the soil moisture content was close to, or at, field capacity for most of the experimental period (Table 4.1).

During the 19-week monitoring period of the Early-autumn grazing experiment (30th March to 11th August 2022), the total rainfall was 508 mm. The highest weekly rainfall was recorded in the eleventh week of the experiment (107 mm) and the lowest weekly rainfall was in the fourteenth week (5 mm). From the fifteenth week to the nineteenth week (the last week of the experiment), the weekly rainfall ranged from 15 to 69 mm (Fig 4.8). During the first 40 days after urine application, the soil moisture content was relatively dry, ranging from 22-30% (WFPS of 40-56%). From Day 41 to Day 49, the soil moisture content increased from 22 to 40% and then stayed above 40% (WFPS of 72%) for much of the remainder of the experiment. Therefore, the soil moisture content was only close to, or at, field capacity, after the first 40 days of the experimental period.

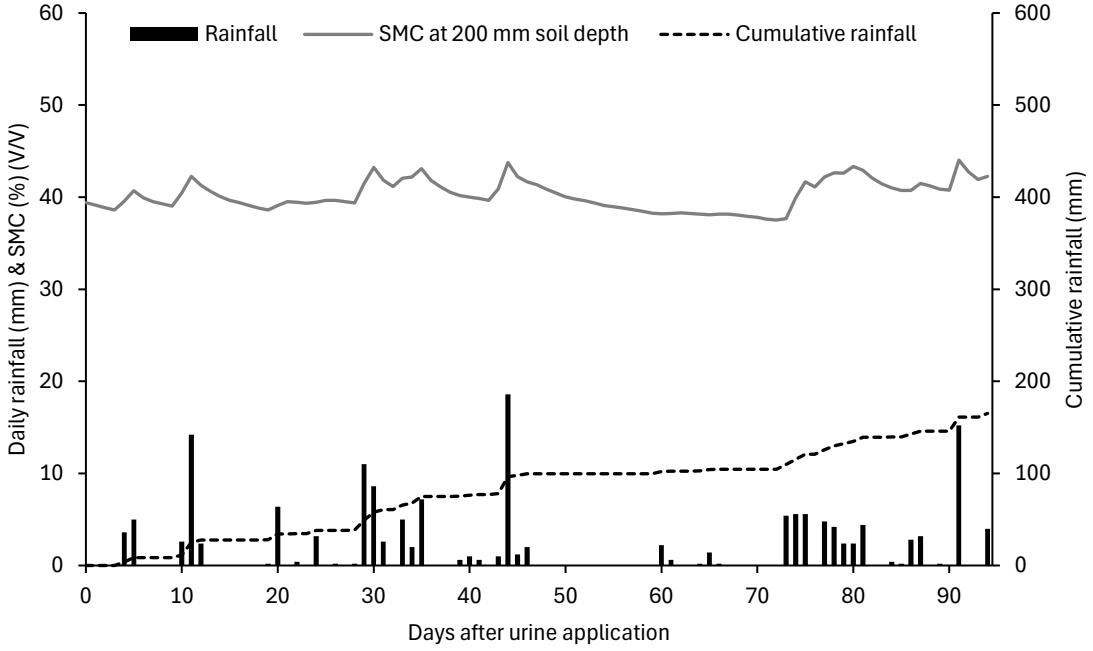


Figure 4.7: Daily rainfall, cumulative rainfall and volumetric soil moisture content following urine application on 8 June 2020 (Early-winter experiment).

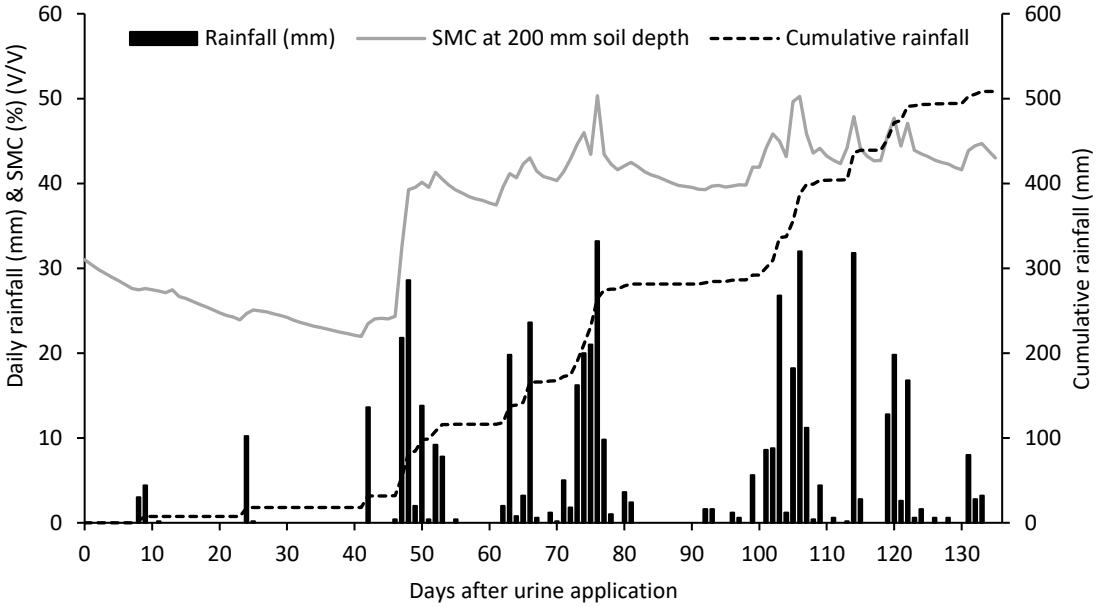


Figure 4.8: Daily rainfall, cumulative rainfall and volumetric soil moisture content following urine application on 29 March 2022 (Early-autumn experiment).

In the Early-winter experiment, the soil temperature ranged from 8-12.7°C (Fig 4.9). In comparison, the soil temperature range was wider for the Early-autumn experiment, being 4.9-18.3°C (Fig 4.10).

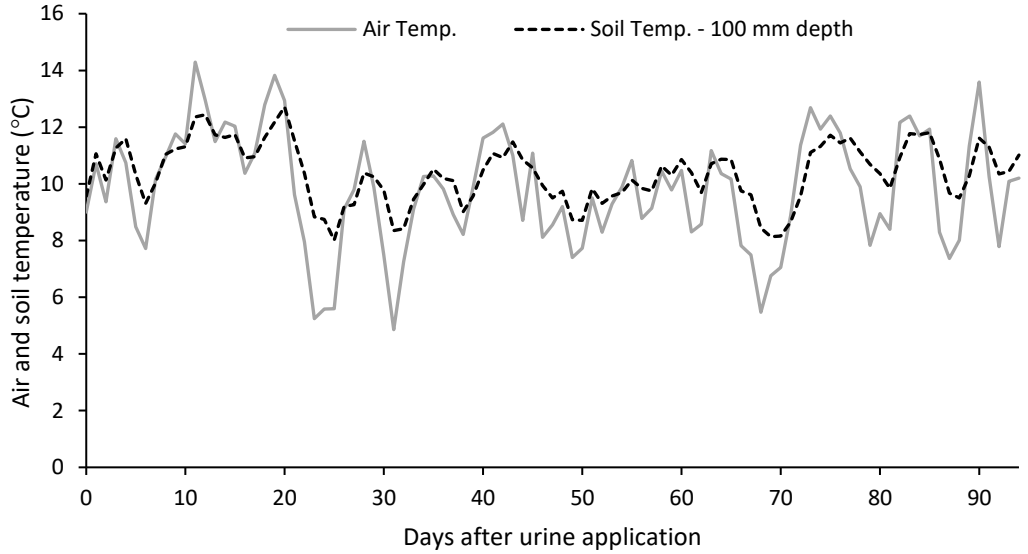


Figure 4.9: Air and soil temperatures at 100 mm soil depth (Early-winter experiment).

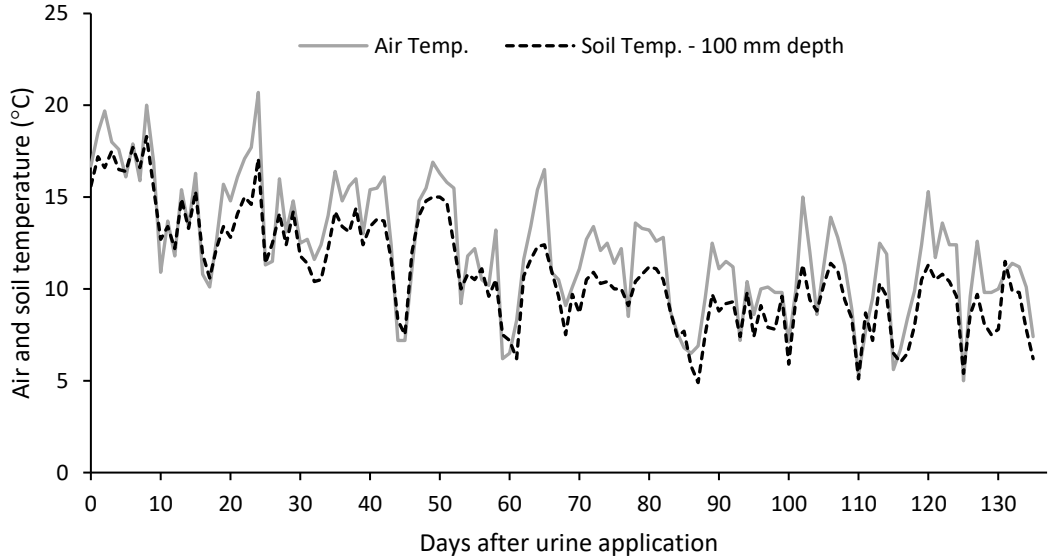


Figure 4.10: Air and soil temperatures at 100 mm soil depth (Early-autumn experiment).

4.3.2 Nitrous oxide emissions

4.3.2.1 Daily Nitrous oxide emissions

Urine application increased daily N_2O emissions for all the urine treatments, during both the Early-winter and Early-autumn experiments (Fig 4.11). The daily N_2O fluxes showed temporal variations and varied responses to rainfall events in both experiments after the background emissions (control no-urine values) were subtracted. During the Early-winter experiment, N_2O flux was 0.55, 0.50 and 0.80 g N_2O-N day^{-1} kg N $applied^{-1}$ on Day 1 for the 0.25, 0.5 and 1 m^2 (extrapolated, hereafter referred to as 1 m^2) urine patch area treatments, respectively. On Day 4, the N_2O flux showed a small further increase to 0.53 g N_2O-N day^{-1} kg N $applied^{-1}$, for the 0.5 m^2 urine patch area, whilst the fluxes declined to 0.40 and 0.53 g N_2O-N day^{-1} kg N $applied^{-1}$ for the 0.25 and 1 m^2 urine patch area treatments, respectively. After Day 4, emissions declined in all urine patch treatments. A second peak in daily N_2O fluxes was observed on Day 11 with 0.39, 0.61 and 0.81 g N_2O-N day^{-1} kg N $applied^{-1}$ for the 0.25, 0.5 and 1 m^2 urine patch areas, respectively. This increase in N_2O-flux values can be attributed to rainfall on Days 9, 10 and 11, which increased the soil moisture content from 39% to 42% in the 200 mm soil depth (Fig 4.6). After the peak on Day 11, the N_2O flux for all the urine patch treatments gradually declined, with a series of smaller peaks on Day 21, Day 28, Day 35, and Day 45. These smaller peaks were also the result of rainfall events increasing the soil moisture content from 39-44%.

After the N_2O fluxes peaked on Day 45, they gradually declined close to background levels, with only a very minor peak on Day 80, even though regular rainfall maintained moisture content close to, or at, field capacity (Fig 4.11 a & Fig 4.7). This indicated that by this time a diminished urinary N supply was likely to be limiting further substantive increases in N_2O emissions.

During the Early-autumn experiment (Fig 4.11 b), N_2O fluxes peaked at 0.36, 0.27 and 0.21 g N_2O-N day^{-1} kg N $applied^{-1}$ on Day 1 for the 0.25, 0.5 and 1 m^2 urine patch area treatments, respectively. The fluxes declined subsequently to low levels until Day 24, when there were small peaks in the N_2O flux of 0.10, 0.04 and 0.07 g N_2O-N day^{-1} kg N $applied^{-1}$, for the 0.25, 0.5 and 1 m^2 urine patch areas, respectively. This was likely due to the rainfall event of 10.2 mm on Day 24 (Fig 4.8). Subsequent smaller peaks in the

N_2O flux were attributed to the rainfall events after Day 42, which resulted in an increase in soil moisture content (Fig 4.8). It is interesting to note that on Day 70, there was a higher peak in the N_2O flux (0.24 g N_2O-N day^{-1} kg N $applied^{-1}$) for the 0.25 m^2 treatment, compared to the 0.5 and 1 m^2 treatments (0.05 and 0.02 g N_2O-N day^{-1} kg N $applied^{-1}$, respectively), which coincided with high soil moisture contents which ranged from 40 to 41% leading up to this day. Smaller peaks in N_2O fluxes followed and remained close to background levels after Day 94, even though soil moisture content remained high (Fig 4.11 b & Fig 4.8). This indicated that the availability of urinary N supply in the soil was likely to have become a limiting factor at this stage.

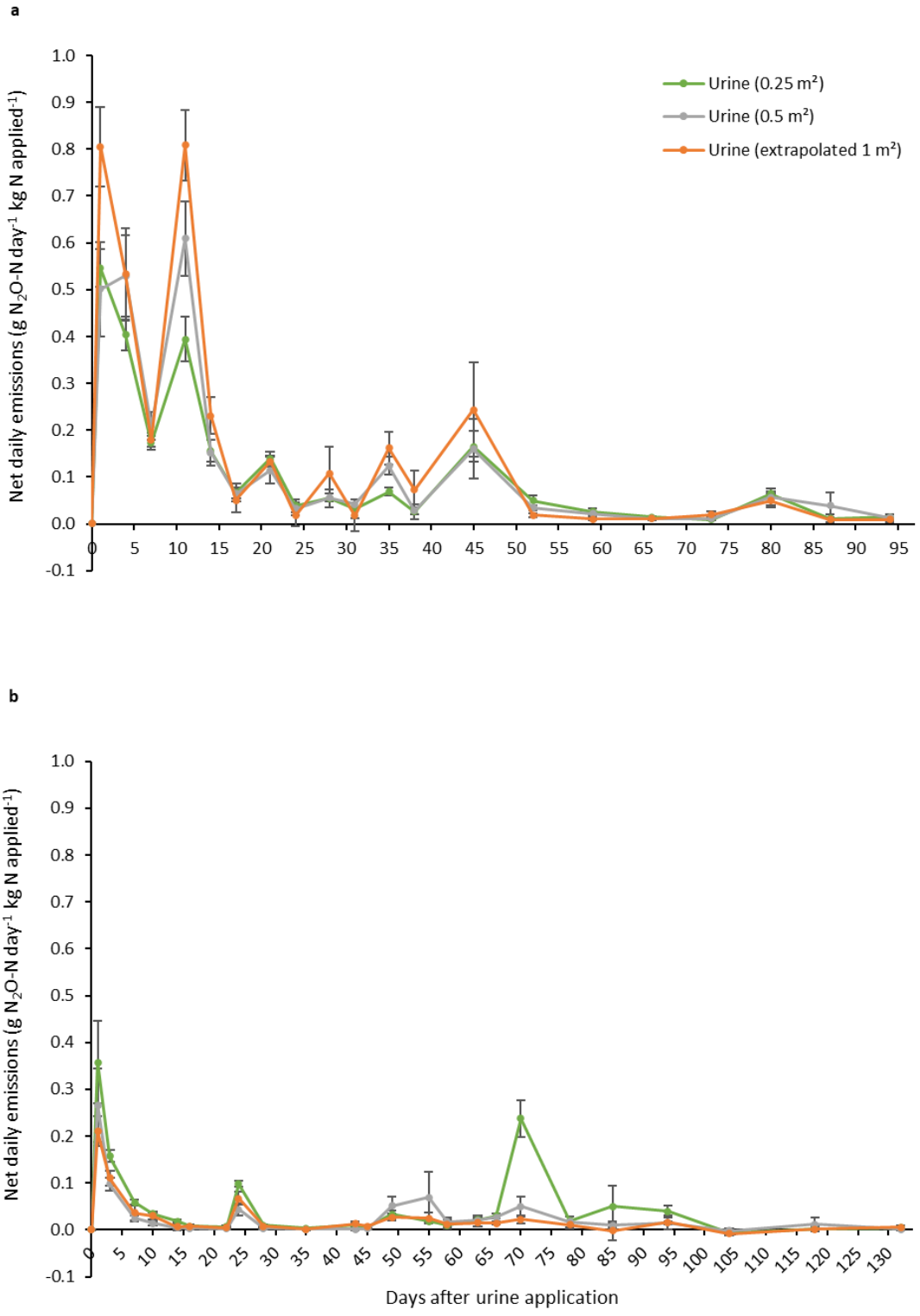


Figure 4.11: Net daily N_2O emissions (a) Early-winter experiment and (b) Early-autumn experiment. The error bars indicate standard errors of the mean (SEM).

4.3.2.2 Total N_2O emissions

For the Early-winter experiment, most of the increases in net cumulative N_2O emissions occurred in the first 52 days after urine application, with the highest rate of losses occurring during the first 14 days (Fig 4.12 a). Most of the differences between the urine patch size treatments also occurred in the first 52 days. Over the whole experimental period, the total net cumulative N_2O emissions for the 0.25, 0.5 and 1 m^2 (extrapolated) urine patch treatments were estimated to be 9.26, 10.41 and 12.84 g N_2O-N kg N $applied^{-1}$, respectively (Table 4.5). While there was a trend of N_2O emissions increasing with an increase in urine patch size, the differences between the treatments were not large enough to be of statistical significance ($P>0.05$). However, for first 14 days of total N_2O emissions, the 1 m^2 urine patch treatment was statistically different ($P<0.05$) from the 0.25 m^2 urine patch treatment.

For the Early-autumn experiment, like with the Early-winter experiment, there was an initial decrease then increase in net cumulative N_2O emissions over the first 14 days (Fig 4.12 b). Following this, the net cumulative N_2O emissions for the two larger urine patch treatments showed no increases over the remainder of the experiment. The smallest (0.25 m^2) urine patch treatment also showed no increase in emissions up to 66 days after urine application but then exhibited an increase in the rate of emissions between Days 66 and 78, before returning to a more gradual rate of emissions. The total net cumulative N_2O emissions over the experimental period for the 0.25, 0.5 and 1 m^2 urine patch treatments were 5.04, 2.91 and 2.21 g N_2O-N kg N $applied^{-1}$, respectively (Table 4.5). In contrast to the Early-winter experiment, the total net cumulative N_2O emissions showed a trend of decreasing with increasing urine patch area. The N_2O emissions for the 1 m^2 urine patch treatment was only significantly different ($P<0.05$) from the 0.25 m^2 urine patch treatment, with losses that were 56% lower compared to the more 'typical' urine patch area.

In Figure 4.12, there is a clear trend of higher net cumulative N_2O emissions for all the urine patch treatments during the Early-winter compared to the Early-autumn. In addition, during the Early-winter experiment, the net cumulative N_2O emissions increased when the urine patch areas were increased. In contrast, during the Early-autumn experiment the net cumulative N_2O emissions decreased with increasing urine patch area.

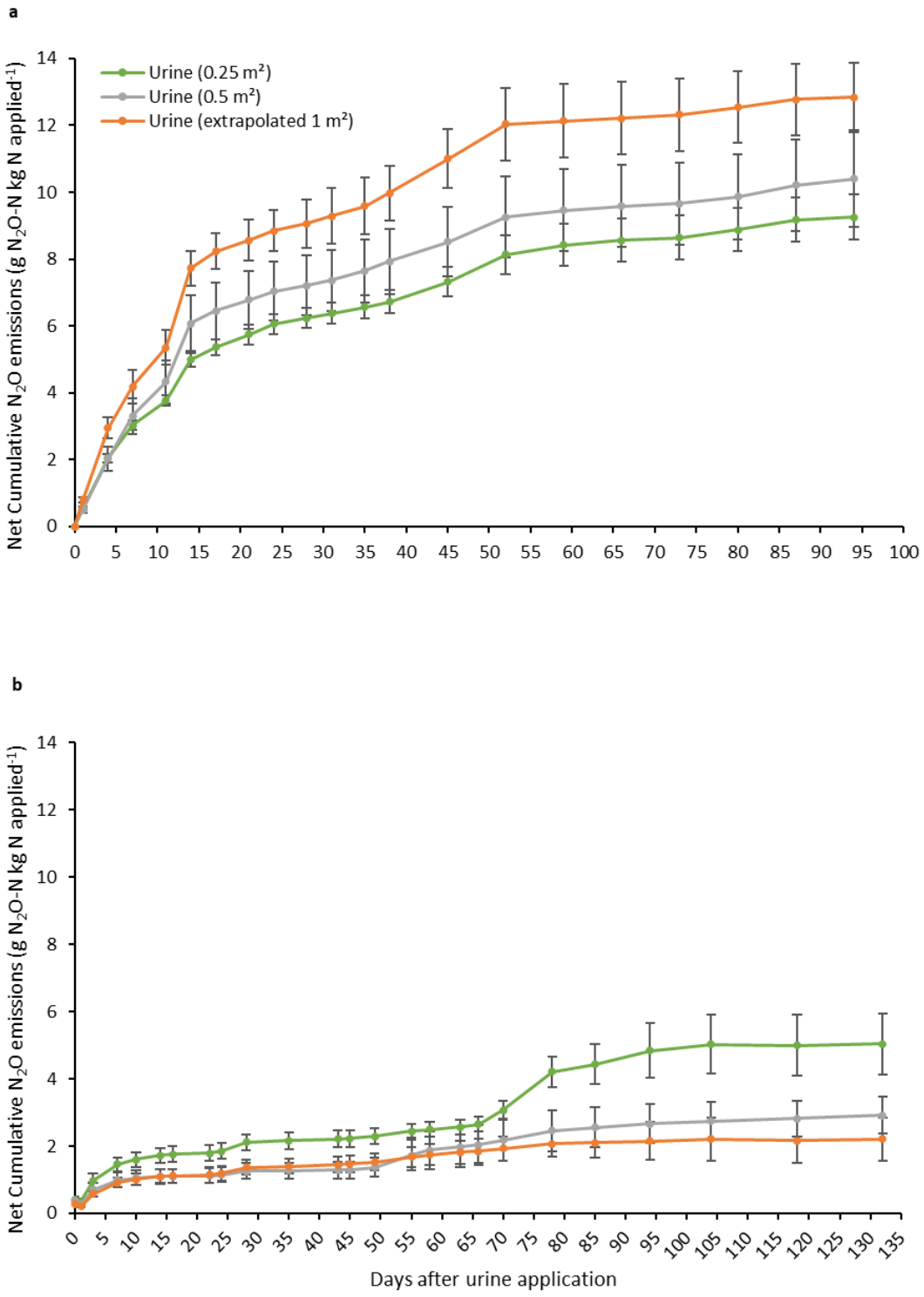


Figure 4.12: Net cumulative N_2O emitted for the three urine patch areas during the (a) Early-winter experiment and the (b) Early-autumn experiment. The error bars indicate standard errors of the mean (SEM).

Nitrous oxide emission factors (EF_3) for the Early-winter experiment are presented in Table 4.5 for each of the urine patch treatments. The EF_3 values for the urine patch areas 0.25, 0.5 and 1 m^2 were 0.93, 1.04 and 1.28%, respectively. For the Early-autumn

experiment, the EF_3 values for the urine patch areas 0.25, 0.5 and 1 m^2 were 0.50, 0.29 and 0.22%, respectively. While the EF_3 values for the three treatments during the Early-winter experiment were close to or above a value of 1%, for the Early-autumn experiment, the EF_3 values were all below 1%.

Table 4.5: Emission factors from each of the urine patches (Early-winter and Early-autumn experiments).

Experiments	Urine patch area (m^2)	Net N_2O-N emissions (g N kg N^{-1})	EF_3 (%)
Early-winter	0.25	9.26[a] (± 0.68)	0.93
	0.5	10.41[a] (± 1.45)	1.04
	1	12.84[a] (± 1.04)	1.28
Early-autumn	0.25	5.04[a] (± 0.92)	0.50
	0.5	2.91[ab] (± 0.55)	0.29
	1	2.21[b] (± 0.65)	0.22

Note: values with the same superscript letters indicate that the treatments are not significantly different.

4.3.3 Soil inorganic N

4.3.3.1 Soil ammonium

Early-winter experiment

In the 0-50 mm soil depth, net NH_4^+-N concentration for the 10, 5 and 2.5 mm urine application depth treatments peaked at 436, 318 and 266 mg N kg $soil^{-1}$, respectively, on Day 1 and decreased thereafter to background levels by Day 31 (Fig 4.13 a). These peak concentrations equate to 5,337, 7,795 and 13,011 mg NH_4^+-N $patch^{-1}$ when extrapolated to the urine patch areas of 0.25, 0.5 and 1 m^2, respectively (Fig 4.13 b). The highest (10 mm) urine application treatment had the highest NH_4^+-N concentration in the surface soil (mg N kg $soil^{-1}$), however, when extrapolated to the urine patch areas they represent, the smallest application depth (2.5 mm) had a higher proportion of urinary N retained in the surface soil but spread over a larger area (extrapolated to 1 m^2).

In the 50-100 mm soil depth, net NH_4^+-N concentration for the 10, 5 and 2.5 mm urine treatments peaked at 195, 75 and 52 mg N kg $soil^{-1}$, respectively, on Day 1 after urine

application (Fig 4.13 c). These peak net NH_4^+-N concentrations equate to 2,655, 2,056 and 2,825 mg N patch^{-1} when extrapolated for the urine patch areas of 0.25, 0.5 and 1 m^2, respectively, and decreased thereafter to background levels (Fig 4.13 d).

In the 0-100 mm soil depth, the combined NH_4^+-N in the 0.25 and 1 m^2 urine patch areas were 7,992 and 15,836 mg N patch^{-1}, respectively, on Day 1. Therefore, in the 0.25 and 1 m^2 urine patch areas, the equivalent of 55 and 109 %, respectively, of total urine N applied was measured in the 0-100 mm soil depth approximately 24 hours after urine application. The higher proportion of urinary N recovered in the surface soil for the larger urine patch area could contribute to it having a higher potential for NH_3 volatilisation.

Early-autumn experiment

In the 0–50 mm soil depth, net NH_4^+-N concentrations peaked at 201, 107 and 117 mg kg soil^{-1} for the 10, 5 and 2.5 mm urine application treatments, respectively, on Day 1 and gradually decreased thereafter to be close to or at background levels by Day 14. These peak concentrations equate to 2,467, 2,629 and 5,709 mg N patch^{-1} when extrapolated to urine patch areas of 0.25, 0.5 and 1 m^2, respectively (Fig 4.14 a & b). As for the Early-winter experiment, the highest (10 mm) urine application depth treatment had the highest NH_4^+-N concentration in the surface soil, however, when extrapolated to the urine patch areas they represent, the smallest application depth (2.5 mm, extrapolated to 1 m^2) had a higher proportion of urinary N retained in the surface soil.

In the 50-100 mm soil depth, net NH_4^+-N concentrations also peaked on Day 1 after urine application. At this soil depth, the net NH_4^+-N concentrations were lower and more similar between treatments, compared to the 0-50 mm soil depth, being 63, 47, 59 mg kg soil^{-1} for the 10, 5 and 2.5 mm urine application depth treatments, respectively (Fig 4.14 c). These peak net NH_4^+-N concentrations equate to 865, 1,274 and 3,236 mg N patch^{-1} when extrapolated for urine patch areas 0.25, 0.5 and 1 m^2, respectively (Fig 4.14 d).

In the 0-100 mm soil depth, the combined NH_4^+-N in the 0.25 and 1 m^2 urine patch areas were 3,332 and 8,945 mg N patch^{-1}, respectively, on Day 1. Therefore, in the 0.25 and 1 m^2 urine patch areas, 33 and 89%, respectively, of total urine N applied was recovered. This showed that in the small urine patch, less urinary N was recovered compared to the large urine patch. This indicated that there is a potential for high NH_3 volatilisation from the large urine patch.

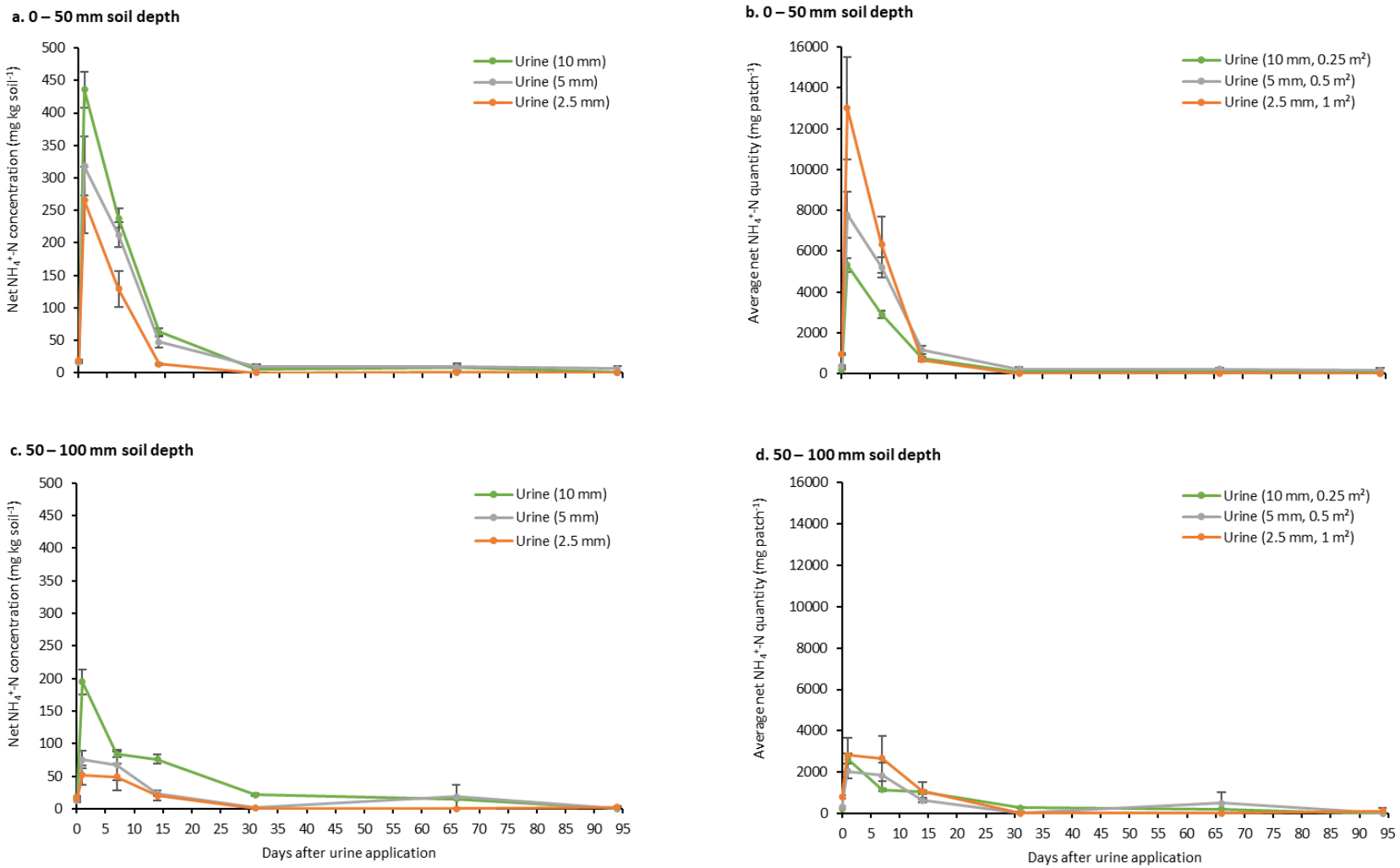


Figure 4.13: Soil net NH_4^+-N concentrations (a and c) and equivalent urine patch quantity of net NH_4^+-N (b and d) at two soil depths for each urine application depth treatment for the Early-winter experiment. The error bars indicate standard errors of the mean (SEM).

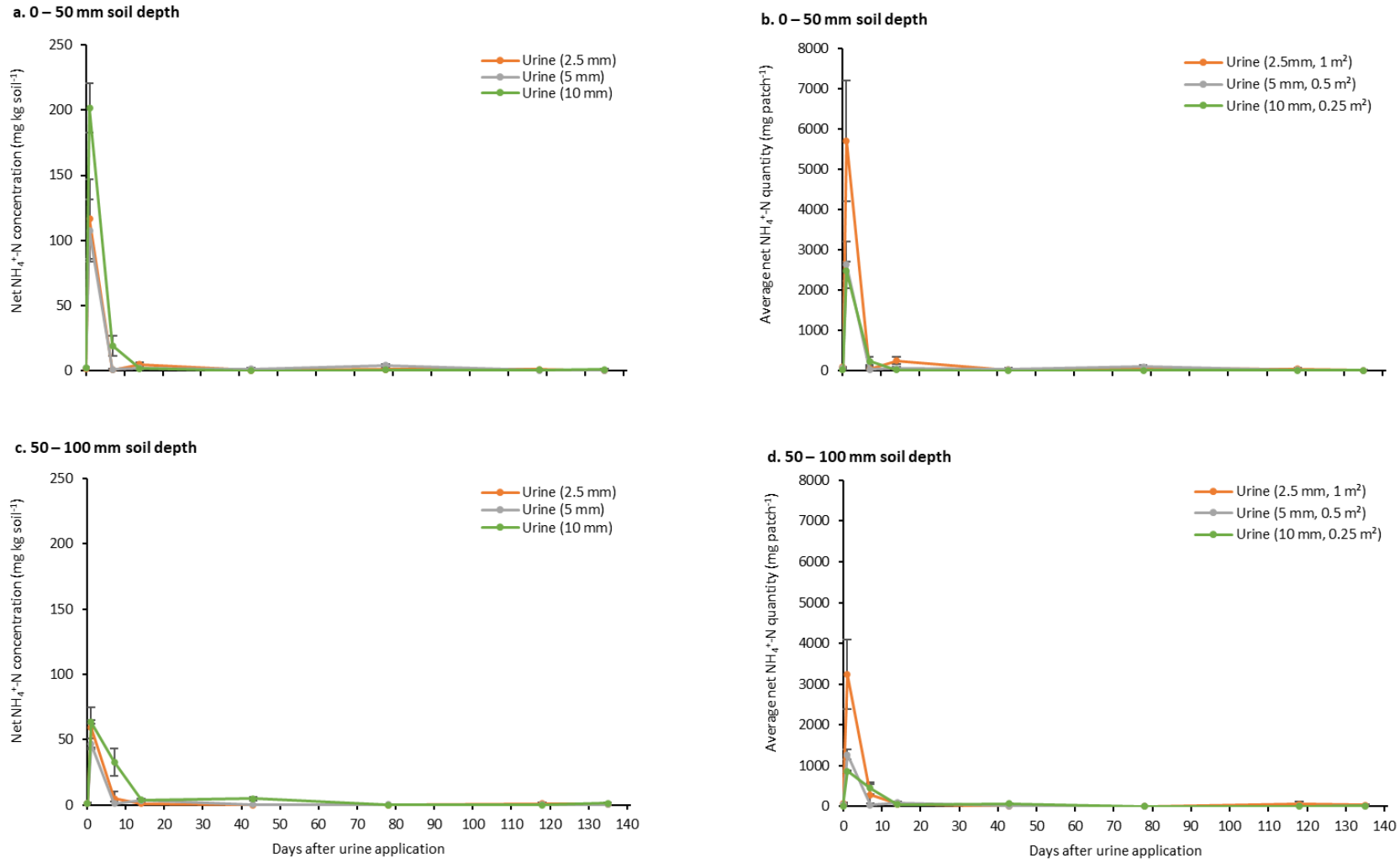


Figure 4.14: Soil net NH_4^+-N concentrations (a and c) and equivalent urine patch quantity of net NH_4^+-N (b and d) at two soil depths for each urine application depth treatment for the Early-autumn experiment. The error bars indicate standard errors of the mean (SEM).

4.3.3.2 Soil nitrate

Early-winter experiment

In the top 0-50 mm soil depth, there was a peak in the net NO_3^--N concentration on Day 14 of 163, 110, and 98 mg N kg soil^{-1} for the 10, 5 and 2.5 mm urine depth treatments, respectively (Fig 4.15 a). At this time, these net NO_3^--N concentrations were equivalent to 1,993, 2,683 and 4,785 mg N patch^{-1}, when the 10, 5 and 2.5 mm urine application depth treatment values were extrapolated to urine patch areas of 0.25 m^2, 0.5 m^2 and 1 m^2, respectively (Fig 4.15 b). After the peak on Day 14, the net and average NO_3^--N decreased thereafter, to be close to background levels by the end of the trial (Day 94).

In the 50-100 mm soil depth, net NO_3^--N concentrations also peaked on Day 14 for the urine treatments and then declined at subsequent sampling times. At Day 14 the net NO_3^--N concentrations were 124, 77 and 60 mg N kg soil^{-1} for the 10, 5 and 2.5 mm urine depth treatments, respectively (Fig 4.15 c), which were equivalent to 1,690, 2,089 and 3,291 mg N patch^{-1}, for urine patch areas of 0.25 m^2, 0.5 m^2 and 1 m^2, respectively (Fig 4.15 d).

In the 0-100 mm soil depth, the 10 mm urine depth treatment had higher NO_3^--N concentrations over 14 days compared to the 2.5 mm urine depth treatment. These concentrations in the 10 mm treatment decreased after Day 14 but remained high until the end of the experiment (Fig 4.15 a and c). When the NO_3^--N concentrations were extrapolated to 0.25 m^2 (10 mm) and 1 m^2 (2.5 mm), the net NO_3^--N quantity for the latter remained high in the first 30 days and decreased thereafter to be like the small urine patch until the end of the experiment (Fig 4.15 b and d).

Early-autumn experiment

In the top 0–50 mm soil depth, net soil NO_3^--N concentrations peaked on Day 7 after urine application with values of 215, 176 and 119 mg kg soil^{-1} for the 10, 5 and 2.5 mm urine treatments, respectively (Fig 4.16 a). At this time, these net concentrations were equivalent to 2,634, 4,311 and 5,845 mg N patch^{-1}, when extrapolated to urine patch areas of 0.25, 0.5 and 1 m^2, respectively (Fig 4.16 b). After the peaks on Day 7, the net soil NO_3^--N concentrations decreased to background levels at the end of the trial (Day 135) for the 0.25 and 0.5 m^2 urine patch areas.

In the 50–100 mm soil depth, net NO_3^--N concentrations peaked at 42 and 84 mg kg soil^{-1} for the 2.5 and 5 mm urine treatments, respectively, on Day 7 (Fig 4.16 c). Whilst net NO_3^--N concentrations for the 10 mm urine treatment peaked at 138 mg kg soil^{-1} on Day 14. At these times, these net concentrations were equivalent to 2,272, 2,285 (Day 7) and 1,885 (Day 14) mg N patch^{-1} when the 2.5, 5 and 10 mm urine application treatment values were calculated for the urine patches of 1, 0.5 and 0.25 m^2, respectively. After the peaks on Day 7 and 14, the NO_3^--N concentrations decreased to background levels and then slightly increased after Day 118 until the end of the experiment (Day 135) for all the urine patch areas (Fig 4.16 c & d).

In the 0-100 mm soil depth, the 10 mm urine depth treatment had higher NO_3^--N concentrations over a long period of time compared to the 2.5 mm urine depth treatment. It remained high in the first 14 days and decreased thereafter until the end of the experiment (Fig 4.16 a and c). When the NO_3^--N concentrations were extrapolated to 0.25 m^2 (10 mm) and 1 m^2 (2.5 mm), the net NO_3^--N quantity for the latter increased until Day 7 and decreased thereafter to be like the small urine patch until the end of the experiment (Fig 4.16 b and d). In addition, the NO_3^--N concentrations for the 5 and 2.5 mm urine depth treatments decreased more quickly in autumn than in winter.

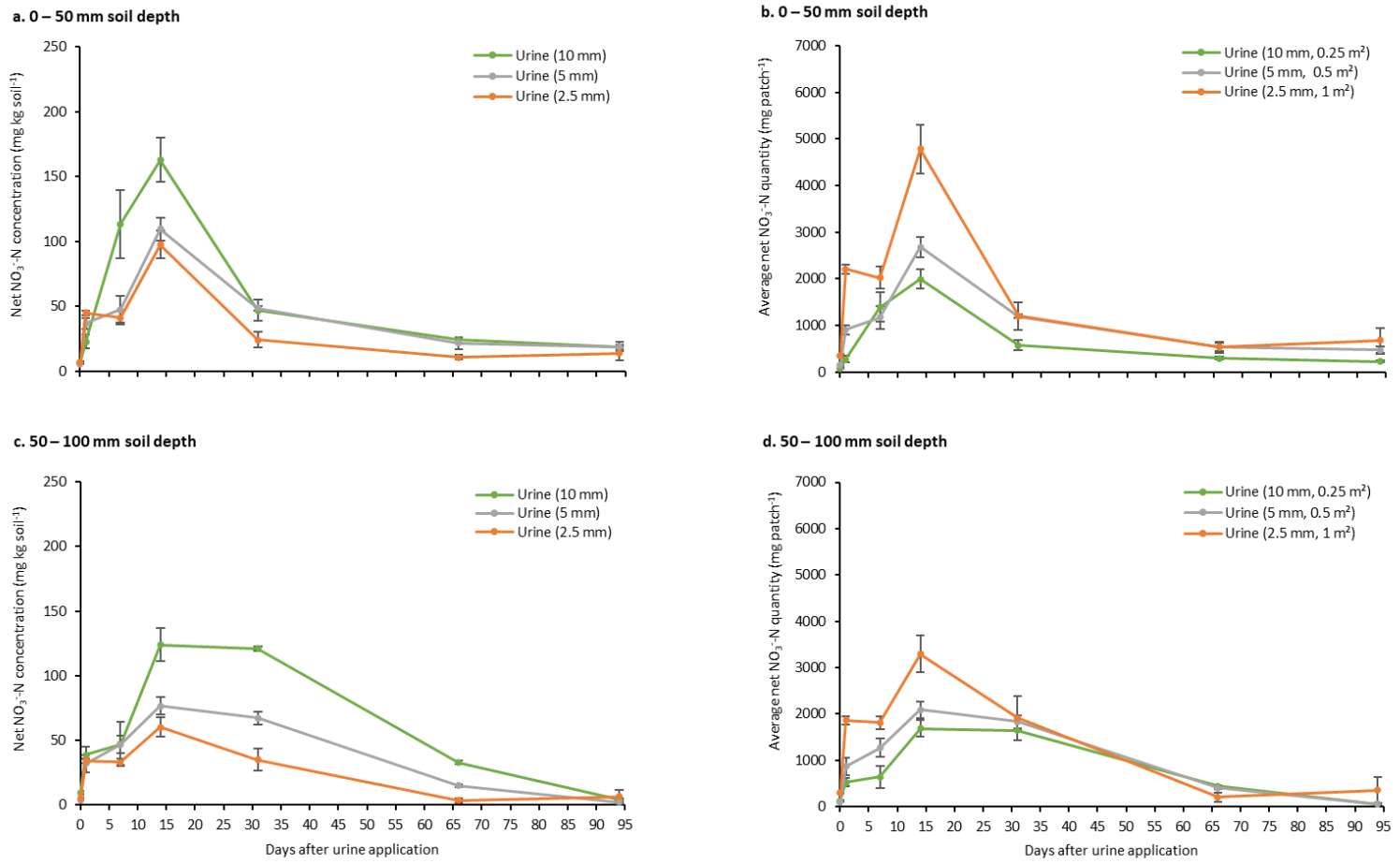


Figure 4.15: Soil net NO_3-N concentrations (a and c) and equivalent urine patch quantity of net NO_3-N (b and d) at two soil depths for each urine application depth treatment for the Early-winter experiment. The error bars indicate standard errors of the mean (SEM).

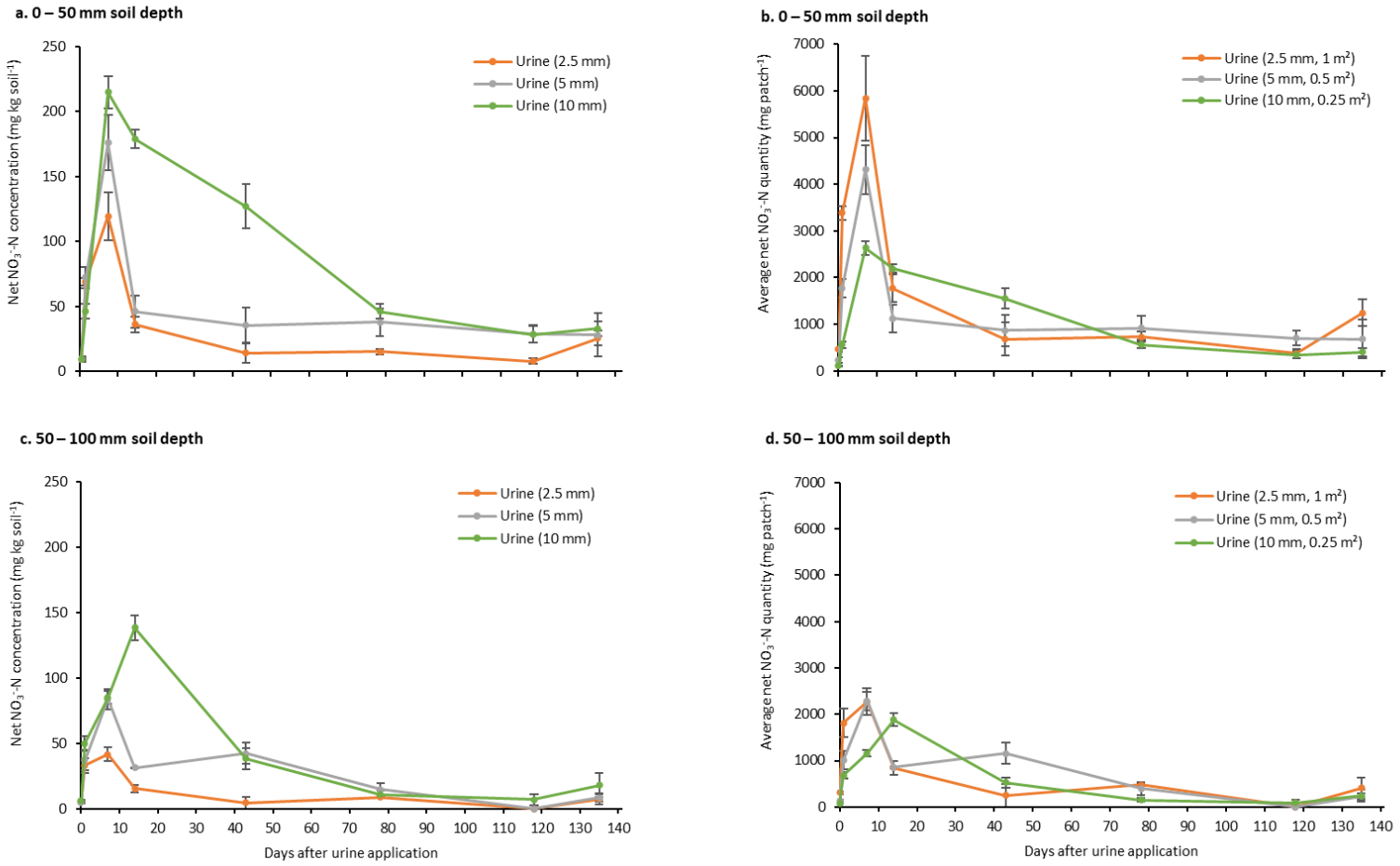


Figure 4.16: Soil net NO_3-N concentrations (a and c) and equivalent urine patch quantity of net NO_3-N (b and d) at two soil depths for each urine application depth treatment for the Early-autumn experiment. The error bars indicate standard errors of the mean (SEM).

4.3.3.3 Changes in net inorganic N with soil depth

On Day 14 after urine application in the early-autumn experiment, the highest urine application depth (10 mm) treatment had the highest soil net inorganic N concentrations at all sampling depths down to a soil depth of 300 mm (Fig 4.17 a). This treatment had soil net inorganic N concentrations of between 81 and 179 mg N kg^{-1}, with the highest concentration being in the surface (0-50 mm). In comparison, the soil net inorganic N concentrations for the other two treatments remained below 50 mg N kg^{-1} in all soil depths measured. When these soil concentrations were extrapolated to the urine patch areas represented by each treatment, the quantity of net inorganic N per patch was estimated to be highest for the 10 mm application depth (0.25 m^2 urine patch area) treatment, being 4,109 mg N $patch^{-1}$ in the top 0-100 mm soil depth (Fig 4.17 b). There was a similar quantity of N per patch in the 100-200 mm soil depth but then decreased to 3,041 mg N $patch^{-1}$ in the 200-300 mm soil depth. The quantities of net inorganic N per patch were lower for the other two treatments at all three soil depths. The 2.5 mm application depth (1 m^2 extrapolated urine patch area) treatment had the second highest quantity of N per patch in the 0-100 and 100-200 mm soil depths, being 2,842 and 2010 mg N $patch^{-1}$. The net inorganic N per patch for this treatment then decreased to 799 mg N $patch^{-1}$ in the 200-300 mm soil depth, which was lower than the values for the two smaller urine patch treatments. Having more inorganic N in the soil at this depth has potential to increase the risk of nitrate leaching, as there will be less opportunity for recovery of this N by plant up take.

On Day 78 after urine application, all treatments had net inorganic N concentrations below 50 mg N kg^{-1} in the 0-50 m soil depth and below 15 mg N kg^{-1} at all other soil depths (Fig 4.17 c). This is a reduction in inorganic N concentrations, compared to the previous sampling (Day 14). At this time, when the 2.5 mm urine application depth treatment was extrapolated to 1 m^2, the estimated quantity of soil net inorganic N remained relatively constant down the soil profile up to 300 mm, ranging from 1,276 mg N $patch^{-1}$ (0-100 mm soil depth) to 748 mg N $patch^{-1}$ (100-200 mm soil depth). In comparison, the 10 mm urine application depth treatment (0.25 m^2) resulted in lower quantities of N in the 0-200 mm soil depth, ranging from 674 mg N $patch^{-1}$ (0-100 mm soil depth) to 52 mg N $patch^{-1}$ (100-200 mm soil depth) (Fig 4.17 d). Over the 64 day

period between sampling times, the amount of net inorganic N measured in the 0-300 mm soil depth decreased by 10,082 mg N patch^{-1} for the 0.25 m^2 compared to only 2,756 mg N patch^{-1} for the 1 m^2. The larger loss of inorganic N for 0.25 m^2 urine patch treatment could be due to a number of losses including plant uptake, soil immobilisation, nitrous oxide emissions and nitrate leaching.

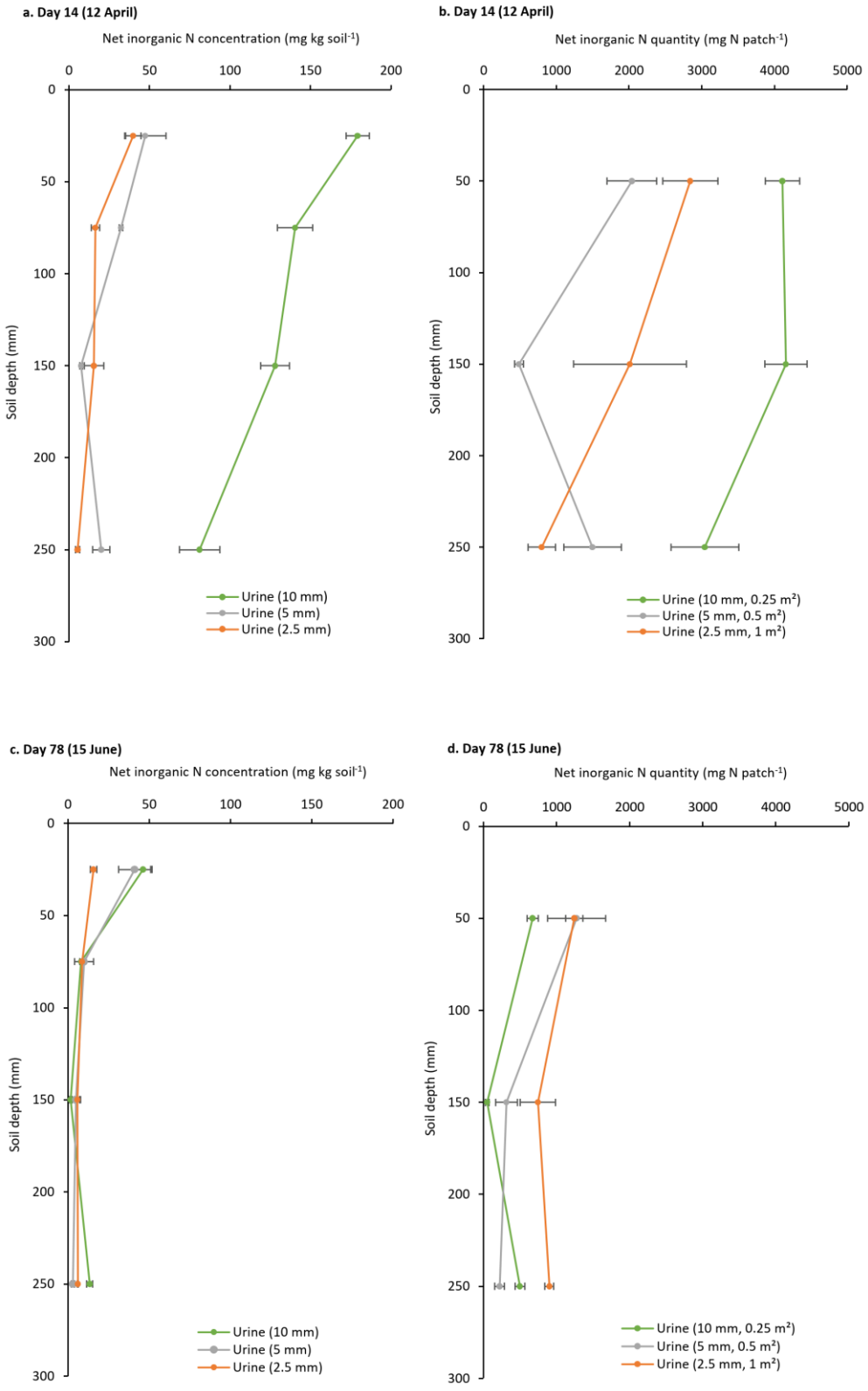


Figure 4.17: Soil net inorganic N (nitrate and ammonium) concentrations (a and c) and equivalent urine patch quantity of net inorganic N (b and d) at four soil depths (0-50, 50-100, 100-200, 200-300 mm) for each urine application depth treatment during the Early-autumn experiment. The error bars indicate standard errors of the mean (SEM).

4.3.4 Pasture yield and N uptake

During the Early-winter experiment, the cumulative pasture dry matter (DM) yield (g patch^{-1}) at Days 25, 63 and 95 after urine application for each treatment were not significantly different from each other (P>0.05) (Table 4.6). The contribution of each urine patch treatment, from a single grazing event to total DM accumulation on a per hectare basis was estimated over 95 days. This was calculated assuming 2400 urine patches per hectare for a 24 hour grazing event (see details of assumption in Appendix B). This approach estimated that the contribution from the 0.25, 0.5 and 1 m^2 patch areas would be 148, 98 and 138 kg DM ha^{-1}, respectively. Therefore, the urine patch contribution from a single grazing was estimated to be minor, with no additional benefit from increasing the urine spread area. It is worth noting that more of the edge effect influence on pasture response would have been included for the 0.25 m^2 urine patch, compared to the two larger urine patch treatments (0.5 and 1 m^2). Therefore, the additional yield responses for the two larger urine patches are likely to be underestimated.

The cumulative N uptake on Day 25 was not significantly different between the three urine patch treatments (P>0.05). On Day 63 and Day 95 (end of the experiment), the cumulative N uptake was only significantly different (P<0.05) between the 0.5 m^2 urine patch and the 1 m^2 (extrapolated) urine patch areas (Table 4.6). By Day 95, the cumulative N recovery in the larger urine patch treatment (extrapolated to 1 m^2) was 3.9 g N patch^{-1}, compared to 2.0 g N patch^{-1} for the 0.5 m^2 urine patch treatment. The cumulative plant N uptake value was equivalent to a urinary N recovery of 25.8% for the 1 m^2 urine patch area, which was about double the recovery of 13.2% for the 0.5 m^2 urine patch area.

Table 4.6: Effect of urine patch areas on cumulative pasture DM, N uptake and estimated urinary N recovery during the Early-winter experiment on Day 25, 63 and 95 after cow urine application.

	Cumulative Dry Matter (g patch^{-1})			Extrapolated Dry Matter (kg ha^{-1})*
Treatments	**Day 25**	**Day 63**	**Day 95**	**95 Day Total**
Urine (0.25 m^2)	3.2 (± 0.9) ^{a}	17.6 (± 4.6) ^{a}	61.8 (± 11.5) ^{a}	148
Urine (0.5 m^2)	-0.6 (± 4.5) ^{a}	12.5 (± 9.2) ^{a}	40.9 (± 10.7) ^{a}	98
Urine (1 m^2)	9.7 (± 4.9) ^{a}	30.6 (± 11.2) ^{a}	57.6 (± 11.6) ^{a}	138
	Cumulative N uptake (g patch^{-1})			
Urine (0.25 m^2)	0.2 (± 0.02) ^{a}	1.2 (± 0.2) ^{ab}	3.5 (± 0.5) ^{ab}	
Urine (0.5 m^2)	0.1 (± 0.2) ^{a}	0.9 (± 0.4) ^{b}	2.0 (± 0.3) ^{b}	
Urine (1 m^2)	0.7 (± 0.2) ^{a}	2.0 (± 0.2) ^{a}	3.9 (± 0.5) ^{a}	
	Cumulative N Recovery (%)			
Urine (0.25 m^2)	1.6	7.9	23.2	
Urine (0.5 m^2)	0.8	6.0	13.2	
Urine (1 m^2)	4.7	13.4	25.8	

Values are mean ± standard error of mean (SEM). Treatments with the same superscript letters in each column are not significantly different.
*****Refer to Appendix B for how pasture DM was extrapolated from g patch^{-1} to kg ha^{-1}.

In the Early-autumn experiment, the cumulative pasture DM yield (g patch^{-1}) on Day 23 was not significantly different between the 0.25 and 0.5 m^2 urine patch treatments, but the 1 m^2 urine patch treatment was significantly different from the two smaller urine patch areas ($P<0.05$) (Table 4.7). However, by Days 66 and 135, any differences between treatments were no longer statistically significant ($P>0.05$). The contribution of each urine patch treatments, from a single grazing event to total DM accumulation on a per hectare basis was estimated over 135 days. This was calculated assuming 1000 urine patches per hectare for a 10-hour grazing event (see details of assumption in Appendix 1). The approach estimated that the contribution of the 0.25, 0.5 and 1 m^2 patch areas would be 82, 102 and 128 kg DM ha^{-1}, respectively (Table 4.7). As with the Early-winter experiment, the urine patch contribution from a single grazing is small, with negligible benefit from increasing the urine spread area. .

The effect of treatments on cumulative N uptake on Day 23, was only significantly different between the extrapolated 1 m^2 urine patch area (2.2 g N $patch^{-1}$) and 0.25 m^2 urine patch area (1.0 g N $patch^{-1}$) ($P<0.05$) (Table 4.6). However, by Days 66 and 135, there were no longer any significant difference between treatments ($P>0.05$). Overall, by Day 135 the estimated urinary N recovery was higher for this experiment, compared to the Early-winter experiment, with values ranging from 32.5% for the 0.25 m^2 urine patch area to 42.1% for the 0.5 m^2 urine patch area. The recovery for the extrapolated 1 m^2 urine patch area was estimated to be 40.2%, which was similar to that of the 0.5 m^2 urine patch area.

Table 4.7: Effect of urine patch areas on mean cumulative pasture DM, N uptake and estimated urinary N recovery during the Early-autumn experiment on Day 23, 66 and 135 after cow urine application.

	Cumulative Dry Matter (g $patch^{-1}$)			**Extrapolated Dry Matter (kg ha^{-1})***
Treatments	**Day 23**	**Day 66**	**Day 135**	**135 Day Total**
Urine (0.25 m^2)	21.5 (± 2.2) ^{b}	71.6 (± 3.2) ^{a}	81.9 (± 10.0) ^{a}	82
Urine (0.5 m^2)	23.7 (± 3.0) ^{b}	82.8 (± 11.8) ^{a}	102.2 (± 23.6) ^{a}	102
Urine (1 m^2)	46.7 (± 5.6) ^{a}	104.7 (± 11.5) ^{a}	127.7 (± 33.4) ^{a}	128
	Cumulative N uptake (g $patch^{-1}$)			
Urine (0.25 m^2)	1.0 (± 0.4) ^{b}	3.0 (± 0.4) ^{a}	3.2 (± 0.5) ^{a}	
Urine (0.5 m^2)	1.4 (± 0.1) ^{ab}	3.8 (± 0.4) ^{a}	4.2 (± 0.7) ^{a}	
Urine (1 m^2)	2.2 (± 0.1) ^{a}	3.7 (± 0.3) ^{a}	4.0 (± 0.9) ^{a}	
	Cumulative N Recovery (%)			
Urine (0.25 m^2)	9.9	29.7	32.5	
Urine (0.5 m^2)	14.2	38.4	42.1	
Urine (1 m^2)	21.5	37.4	40.2	

Values are mean ± SEM. Treatments with the same superscript letters in each column are not significantly different.
*****Refer to Appendix B for how DM was extrapolated from g $patch^{-1}$ to kg ha^{-1}.

4.4 Discussion

As expected, there were increased daily N_2O flux for all the cow urine treatments, compared to a no-urine Control treatment in both the Early-winter and Early-autumn experiments, as a result of the increased soil inorganic N concentrations measured after urine application (Chadwick et al., 2018; Luo et al., 2019; van der Weerden et al., 2017a).

Overall, N_2O emissions were higher from urine application to the soil in Early-winter compared to Early-autumn. For example, the total N_2O emissions measured during the Early-winter experiment for the 'typical' urine patch area treatment of 0.25 m^2 was 9.3 g N_2O-N kg N applied^{-1}. This loss was 84% higher than the same treatment in Early-autumn (5.0 g N_2O-N kg N applied^{-1}). The lower N_2O emissions from urine applied in the Early-autumn experiment is likely to be due to drier soil moisture conditions during the first 40 days after urine application and lower urea N concentrations in the urine, compared to the Early-winter experiment (Di et al., 2014). Both of these differences would have allowed for more opportunity for N to be removed from the urine patch (e.g. via NH_3 volatilisation and plant uptake), before the onset of wetter soil moisture conditions that were more conducive to denitrification occurring (de Klein et al., 2020; Moir et al., 2016). The Early-winter experiment results are consistent with those of Luo et al. (2019) who found high total N_2O emissions in winter (2.9 to 12.7 g N_2O-N kg N applied^{-1}) from the Makotuku fine sandy loam soil in the Manawatu. On the contrary, the Early-autumn experiment result was lower than the findings by Rodríguez Gelós (2020), who found the total N_2O emissions in autumn/winter from the same soil type (Tokomaru silt loam) was 9.4 g N_2O-N kg N applied^{-1}. This is attributed to drier soil moisture conditions during our Early-autumn experiment.

The effect of increasing the urine patch area (i.e. extrapolated 1 m^2 urea patch) on N_2O emissions, compared to 'typical' urine patch area treatment of 0.25 m^2, differed between the Early-winter and Early-autumn experiments. In the Early-winter experiment, the 1 m^2 urine patch area was estimated to have increased N_2O emissions by 39%. While there was insufficient evidence to support this being statistically significant, there was no reduction in N_2O emissions from increasing the urine patch spread area at this time of year. In contrast, in the Early-autumn experiment increasing the urea spread area from 0.25 to 1 m^2 is estimated to decrease N_2O emissions by 56%. This different effect of increasing the spread area of urine in the two different seasons, was likely influenced by the differences

in soil moisture conditions. Spreading the urine over a larger area, initially retains a higher proportion of the urinary N in the surface soil. In both experiments the quantity of nitrate and ammonium per patch in the surface soils (0-50 mm) were initially highest for the extrapolated 1 m^2 treatment. In the Early-winter experiment, which had higher and more favourable soil moisture conditions for dentification from the start of the experiment, the greater quantity of urinary N in the surface soil would have contributed to higher N_2O emissions (Bolan et al., 2004; de Klein & van Logtestijn, 1994; Di et al., 2014; Saggar et al., 2009; Uchida & Clough, 2015).

In contrast, the Early-autumn experiment initially had lower soil moisture conditions during the first 40 days after urine application, which would have provided time for other soil processes (e.g., immobilisation) to reduce soil urinary N before the soil moisture conditions increased to levels that were more favourable for N_2O emissions. Furthermore, at this time of the year increasing the urine patch area can also increase NH_3 volatilisation losses, as was observed in the experiment described in Chapter 3. This would also decrease the proportion of urinary N that remained in the soil after the first day following urine application. Therefore, increasing the urine spread in Early-autumn has the potential as a mitigation for reducing N_2O emissions from grazing events. In contrast, it would not be advisable in Early-winter to increase the urine patch area due to the risk of increasing these emissions.

For the Early-autumn experiment on Day 14, the 'typical' urine patch area of 0.25 m^2 had a net inorganic N concentration in the 200-300 mm soil depth that was about fifteen times higher than the concentration in the extrapolated 1 m^2 urine patch treatment. When this net inorganic N concentration was calculated on a per patch basis, the smaller urine patch still had almost four times the quantity of net inorganic N in the 200-300 mm soil depth than the larger urine patch area. Higher inorganic N concentrations at this depth in the soil in autumn poses an increased risk of N leaching, because it reduces the likelihood of this N being recovered by plant uptake prior to the onset of the drainage season. By Day 78, the net inorganic N quantity per patch for the 0.25 m^2 urine patch treatment had decreased by about 70%, compared to only a slight change for the 1 m^2 treatment. A number of processes, including plant uptake, denitrification, immobilisation and N leaching, could have contributed to the loss of net inorganic N quantity in the small urine patch area (Cameron et al., 2013; Saggar et al., 2013; Selbie et al., 2015a). Out of these processes, N leaching losses were expected because this soil sampling was conducted in

early winter (15 June), prior to which soil moisture conditions and rainfall indicate the occurrence of days with saturated soil conditions and the likelihood of N leaching during the larger rainfall events (Fig 4.7). In a similar study, Ramirez (2017) applied 2.1 L of cow urine to areas of 0.2 m^2 and 1 m^2 in early Autumn. At the third soil sampling, which was 53 days after urine application (and 24 days after the commencement of the drainage), the net inorganic N in the 45-120 cm for the 1 m^2 urine patch treatment was 64% lower than for the 0.2 m^2 urine patch treatment. This suggested that increasing the urine spread area is likely to have reduced the quantity of N that was susceptible to leaching, compared to the more typical urine patch area of 0.2 m^2.

In this study, increasing the urine spread area from 0.25 to 1 m^2 increased the EF_3 for urine deposited to pasture in Early-winter from 0.93 to 1.28%. In contrast, the increasing urine spread area decreased the EF_3 for urine deposited in Early-autumn from 0.50 to 0.22%. For the Early-winter experiment, the EF_3 for the 0.25 m^2 urine patch (typical patch size) was similar to the current average NZ-specific N_2O EF_3 (0.98) (van der Weerden et al., 2020). However, when the urine patch area was spread to 1 m^2, the EF_3 was greater than the current NZ-specific N_2O EF_3. In comparison, the EF_3 for all the urine patch areas during the Early-autumn experiment were lower than the current NZ-specific N_2O EF_3.

4.5 Conclusions

This study found that increasing the size of the urine patches from 0.25 to 1 m^2 in early-winter did not decrease N_2O emissions and could potentially increase N_2O emission by up to 39%, though there was insufficient statistical evidence to support this. However, this highlights that caution needs to be taken with any mitigation used to increase urine patch area during winter when soil moisture conditions are close to or at saturation. In contrast, increasing the size of the urine patches from 0.25 to 1 m^2 in early-autumn decreased N_2O emissions and EF_3 by 56%. This mitigation was effective during the autumn due to an extended period of about 40 days after urine application with drier soil conditions. In addition, the higher NH_3 losses resulting from increasing the urine spread at this time of year (Chapter 3), may have also resulted in less urinary-N being present in the urine patch and, thus, less risk of direct N_2O emissions. However, high NH_3 emissions can contribute to indirect N_2O in the atmosphere. Therefore, it is also important to include the effect of increasing urine spread on indirect N_2O for the overall impact on GHG

emissions, which will be discussed in Chapter 6. In addition, the effect of increasing urine spread in early-autumn decreased the urinary-N present in the soil when the drainage season starts. Therefore, there is a potential in reducing the risk of NO_3^- leaching. The effect of a urine spreading device, worn by dairy cows, on NO_3^- leaching was assessed in an experiment that is presented in the next chapter (Chapter 5).

Chapter 5

Effect of a device that increases cow urine patch area on nitrate leaching and pasture accumulation in a pasture soil

5.1 Introduction

Dairy farming's role in declining water quality in New Zealand is one of the most pressing environmental issues currently facing the industry. The main impact that dairying has on declining water quality is from nitrate leaching, which is mostly caused by the high concentrations of nitrogen (N) returned in cow urine to relatively small areas of pasture (approximately 2-3% of the grazed area at each grazing; with typical loading rates of nitrogen equivalent to 400-800 kg N ha^{-1}). Specifically, urine deposited on pastures during grazings from the mid-summer to early-winter period has been identified as the main contributor to nitrate leaching (Christensen, 2013; Shepherd et al., 2011). This is because dry conditions and/or insufficient time prevent pasture from taking up and removing most of the nitrate in these late lactation urine patches before the start of the subsequent drainage season. Typically, drainage starts in late-autumn/early-winter in most regions of New Zealand.

By increasing the area of pasture covered by each urination, it is expected that plant uptake of nitrate from these urine patches will increase, thereby, reducing the accumulation of soil nitrate available at the time drainage commences. Ramirez (2017) compared changes in the inorganic N concentrations in soil under urine patches of different sizes. Cow urine was spread to pasture plots in early autumn and the treatments included: no-urine, urine applied to 0.2 m^2 and urine spread over 1 m^2. The urine (0.2 m^2) treatment was used to represent a typical urine patch area. The two urine treatments received 2.1 L of real dairy cow urine and soil inorganic N was measured on three occasions: 15, 36 and 53 days after urine application. At the third soil sampling date, 24 days after the estimated start of drainage, the quantity of net inorganic N below the root

zone (i.e., in the 45-120 cm soil depth) of the Urine (1 m^2) was 64% less than the Urine (0.2 m^2) treatment. Other potential benefits of spreading urine over a larger area include increased pasture growth and improved nitrogen use efficiency (Cichota et al., 2018; Moir et al., 2011). A study that was conducted during summer with minimal rainfall by Orwin et al. (2009) found that large urine patches retain a larger amount of N which enables greater plant growth and N uptake compared to small urine patches.

Novataro Ltd have developed a urine-spreading device that is designed to be worn by cows to increase the size of urine patches and, thereby, reduce nitrate leaching. The aim is to achieve a uniform spread of urine over a wetted area of about 1 m^2, compared with the more typical urine patch area of approximately 0.20-0.25 m^2 (Selbie et al., 2015a). The intention is to use the device on dairy cows for up to about 5 months each year, during the mid-summer and autumn period, to increase the spread of urine patches.

Novataro Ltd commissioned Massey University to assess the performance of the device on a small number of cows in initial evaluation studies; Stage 1, (Hanly et al., 2017b); Stage 2, (Hanly et al., 2018). More recently (March/April 2021), another evaluation study (Stage 3) assessed the effect of an improved version of the urine-spreading device on urine spread at Massey University's Dairy Farm 4. The device was used on two dairy cows and the urine spread was determined using thermal imaging and soil sampling (Fig 5.1). The results of the study showed that the device was effective at increasing the spread of urine and retaining more of the urinary N in the top 0-10 cm soil depth. Further research is required to determine the effect of the device on nitrate leaching and pasture production.

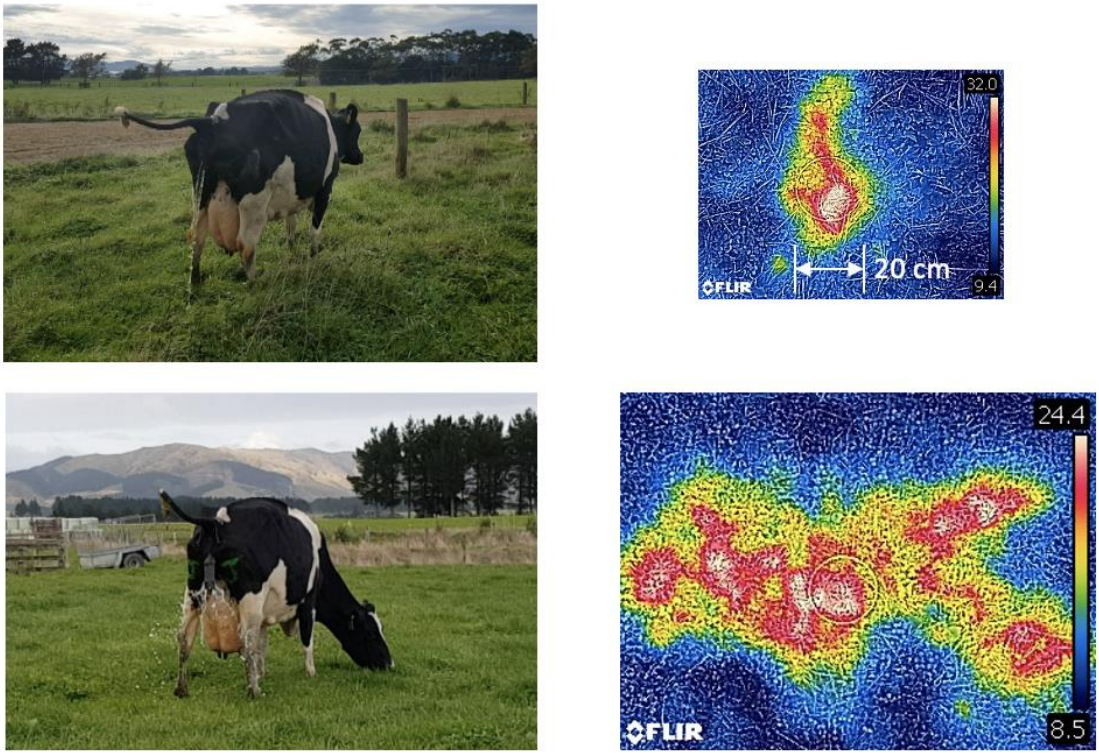


Figure 5.1: Comparison of urine spread for a cow with (bottom) and without (top) the urine spreading device (Stage 3, April 2021). *The thermal images show differences in urine spread (20 cm diameter aluminium ring used to achieve the same scale for both images; values on the right of each thermal image are temperatures ranges in ˚C).*

This research presents the results of a large-scale grazed plot drainage field trial conducted at Massey University's Dairy Farm 4 in 2022. The objectives of this study were to quantify the effect of the urine spreading device, worn by cows during late-summer and autumn grazings, on; the pattern of urine spread onto and in soil, nitrate and total N leaching, and pasture accumulation.

5.2 Materials and Methods

5.2.1 Experimental site

This study commenced in February 2022 on Massey University's Dairy Farm 4 near Palmerston North, Manawatu Region (40°23'48.4"S 175°36'37.8"E). The average rainfall (2002-2022) at this site is 980 mm which is evenly distributed throughout the year. The

driest months are January to March. The mean annual soil temperature at 10 cm is 13°C and the coldest and the warmest months are July (9°C) and January/February (19°C).

The farm has an area of 250 ha with an effective area of 221 ha which is subdivided into approximately 80 x 1.5–3.5 ha paddocks. The experimental site was located on one of the paddocks (paddock 35) with an area of 3.07 ha and consisted of twelve pasture plots measuring 800 m^2 per plot (Fig 5.2). The soil type in the paddock is the Tokomaru silt loam soil, which is an Argillic-fragic Perch-gley Pallic soil (Hewitt, 2010), and is formed from windblown loess. Soil characteristics are defined in Table 5.1.

Table 5.1: Physical and chemical characteristics of soil at 0-100 mm. Adapted from Pereira et al. (2019) and the results from the current study.

Depth (mm)	Soil pH (water)	Total C (%)	Total N (%)	CEC (meq 100 g^{-1})	Total porosity (%)	Field capacity (%)	Bulk density (Mg m^{-3})
0-100	6.0	3.8	0.4	12.1	54.7	45	1.2

The pasture at the experimental site consisted of a mixture of predominantly ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) with some weed species. Prior to the trial in February 2022, the pasture plots were unfenced and were grazed as part of the farm's normal grazing rotation.

5.2.2 Urine spreading device

The prototype urine-spreading device design that was used in this study is shown in Figure 5.4. The device is worn by a cow using flexible elastic cords attached to a rubber pad, which is fixed by an adhesive (KAMAR® adhesive) onto the cow's back. The method used to hold the rubber pad on the cow is similar to that used to secure heat detection pads on cows. This research was approved by the Massey University Animal Ethics Committee, Protocol No. 21/75.

Figure 5.2: Different views of the urine spreading device attached to a cow at Massey University's Dairy Farm 4.

5.2.3 Experimental design and treatments

The twelve pasture plots consisted of two treatments and six replicates of each treatment. The plots were individually fenced in January 2022 and each plot had a mole and pipe drainage systems, which was used to monitor drainage volumes, nitrate, and total N concentrations (Fig 5.2). The treatments were cows wearing urine-spreading devices ('Device' treatment) and cows without the device ('Control' treatment). There were four grazing events over the late summer and autumn periods in 2022: Grazing 1 (8-10 February), Grazing 2 (7-9 March), Grazing 3 (11-13 April), and Grazing 4 (25-27 May). After Grazing 4 the plots were, again, grazed as part of the farm's normal grazing rotation.

At each grazing event, four different plots (two Control and two Device treatment) were grazed per day, which enabled all twelve plots to be grazed over three consecutive days. Grazing in this manner, minimised the number of cows needed for the study, which was required by the University's animal ethics protocol requirement. This also reduced the time needed to put the devices on the cows before each grazing and enabled the cow grazing observations to be made by two people. Each plot was grazed by twelve cows at all four grazing events. Grazing 1 and 2 were morning grazing events, which were approximately 6 hours in duration. Grazing 3 and 4 were overnight grazing events. The grazing duration at Grazing 3 was approximately 13.5 hours and at Grazing 4 it was approximately 18 hours.

Figure 5.3: Layout of the 12 pasture plots (each ~800 m^2) used for the study on Massey University's Dairy Farm 4 (Google Maps).

At each grazing event, two observers recorded when cows were seen urinating, and the observers also ranked how well the spreading device intercepted and spread the urinations (Fig 5.3). At Grazing 1 and 2, observations were made during the total duration of each grazing (6 hours). At Grazing 3 and 4, observations were made for approximately the first 2.5 hours of each grazing while there was still daylight.

Figure 5.4: Manual observations and recording of cow urinations at a grazing event on the drainage plots.

5.2.4 Drainage water monitoring and water analyses

Drainage water from each plot was channelled through a drainage pipe into individual tipping-bucket flow meters located in sampling pits nearby (Fig 5.5). Drainage volumes were measured using automated tipping bucket flow meters (~5 L tip^{-1}) and volume proportioned samples were collected during each drainage event for nitrate and total N (TN) analysis. Drainage water samples were filtered through a 0.45 μm filter and stored frozen until analysed. Filtered samples were analysed for NO_3^--N using an Ion Chromatograph (IC, Dionex Aquion). Total N was determined in unfiltered samples using the persulphate digestion method of Hosomi and Sudo (1986), which converts the various forms of N in the sample to NO_3^-, which was then analysed using a colorimetric method on a Technicon Auto Analyser (Blakemore et al., 1987).

Figure 5.5: An example of a drainage tipping bucket flow rate meter with sample collector standing on a concrete base.

5.2.5 Pasture accumulation measurements

A rising plate pasture height meter (Jenquip) was used to estimate pasture accumulation before and after each grazing event. Pasture accumulation between grazing events was calculated as the difference between pasture mass at grazing minus the post-grazing pasture mass at the previous grazing. The pasture height value from each plot was an average of approximately sixty individual measurements. The plan was to assess pasture

accumulation over a 12-month period, however, in late December 2022 a grazing event inadvertently occurred before pasture height measurements were made. In addition, because research staff were not notified of the grazing event, post-grazing pasture height measurements were also not taken. This resulted in pasture accumulation values only being available for approximately the first 9 months of the study (February to November 2022).

Pasture height was converted to estimated pasture cover (kg DM ha^{-1}) using the following equation (standard winter calibration equation provided by Jenquip):

Pasture cover (kg DM/ha) = (Pasture height x 140) + 500

5.2.6 Cow dirtiness assessment

At the fourth grazing event, the 24 Device treatment cows grazed two plots per event (12 cows per plot) for three consecutive days (~18 hours day^{-1} on 25, 26 and 27th May 2022). The device was removed from the cows and cleaned between each of the three grazing times. After the completion of the third grazing event, on the 28th of May 2023, the Device treatment cows were photographed to provide a record of dirtiness. The photographs were then visually assessed, and each cow was assigned a level of dirtiness. This was measured to evaluate whether the device had an influence on the level of dirtiness on cows after grazing, which could have a negative impact on milk quality.

5.2.7 Assessment of urine patch spread

On the 30th of June 2022, ten dairy cows were put into a paddock on Dairy Farm 4 from 7.30 am to 9.30 am. Five of the cows were without a device and the other five were with the urine spreading device. Immediately after each urination, the centre of the urine patches was marked with a metal ring, and an infrared thermal image was taken. This was done straight after the cows urinated to assess the initial spread of each urine patch. The urine patch areas then were left for approximately six hours for the urine to infiltrate into the soil.

Soil samples were taken from each of the urine patch areas to see how far the cow urine had moved down into the soil and how far it had spread from the centre of the urine patch. A soil corer was used to sample the soil at the depths 0–10 and 10–30 cm at the distances 0 (the centre), 10, 20, 30, 40, and 50 cm to the left from the centre (Fig 5.6). Each sample

was a composite of three cores, a core from the centre line and a core from approximately 10 cm from each side of the line. After sampling, the soil samples were stored at room temperature (22°C) for up to 7 days, to ensure that all the urine urea N had converted to either NO_3^- or NH_4^+. The fresh soil samples were mixed by hand and then analysed for inorganic N (NH_4^+-N and NO_3^--N) concentrations in the laboratory. Five grams of fresh soil was weighed and 30 ml of KCl was added to extract the NO_3^--N and NH_4^+-N (Refer to Chapter 3 for further details of the method). Approximately 19 g of fresh soil of each sample was weighed, and oven dried to determine gravimetric soil moisture content.

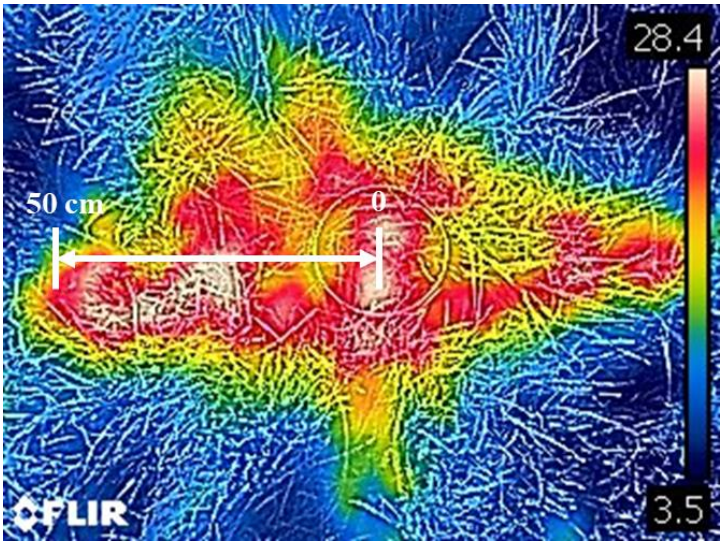


Figure 5.6: A thermal image of a urine patch from a cow wearing a urine spreading device, showing the distance from 0 (centre of urine patch) to 50 cm to the left, which was the zone used for soil sampling. The diameter of the ring is 20 cm.

5.2.8 Climate Data

Rainfall and daily soil water deficit for the trial period were taken from the NIWA/AgResearch weather station, which is approximately 3 km from the trial site.

5.2.9 Data and statistical analysis

The mean values and standard error of the means for drainage water depth, NO_3^--N, Total N (TN) concentrations, and pasture accumulation were calculated based on the six replicates for each treatment using Microsoft Excel for Microsoft 365. The least significant differences and p values were calculated using one-way analysis of variance

(ANOVA) to detect any significant difference and different treatment means were compared using Tukey Pairwise Comparisons in Minitab 19.

5.3 Results

5.3.1 Climate Data

The annual rainfall for 2022 was 1,136 mm. The total rainfall throughout the grazing period (8th February to 6th October 2022) was 784 mm (Fig 5.7). The highest monthly rainfall was recorded in July (209 mm) and the lowest rainfall was recorded in April (18 mm). During the trial, the soil water deficit ranged from 0–123 mm. From the beginning of March to the end of May the soil water deficit was large (ranging from 33–123 mm). In contrast, from the beginning of June to the end of September, the soil water deficit was small (ranging from 0–14 mm), being at or close to field capacity. The small soil water deficit from June to September was due to high rainfall over that period (a total of 532 mm) and small evapotranspiration rates. As the rate of evapotranspiration increased in spring, the soil water deficit also started increasing through to the final grazing (6th of October; Fig 5.7).

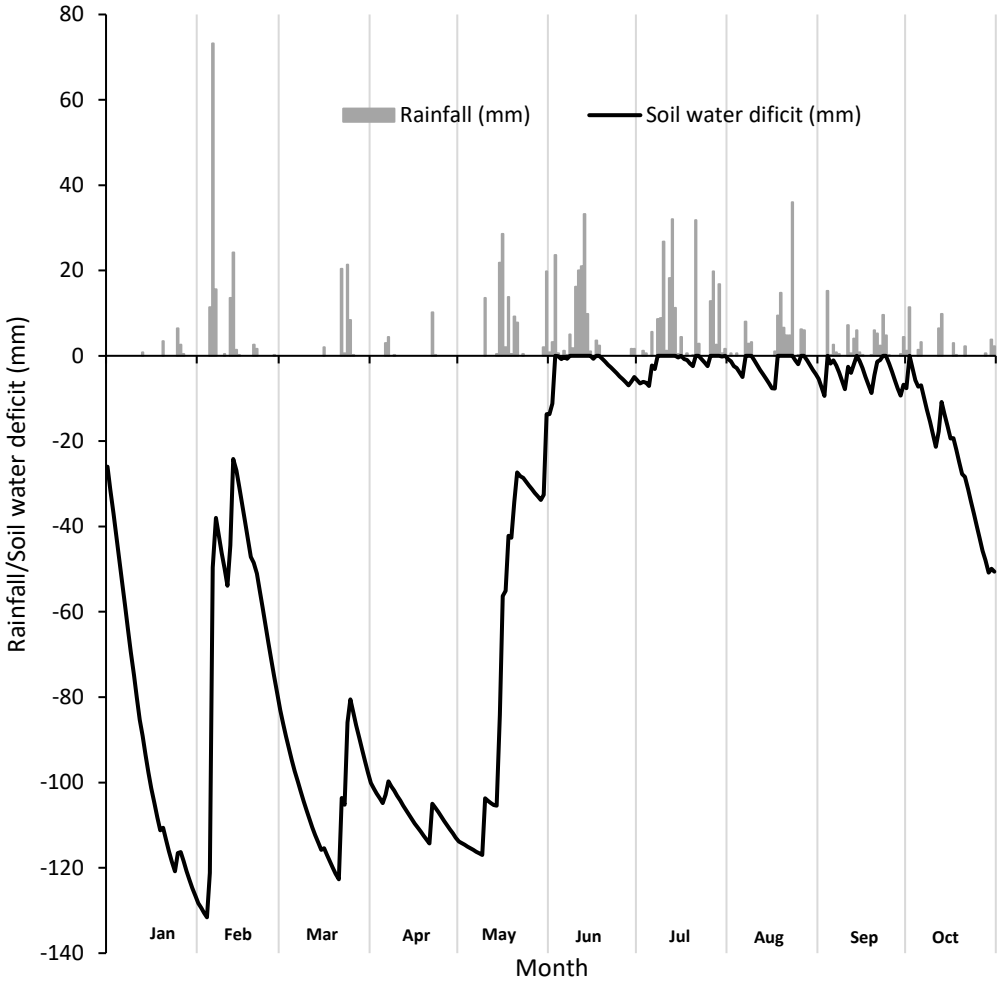


Figure 5.7: Daily rainfall and soil water deficit at Dairy Farm 4 from January to October of 2022.

5.3.2 Urine spreading device performance

Cow observations were used to provide an indication, rather than an exact value, of how well the spreading devices worn by cows (Device treatment) were intercepting and spreading urinations (Table 5.3). This is because it was not possible to observe all the urinations that occurred during the observation period. It was more difficult to monitor cows when they were in groups or grazing further away from the observer. Furthermore, the last two grazing events occurred mostly at night, so only the initial part of these grazings was observed.

At the first grazing event in February, approximately 28% of the observed urinations were not intercepted by the spreading device worn by the cows. This was mostly due to the

urination stream passing to one side or over the top of the splash plate. This decreased to only approximately 15% at the second grazing event in March. For the remaining two grazing events in April and May, the percentage of observed urinations that were not intercepted by the urine spreading device remained at less than 20%.

Table 5.2: Summary of cow observation data for the four grazing events in 2022.

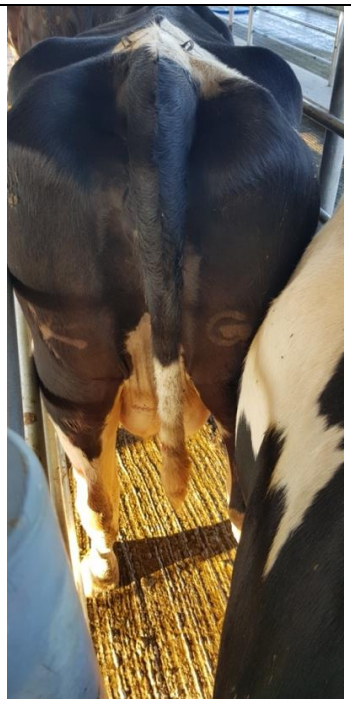
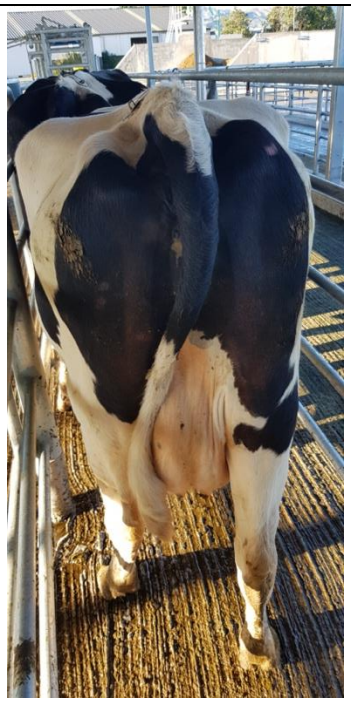
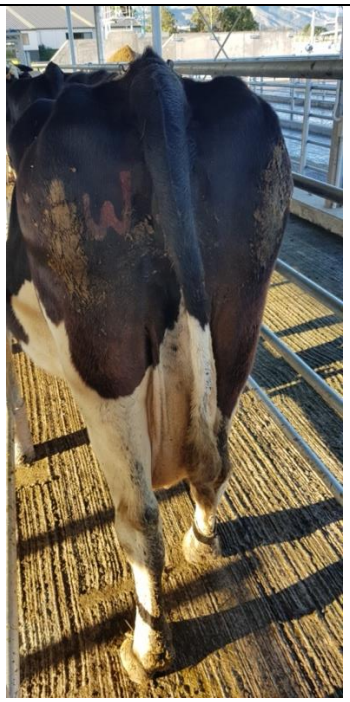
Grazing event (date)	Grazing duration (hours)	Observation duration (hours)	Average number of urinations observed per plot		Average percentage (%) of observed urinations intercepted by device		
			Control treatment	Device treatment	Yes	No	*Not fully seen
1 (8-10 Feb)	~6	~6	30	40	62	28	10
2 (7-9 Mar)	~6	~6	38	47	84	15	1
3 (11-13 Apr)	~13.5	~2.5	15	18	77	18	5
4 (25-27 May)	~18	~2.5	15	19	78	11	11

*Not fully seen means observers only saw the end of urination being intercepted by the device.

At all four grazing events, the number of observed urinations was higher for the Device treatment than the Control treatment. At the first and second grazing events, the number of observed urinations were 33% and 24% higher, respectively, for the Device treatment. The relatively short observation times (~2.5 hours) at the last two grazing events meant the ability to compare the treatments was limited. The reason for this difference is not clear, but it is possible that wearing the device is influencing cow behaviour in a way that results in more frequent urination. However, there was also a large variation in the frequency of observed urination between cows in the Device treatment group, ranging from 1-8 observed urination cow^{-1} over a ~6-hour grazing duration at the first and second grazing events. Therefore, any possible effect of the device on urination frequency was not consistent for all cows.

Photographs of the 24 Device treatment cows taken on the 28th of May 2022, after the fourth grazing event, were grouped into three levels of dirtiness (nil, minor, moderate) (Table 5.4). The assessment showed that wearing the device for 18 hours day^{-1} for three days in a row, resulted in nil or minor dirtiness for 79% of cows, and only 21% of cows showed moderate levels of dirtiness.

Table 5.3: Visual assessments of cow dirtiness for the Device treatment cows after the fourth grazing event on 28th May 2022. Photographs provide examples of each level of dirtiness.

		
Nil dirtiness	***Minor dirtiness***	***Moderate dirtiness***
3 cows (12%)	16 cows (67%)	5 cows (21%)

5.3.3 Drainage water nitrate and total nitrogen

The first drainage sample was collected on 1st June 2022 and the last drainage sample was collected on 6th October. Drainage samples were collected from the experimental plots on 37 occasions. The average total drainage on the Control and Device treatment plots was 264 (± 28) and 277 (± 29) mm, respectively (Fig 5.8).

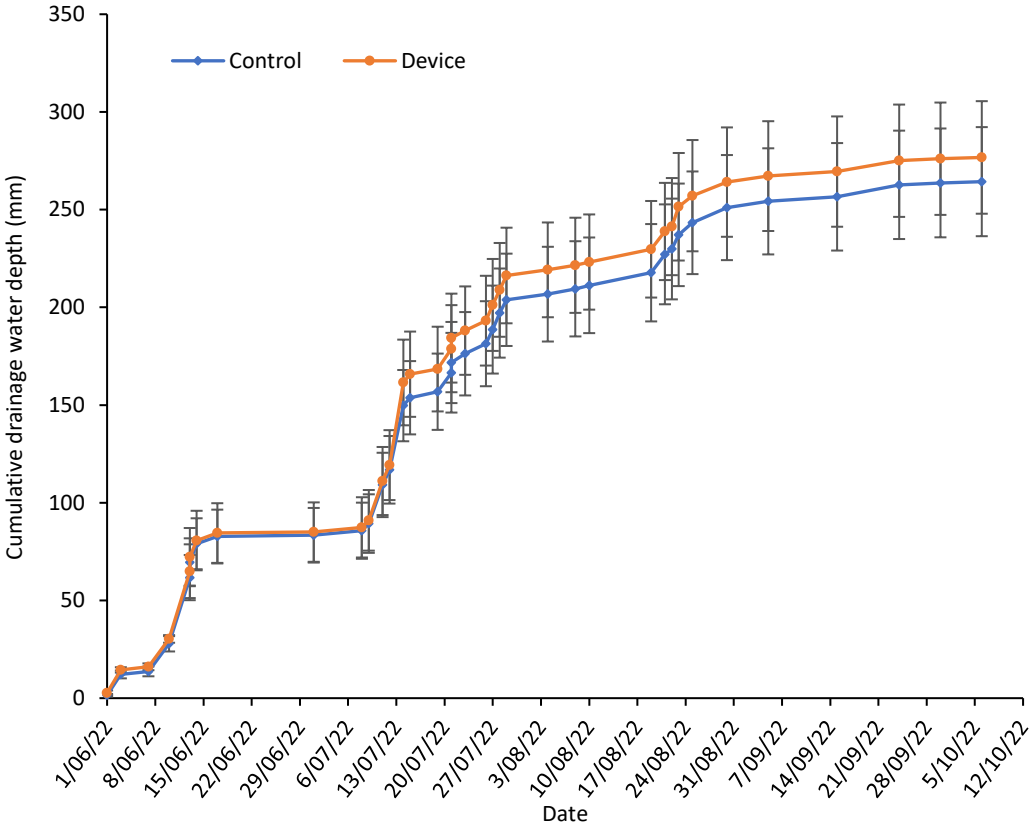


Figure 5.8: Average cumulative drainage water depth for the Control and Device treatment plots. The error bars indicate standard errors of the mean (SEM).

Drainage water nitrate concentrations started high and decreased as the drainage season progressed (Fig 5.9), The Device treatment average nitrate concentration at the first sampling (1st June 2022) was 7.4 (± 1.8) mg NO_3^--N L^{-1}, which was 46% less than the Control treatment value of 13.8 (± 4.4) mg NO_3^--N L^{-1}. The difference in nitrate concentration between the two treatments reduced over the subsequent five sampling times. By the sixth sampling time (13th June 2022) there was less than 1 mg NO_3^--N L^{-1} difference between the average drainage water nitrate concentrations for the two treatments. The differences between treatments were small or negligible for the rest of the drainage season. For both treatments, the nitrate concentrations remained below 1 mg NO_3^--N L^{-1} from the end of July until the end of the drainage season on the 6th of October 2022.

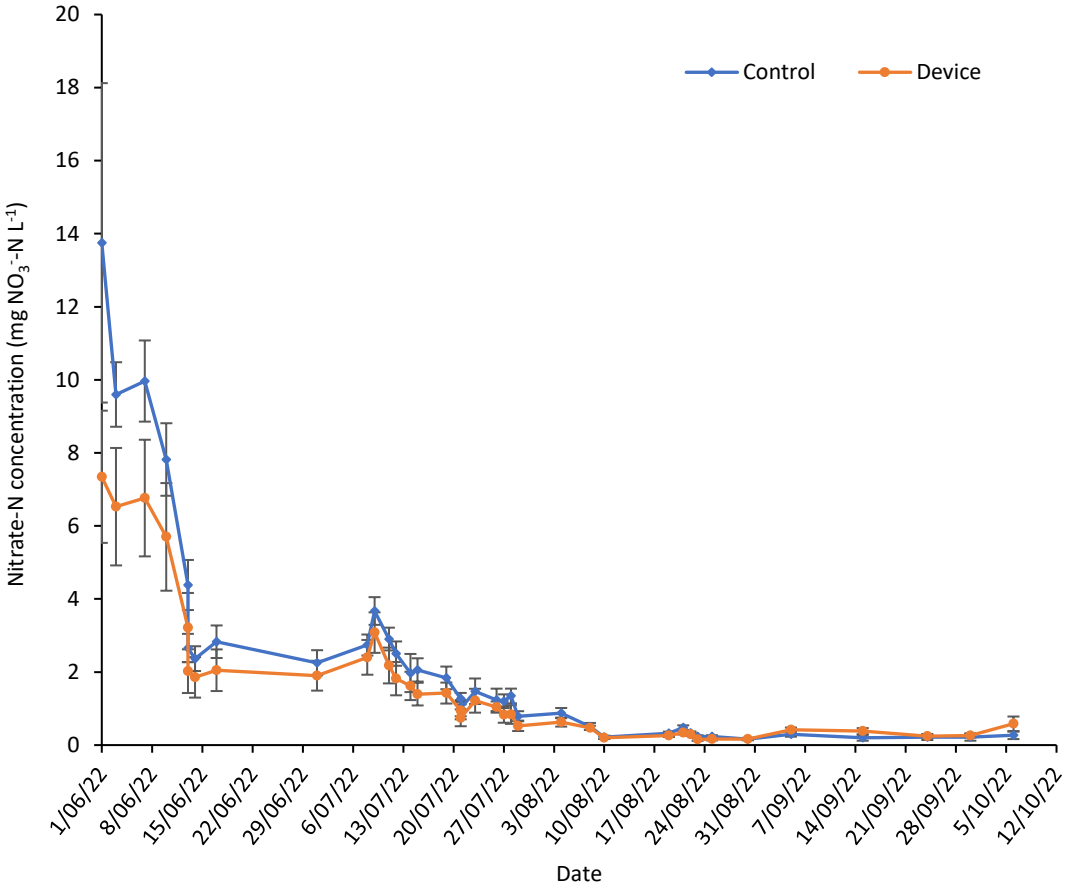


Figure 5.9: Average drainage water NO_3^--N concentrations for the Control and Device treatment plots. The error bars indicate standard errors of the mean (SEM).

On average, the total cumulative nitrate leaching in drainage from the Device treatment was 5.7 (± 1.8) kg NO_3^--N ha^{-1} $year^{-1}$, which was 12% lower than the Control treatment value of 6.5 (± 1.0) kg NO_3^--N ha^{-1} $year^{-1}$ (Fig 5.10). However, due to the variation between the replicate plots for both treatments, this difference between the treatments was not statistically significant ($P>0.05$). Furthermore, for the first five events combined for each of the treatments, there was no statistically significant difference between the treatments ($P>0.05$).

The total cumulative Total N (TN) leaching in drainage from the Device treatment was 7.2 (± 1.7) kg N ha^{-1} $year^{-1}$ on average, which was 8% lower than the Control treatment value of 7.8 (± 0.9) kg N ha^{-1} $year^{-1}$ (Fig 5.11). As with nitrate leaching, the differences in TN leaching between treatments were not statistically significant ($P>0.05$).

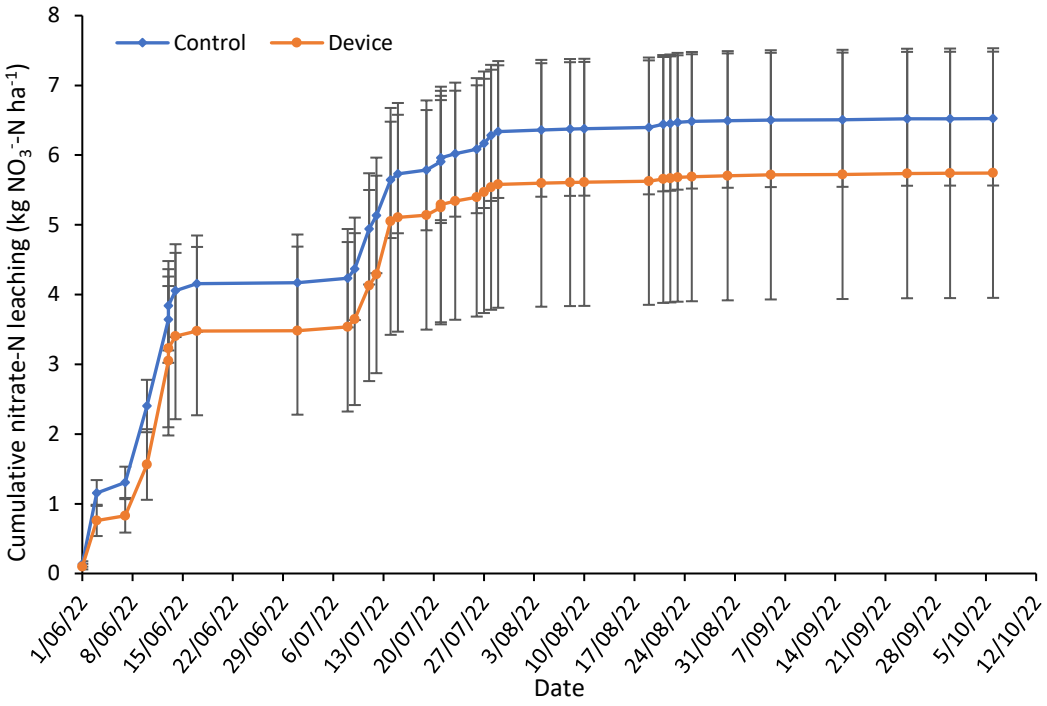


Figure 5.10: Average cumulative nitrate-N leaching for the Control and Device treatment plots. The error bars indicate standard errors of the mean (SEM).

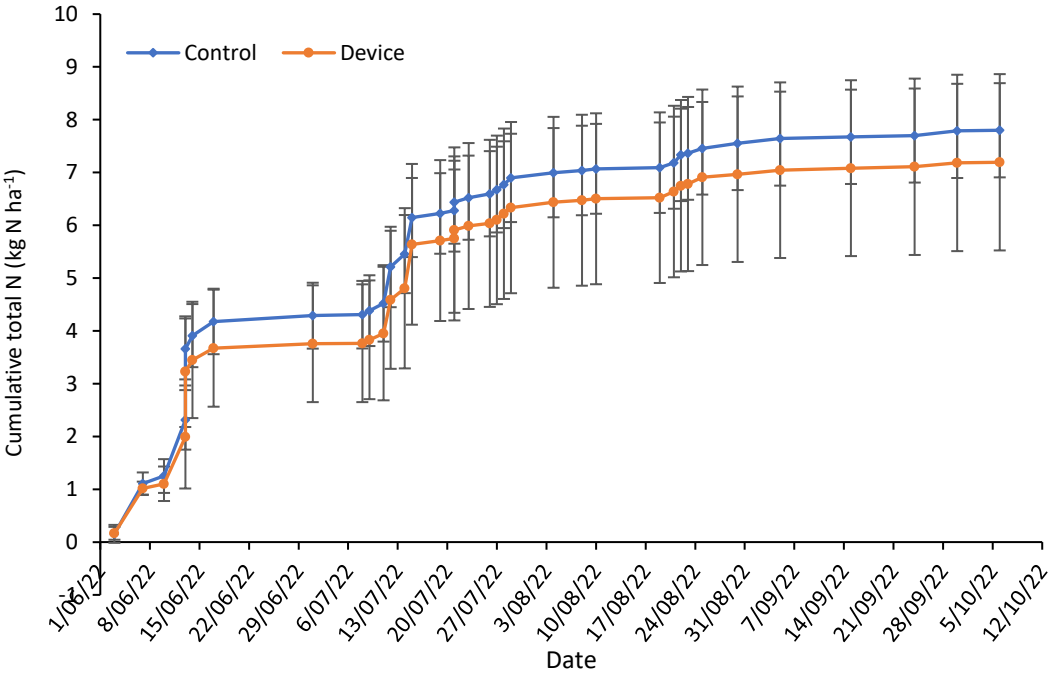


Figure 5.11: Average cumulative total N leached for the Control and Device treatment plots. The error bars indicate standard errors of the mean (SEM).

5.3.4 Pasture accumulation

There were seven pasture accumulation periods assessed over a duration of 9 months (Fig 5.12). The total pasture accumulation for the Control treatment was 8,317 kg DM ha^{-1}, which is similar to the value of 8,540 kg DM ha^{-1} for the Device treatment. The largest difference between treatments occurred in the late autumn accumulation period (14 April to 26 May), where the average pasture accumulation for the Device treatment was 22% higher than the Control treatment value. However, differences between the two treatments for any of the individual accumulation periods and for the total 9-month period, were not large enough to be statistically significant (P>0.05).

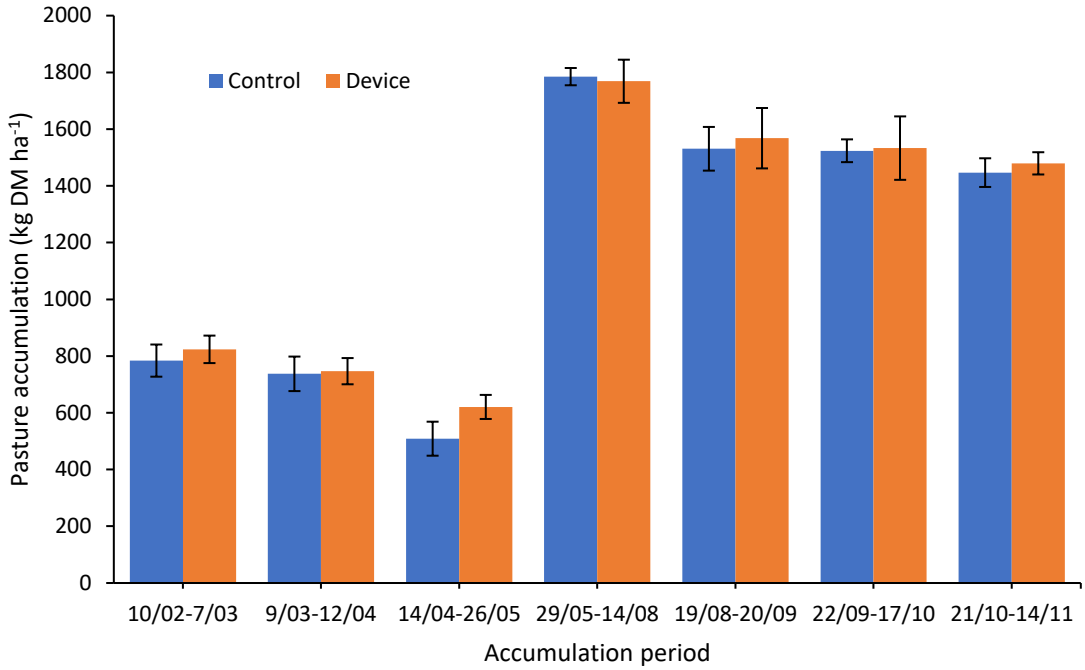


Figure 5.12: Average estimated pasture accumulation for the Control and Device treatment plots (10 February to 14 November 2022). Error bars are ± SEM.

5.3.5 Urine Spreading Device (Device) and urine patch configuration.

Six hours after a urination event, the soil moisture content (SMC) in the 0-10 cm soil depth ranged from 23 to 26% for the Control and Device treatments and was higher than the SMC in the 10-30 cm soil depth, which ranged from 17 to 18% for the Control and Device treatments (Fig 5.13). The SMC in both soil depths slightly decreased as the distance from the centre of the cow urine increased.

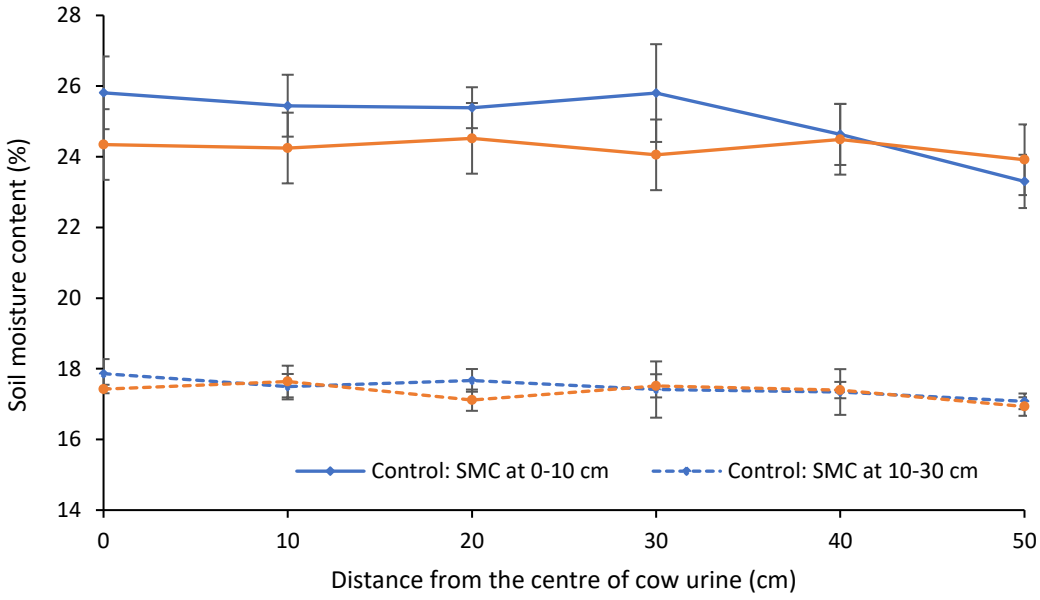


Figure 5.13: Soil moisture content at 0-10 cm and 10-30 cm soil depths at different distances from the centre of the cow urine patch for the Control and Device treatments.

Figure 5.14 shows thermal images of urine patches taken immediately after the completion of each urination. Five images were taken of urine patches from cows not wearing a spreading device (Control treatment) and another five images were of urine patches from cows wearing a spreading device (Device treatment). Caution must be taken when comparing the images because they were taken at various heights from the ground, so they are of different sizes and scales. However, a 20 cm diameter ring was used to provide a reference to allow comparisons. In addition, the temperature ranges also varied between the images, which are shown on the right side of each image. The other consideration is that urine volume for each urination is also likely to vary, but this was not measured as part of this assessment. Overall, the images show that the initial areas of the urine patches were consistently smaller for the Control treatment, with the majority of each urination area from this treatment being within a diameter of approximately 40 cm (i.e. about twice the diameter of the ring). In contrast, the initial urine patch area of the urinations for the Device treatment area appears to be a least 2-3 times larger. While this assessment did not provide a quantitative difference between the treatments, it helps explain differences observed between the urine patch soil inorganic N concentrations.

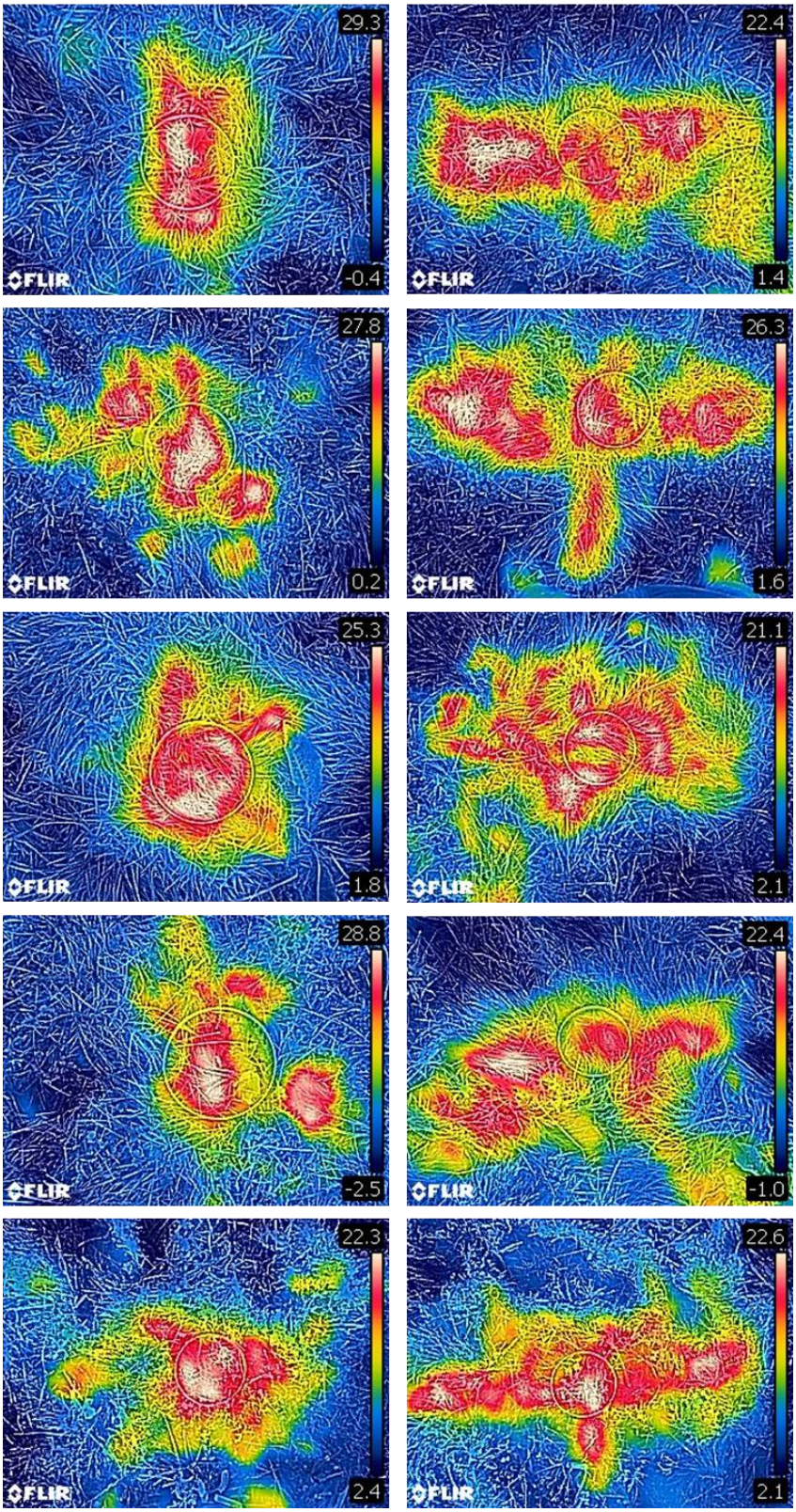


Figure 5.14: Thermal imagery of urine patch sizes for the Control treatment (images on left) and Device treatment (images on right). In all images, the circle in the centre of the urine patch has a diameter of 20 cm, to provide a scale comparison. The values on the right provide the range of temperature (^{o}C) values.

In the 0–10 cm soil depth, both treatments showed a trend of decreasing average inorganic N concentrations with distance from the urine patches (Fig 5.15). The average inorganic N concentrations for the Control treatment urine patches decreased from 276 mg N kg soil^{-1} in the centre of the patches to 25 mg N kg soil^{-1} at 50 cm from the centre. In comparison, the average inorganic N concentrations for the Device treatment urine patches decreased from 128 mg N kg soil^{-1} in centre of the patches to 23 mg N kg soil^{-1} at 50 cm from the centre. The average concentration over the 50 cm length for the Control treatment was more than double the average for the Device treatment urine patches. However, there was a large variation between individual urine patch values, especially within 10 cm from the centre of the urine patches. Consequently, there was no statistical difference (P>0.05) between the treatments. In the 10–30 cm soil depth, inorganic N concentrations were similar for the two treatments and were lower than for the surface 0-10 cm soil depth, with average concentrations not exceeding 62 mg kg soil^{-1}. Therefore, for both treatments, most of the influence of urine patches on elevating soil inorganic N concentrations was in the surface 0-10 soil depth and within 40 cm of the centre of the urine patches.

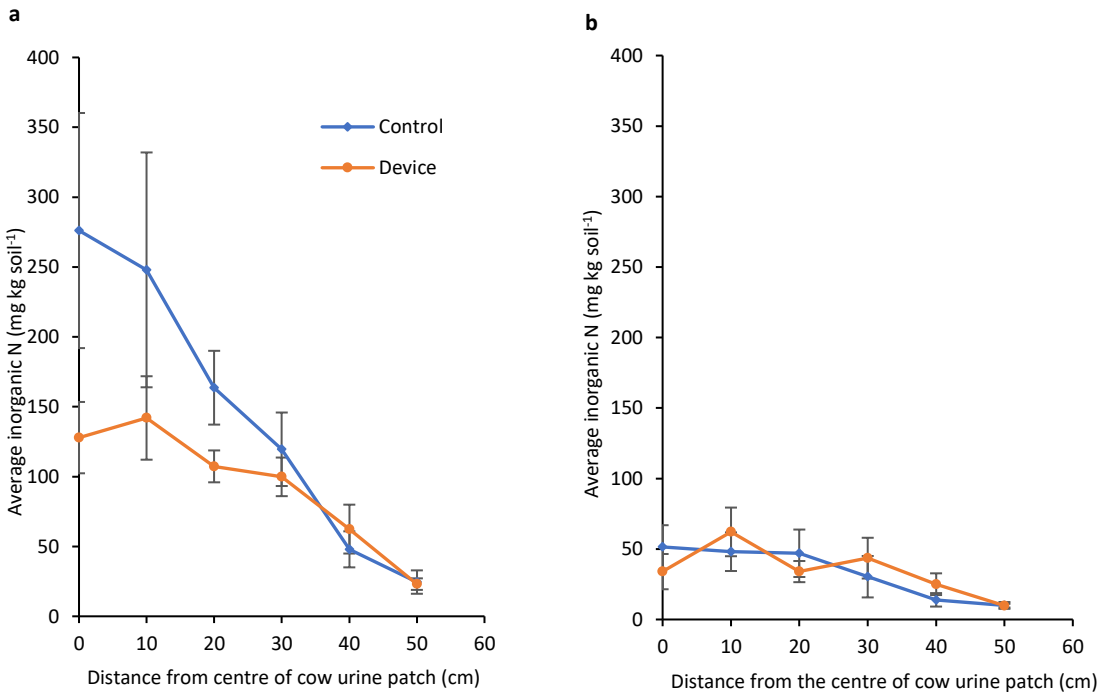


Figure 5.15: Average inorganic N concentrations in (a) 0–10 cm soil depth, (b) 10-30 cm soil depth at different distances from five urinations for each of the Control and Device treatments. The error bars indicate standard errors of the mean (SEM).

5.4 Discussion

5.4.1 Performance of Urine Spreading Device

The extent to which the urine patch is spread depends on the device's ability to intercept and disperse the urine stream. On some cows the device intercepted the urine stream well, but on other cows the device did not work consistently well. The more variable performance of the device on some cows seemed to be due to differences in how the device was positioned on the cows, the back body structure of cows, and how much cows move their tails during urination. Some cows move their tails more to the side during urination, which can move the device sideways and out of the urine stream. At other times, the device was not lifted high enough to intercept the urine stream, causing the urine to go over the top of the splash plate.

As the trial progressed, the adjustments made to the device and removing some of the cows from the Device treatment group for which the device did not fit well, resulted in more consistent performance. This was observed between the first and second grazing events, with an increase in the proportion of observed urinations that were intercepted by the spreading device from 62 to 84%.

At the last grazing event, when the cows wore the devices for 18 hours day^{-1} for three days in a row, 79% of the cows showed nil or minor dirtiness, and only 21% showed moderate levels of dirtiness. The high percentage of nil or minor dirtiness in the cows wearing the device could be attributed to improvements made to the devices throughout the experiment. When the device fits and operates well, then there is less interception of dung, which is intended to go over the top of the device's splash plate.

5.4.2 Nitrate and Total N leaching losses

During this study, drainage water nitrate and TN concentrations started high and decreased as the drainage season progressed, which is a similar trend previously observed at the same site in other studies (Christensen et al., 2019b; Hanly et al., 2017a; Howes, 2019). In the current study, the annual drainage water nitrate and TN drainage losses for the Control treatment were 6.5 and 7.8 kg N ha^{-1} $year^{-1}$, respectively, which is at the lower end of values previously measured at the site. For example, over a combined period of

eight years, from three different studies (Christensen et al., 2019b; Hanly et al., 2017a; Howes, 2019), the annual drainage water TN losses ranged from 6.4 to 26.2 (average of 13.6) kg N ha^{-1} $year^{-1}$. When drainage water N losses are low there is less potential for a mitigation to have a substantive influence on further reductions. However, given that an average of 83% of the TN losses in drainage were as nitrate, for which urine patches are a main source, then there is still potential for mitigations targeting urine patches to influence these losses. This was observed in the first year of the Howes (2019) study, in which the 'Standard grazing' treatment had low drainage water annual nitrate and TN losses of 7.5 and 8.6 kg N ha^{-1} $year^{-1}$, respectively. In that year, the use of targeted duration-controlled grazing (a grazing duration of ca four hours) significantly ($P<0.05$) reduced nitrate and TN losses by 40 and 33%, respectively. However, in the following year of that study, when the 'Standard-grazing' nitrate loss was even lower (5.7 kg N ha^{-1} $year^{-1}$), the treatment difference was not large enough to be statistically different. Therefore, one of the limitations of the current study was only having one year of drainage results. Furthermore, the study year had small drainage N losses.

There were also aspects of the way the current study was conducted that may have influenced the ability to observe treatment differences. One of these was the changes in grazing duration over the four treatment grazings during the Summer to early-Winter period. The first two treatment grazings were short day grazings (~6 hours), which would have had less urine returned compared to the latter two treatment grazings in mid-Autumn (~13.5 hours) and early Winter (~18 hours). With the longer two grazing events being closer to the start of the winter drainage season, especially the last grazing, there would have been less time for plant uptake to remove N from the urine patches. Increased plant uptake of urinary N from a larger spread area is likely to be one of the main mechanisms by which the spreading device is expected to reduce the potential of nitrate leaching from urine patches. However, the process of plant uptake can take weeks to appreciably decrease the nitrate concentrations in soil under urine patches and nitrate leaching potential. In the Ramirez (2017) study, which showed a 64% reduction in net inorganic N below the root zone for the spread urine patch area (1 m^2 cf. 0.2 m^2), there was almost a month between urine application and the commencement of drainage. Therefore, alternating between shorter (e.g. day grazings) and longer grazing duration (e.g. night grazings), which would be more typical of average farm practice, would have been preferable.

Additional improvements to the device to increase the urine spread area may also help reduce leaching losses. At the first treatment grazing event only 62% of the observed urination was intercepted by the urine device. While this increased at subsequent grazing events (77-84%), not having all urination consistently intercepted and spread by the device would have had a bearing on the effectiveness of the Device treatment in reducing drainage water N losses. In addition, although the assessment of spread (Section 5.3.5) using thermal imaging demonstrates that the device does increase the spread area of the urination, compared to no device, it did not result in substantive lower soil inorganic N levels in the urine patch. This is likely because the device did not achieve a uniform application of urine over a larger area (e.g. 1 m^2), as simulated in previous studies (Chapters 3 and 4 in this thesis; (Ramirez, 2017)). Furthermore, Cichota et al. (2018) reported that the lateral movement of urinary N beyond the urine patch (non-device patch) can reduce the N load of urine patches. This contributes to the reduction of differences in soil inorganic N concentrations between the treatments. Overall, before further leaching studies are conducted, improvements in the device in terms of reliability of urine interception and more uniform spread over a larger area are needed.

5.4.3 Pasture accumulation

In this study, there was no difference in pasture accumulation between the Control and Device treatments. Ramirez (2017) also did not observe pasture growth response from an increase in the cow urine patch spread area in autumn. Possible reasons Ramirez (2017) gave for this, were the high clover content of the pasture. In addition, during autumn, background soil inorganic N levels are likely to be better able to supply pasture requirements, which could contribute to pasture not being as responsive to N, or more variable in response, compared to other times of the year (e.g. late-winter and spring) (Cameron et al., 2013; Moir et al., 2011).

Urine patches from any individual grazing event, are deposited on a relatively small proportion of a paddock area per year (i.e. 20-30%), depending on the number of cows (Moir et al., 2011). Therefore, at the paddock or farm levels, the ability of the device to influence total pasture growth by increasing the spread of urine at only four grazing events, will be limited by the relatively small additional area of pasture that is influenced. However, further improvements on the device on urine spread, as discussed above, are needed before further evaluation on pasture production is assessed.

5.5 Conclusions

The objectives of this study were to quantify the effect of a urine spreading device worn by dairy cows during summer and autumn grazing events, on, urine patch area, N leaching and pasture growth accumulation. The differences in N leaching between the Device treatment and the no-device Control treatment were small and not statistically significant. While the device did increase urine spread, further improvements to increase the spread area and the uniformity of spread, may be required. This study was conducted for a single drainage season, which happened to be a season with small overall N leaching losses, making it more difficult to identify treatment differences. Therefore, any further research is recommended to be conducted over multiple drainage seasons.

There was also no statistically significant observed benefit from using the device on pasture production. While improvements to the device to further increase the spread area and uniformity of the urine patch, may be needed for any gains in pasture production, this will depend on the pasture responsiveness to further N at the time of year that the device is used. The device is mainly recommended for use at 4-5 grazing events during the summer and autumn periods to target the urine patches that have the most influence on N leaching. Therefore, the combination of pasture responsiveness to N being more variable at this time of year and the small proportion of the grazed area being influenced by urine patches, may limit the ability of the device to have an important influence on total annual pasture production.

While the dirtiness levels on most of the cows in this study were minor, the device was only on the cows for durations of up to about 18 hours at a time. Therefore, further research evaluating the dirtiness of cows wearing the device over longer durations would also be useful.

Chapter 6

General discussion and recommendations for future research

6.1 Introduction

The expansion and intensification of dairy farming in New Zealand (NZ) over the last few decades is recognised as having a major contribution to the country's greenhouse emissions, and to the nitrogen (N) enrichment of its surface and ground waters. The losses of N from dairy farms, via ammonia (NH_3) volatilisation and nitrous oxide (N_2O) emissions and nitrate (NO_3^-) leaching, are predominantly from cow urinations which deposit N at high concentrations onto pastures in small patches.

The environmental concerns associated with dairy farming have led researchers to investigate potential mitigations to reduce N losses from cow urine patches. Although there are a number of mitigation options available to reduce these N losses to the environment, they vary in their costs and in the difficulty with which they are implemented on different farms. Most involve either reducing the number of urine patches (i.e. reducing grazing duration or stocking rate) or the N concentration of cow urine (i.e. lower N forage crops or supplementary feeds). However, there has been little research conducted on the effect of increasing the spread area of urine patches as a mitigation for N losses. In part, this is due to there being no currently available practical method for increasing the spread area of dairy cow urine patches. The development of a prototype urine spreading device by Novataro Ltd has made the prospect of being able to increase the spread of urine a possibility. The primary aim of this device is to provide a method to decrease NO_3^- leaching from dairy farms by increasing the urine patch area and decreasing the application depth, thereby reducing the N application rate in urine patches. Because this is a novel approach to mitigating NO_3^- leaching, research is required to

quantify the effects of increasing urine spread on N losses from urine patches. Therefore, the main research objectives of this research presented in this thesis are:

- to quantify the effect of increasing the urine patch spread area on NH_3 and N_2O emissions.
- to determine the ability of a proto-type device to spread urine
- to determine the effect of the proto-type device on NO_3^- leaching and pasture production.
- to identify the practical limitations associated with the use of such a device on dairy cows.

This chapter summarises the key findings of the field experiments (Chapters 3-5) conducted to address these objectives. Recommendations for future research is also discussed at the end of this chapter.

6.2 Key findings

6.2.1 Effect of urine spread on ammonia emissions (Chapter 3)

The research presented in Chapter 3 demonstrated that increasing the spread area of urine patches has potential to increase NH_3 emissions. Increasing the urine patch area from 0.25 to 1 m^2 in early autumn increased NH_3 emissions by about 36% and, consequently the emission factor also increased from 25 to 36%. This difference was attributed to the influence that a larger urine patch area has on volatilisation. In this study, a week after the Early-autumn urine application, it was estimated that about three-quarters of the urinary N applied was retained in the 0-50 mm soil depth for the 1 m^2 treatment, compared to only about a quarter for the 0.25 m^2 treatment. As the larger patch retains a greater proportion of urinary N in the surface soil, NH_3 emissions are larger. Although the use of the urine spreading device on dairy cows is mainly intended for use during summer and autumn, rather than year-round, this is also the time of year when the NH_3 emissions from urine patches are expected to be the highest, due to warmer temperatures and drier soil conditions. Therefore, any increase in NH_3 emissions at this time of year is likely to also have a substantial influence on annual emissions.

While NH_3 is not a Greenhouse Gas (GHG), it is considered to be an indirect source of N_2O. New Zealand's N_2O inventory uses the NZIPCC specific emission value of 0.1 for the proportion of livestock manure N that is released into the atmosphere, mainly as NH_3 from urine, to become an indirect source of N_2O (Saggar et al., 2011). Therefore, the increase in NH_3 emissions due to increasing the spread area of early-autumn applied urine, will also increase indirect N_2O emissions. However, the loss of NH_3 from the urine patch also reduces the amount of urinary N that is available for subsequent direct N_2O emissions and NO_3^- leaching.

6.2.2 Effect of urine spread on nitrous oxide emissions (chapter 4)

The research presented in Chapter 4 showed that increasing the size of the urine patches from 0.25 to 1 m^2 in early-winter did not decrease N_2O emissions and could potentially increase N_2O emission by up to 39%, though there was insufficient evidence to support this being a strong statistical difference. In contrast, when this experiment was repeated in early-autumn, increasing the urine patch area from 0.25 to 1 m^2 decreased N_2O emission by 56%. The different effect of increasing the urine patch area in these two different seasons is likely to be attributed to the differences in soil moisture conditions at the time of urine application and the weeks that followed. The lower emissions from increasing the urine spread area in the early-autumn, was likely due to an extended period of about 40 days after urine application of drier soil conditions, which would have increased the opportunity for removing urinary N from the soil via ammonia volatilisation and plant uptake. Spreading urine over a larger area, also initially retains a higher proportion of the urinary N in the surface soil. In both experiments, the quantity of inorganic N per patch in the surface soil (0-50 mm) was initially highest for the 1 m^2 treatment. In the early-winter experiment, which initially had higher soil moisture conditions that favour denitrification, the greater quantity of urinary N in the surface soil contributed to higher N_2O emissions (Bolan et al., 2004; de Klein & van Logtestijn, 1994; Di et al., 2014; Saggar et al., 2009; Uchida & Clough, 2015). Therefore, this research supports that any use of a urine spreading device on dairy cows should be avoided close to or during winter.

This study showed a large reduction in direct N_2O emission could be achieved by increasing the spread of early-autumn applied urine, from 0.25 to 1 m^2. To determine the overall effect of spreading urine on N_2O emissions, it is useful to compare this reduction in N_2O emission with the increase in indirect N_2O emissions from higher NH_3 emissions from the larger spread area of early-autumn urine (Chapter 3). Increasing the urine patch spread area from 0.25 to 1 m^2 is estimated to decrease N_2O emissions by 2.8 g N_2O-N kg N $applied^{-1}$. Whereas increasing urine patch area is also estimated to increase NH_3 emissions by an average of 1.15 g NH_3-N/patch or 102 g NH_3-N kg N $applied^{-1}$. Using the N_2O inventory emission value of 0.1 for indirect N_2O emissions, this increase in NH_3-N emissions has potential to contribute about 10.2 g N_2O-N kg N $applied^{-1}$, which is about 3.6 times higher than the reduction in direct emissions associated with increasing the spread area of early-autumn applied urine. This finding suggests that the overall effect of increasing the spread of early-autumn applied urine also has potential to increase total N_2O emissions, once the indirect emissions of higher NH_3-N emissions are also accounted for. Therefore, whether urine spread is increased in autumn or winter, both practices are expected to result in greater overall accumulation of N_2O in the atmosphere. The device is primarily designed for use during summer and autumn, in order to help reduce the risk of NO_3^- leaching, however, this research highlights even limiting the use of the device to this time of year also has potential to increase a farm's GHG footprint.

This study did not find any improvements in either plant N uptake or pasture DM accumulation from increasing the spread area of the urine patch. However, the impact of the device on pasture could not be fully evaluated in this study, because the edge effects were not included in the measurements for all of the treatments.

6.2.3 Evaluating a urine spreading device and assessing its effect on N leaching and pasture growth (Chapter 5)

The research presented in Chapter 5 demonstrated that differences in N leaching between the Device treatment and no-device Control treatment were small and not statistically significant ($P>0.05$). While the device did increase urine spread, further increases in the spread area and the uniformity of spread may be required if the device is to result in reductions in N leaching. This study was conducted for a single drainage season, which

happened to be a season with smaller N leaching losses, making it more difficult to identify treatment differences.

As for Chapter 4, there was no statistically significant observed benefit from using the device on pasture DM accumulation. While improvements to the device may be needed for any measurable gains in pasture production, any such increase will depend on pasture responsiveness to additional N at the time of year that the device is used. The device is mainly recommended for use at 4-5 grazing events during summer and autumn period to target urine patches that have the most influence on NO_3^- leaching. Therefore, the combination of pasture responsiveness to N being more variable at this time of year and the small proportion of the grazed area being influenced by urine patches, may limit the ability of the device to have an important influence on total annual pasture production.

Further improvements to the device to increase its ability to spread urine to a larger area more uniformly may be required if its use is to result in appreciable reductions in NO_3^- leaching.

In this study, the dirtiness levels on most of the cows wearing the urine spreading device were minor, however, the device was only on the cows for short durations of up to about 18 hours at a time. Therefore, further research is needed to evaluate the dirtiness of cows wearing the device over longer durations.

6.4 Recommendations for future research.

From the results gathered from this research, it is suggested that future research be conducted in the following areas:

- to modify the device so that it can spread urine to patches of greater area in a uniform manner
- determine the ability of an improved device to reduce NO_3^- leaching
- determine the effect of an improved device on N_2O emission from winter grazings
- determine the effect of an improved device on pasture production for multiple drainage seasons with varying drainage and leaching rates.
- determine the effect of different soil types on gas emissions and leaching losses from the urine spreading device.

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Appendices

Appendix A. Climate Data

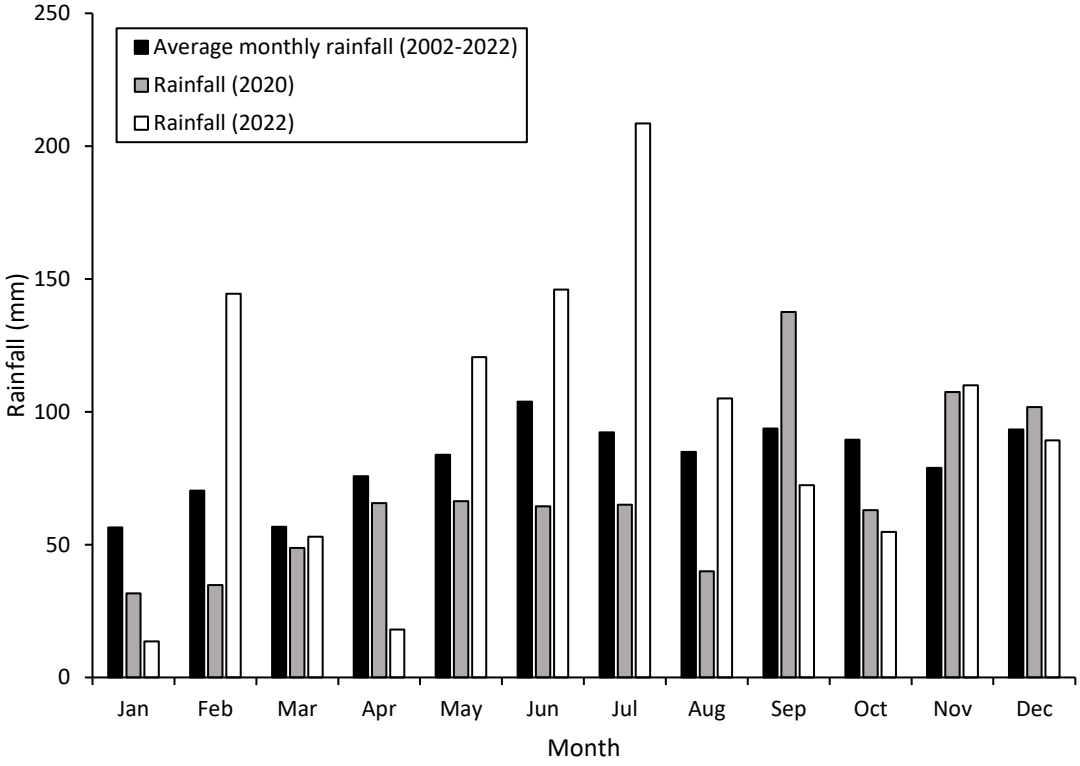

Figure A1: Long-term rainfall averages (2002-2022) and monthly rainfall for the study period in 2020 and 2022.

Appendix B. Net Cumulative Dry Matter

Early-winter experiment (June to September 2020)

*0.25 m^2 patch

- Possible explanation for 0.25 m^2 patch being better at 95 days. All of edge effect likely measured for 0.25 m^2 patch but not for the other two urine patch areas (0.5 and 1 m^2).
- Other two urine patches possibly better if full edge effect was measured.

*Extrapolating DM harvested per patch, measured for each treatment area, to DM harvested per hectare.

0.25 m^2 – 61.81 g DM $patch^{-1}$

0.5 m^2 – 40.94 g DM $patch^{-1}$

1 m^2 – 57.56 g DM $patch^{-1}$

- Extra DM grown ha^{-1} over 95 days in winter from a single grazing event:
  *Assumption: Intake = 8 kg pasture DM cow^{-1} $grazing^{-1}$ (Dry cows).

  24 hour graze

  12 urine patches cow^{-1} $grazing^{-1}$

  Pasture cover 3200 kg DM – Pre-grazing

  1600 kg DM – Post-grazing

  Intake 1600 kg DM cow^{-1}

  Therefore: 1600 kg DM cow^{-1} ÷ 8 kg pasture DM cow^{-1} $grazing^{-1}$ = 200 cows

  *200 cows x 12 urine patch cow^{-1} (1 urine patch per 2 hours) = 2,400 urine patches.

  No. urine patches x g DM $patch^{-1}$ ÷ 1000 = kg DM ha^{-1}

  0.25 m^2 - (2,400 x 61.81) ÷ 1000 = 148.3 kg DM ha^{-1}

  0.5 m^2 - (2,400 x 40.94) ÷ 1000 = 98.3 kg DM ha^{-1}

  1 m^2 - (2,400 x 57.56) ÷ 1000 = 138.1 kg DM ha^{-1}

  *If the edge effect was accounted for, the 1 m^2 urine patch may have been larger than the standard urine patch (0.25 m^2).

Early-autumn experiment (March to August 2022)

* Extrapolating DM harvested per patch, measured for each treatment area, to DM harvested per hectare.

0.25 m^2 – 81.91 g DM $patch^{-1}$

0.5 m^2 – 102.23 g DM $patch^{-1}$

1 m^2 – 127.66 g DM $patch^{-1}$

- Extra DM grown ha^{-1} over 135 days in autumn (Milking cows) from a single grazing event:
  *Assumption: Intake – 8 kg pasture DM cow^{-1} $grazing^{-1}$ (Milking cows). There was 2 grazings per day.

  10 hour graze

5 urine patches cow^{-1} day^{-1}

Pasture cover 3200 kg DM – Pre-grazing

1600 kg DM – Post-grazing

Intake 1600 kg DM cow^{-1}

Therefore: 1600 kg DM/cow ÷ 8 kg DM cow^{-1} grazing^{-1} = 200 cows

*200 cows x 5 urine patch cow^{-1} (1 urine patch per 2 hours) = 1000 urine patches.

No. urine patches x g DM patch^{-1} ÷ 1000 = kg DM ha^{-1}

0.25 m^2 - (1,000 x 81.91) ÷ 1000 = 81.9 kg DM ha^{-1}

0.5 m^2 - (1,000 x 102.23) ÷ 1000 = 102.2 kg DM ha^{-1}

1 m^2 - (1,000 x 127.66) ÷ 1000 = 127.7 kg DM ha^{-1}

*If the edge effect was accounted for, the 1 m^2 urine patch may have been larger than the standard urine patch (0.25 m^2).

- Additional kg DM ha^{-1} from spread
  0.5 m^2 – 102.2 – 81.9 = 20.3 kg DM ha^{-1}

  1 m^2 – 127.7 – 81.9 = 45.8 kg DM ha^{-1}
- Would be potentially more beneficial for pasture production in spring.

Appendix C. Statement of contribution forms (DRC 16)

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

Student name:	May Tana Hedges
Name and title of main supervisor:	Dr. James Hanly
In which chapter is the manuscript/published work?	Chapter 2
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