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EFFECTS OF INCREASING COW URINE DEPOSITION AREA ON SOIL MINERAL NITROGEN MOVEMENT AND PASTURE GROWTH ON A RECENT SOIL IN THE MANAWATU REGION, NEW ZEALAND

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To my parents, Juan Carlos and Melania,
who without expecting anything in return
gave me the best of them.

Abstract

The cow urine patch is a major source of nitrate (NO_3^-) leaching from grazed dairy pasture farms. Increasing the urine deposition area is a direct way of reducing the potential risk of this cause N leaching losses. Research is required to quantity the effectiveness of this mitigation across a range of different soil and climatic conditions. The objective of this study was to determine the effect of increasing the cow urine deposition area on NO_3^- leaching risk and short-term pasture accumulation on Recent soil in the Manawatu Region, New Zealand. A field trial was conducted, which consisted of three treatments evaluated on pasture plots: Urine (1 m^2), Urine (0.2 m^2) and No-urine. The two urine treatments received the same volume of 2.1 L urine/patch. Urine treatments were applied on the 6th of March 2017, and soil inorganic N was measured on three occasions; 15, 36 and 53 days after urine application (DAUA). At the third soil sampling time, which was 24 days after the drainage season was estimated to have commenced, the net inorganic N (inorganic N in the urine treatment minus the value for the No-urine treatment) in the 45-120 cm soil depth was 1.08 g net inorganic N/patch for the Urine (1 m^2) treatment compared to 2.97 g net inorganic N/patch for the Urine (0.2 m2) treatment. Therefore, the Urine (1 m^2) treatment resulted in a 63.6% reduction in the quantity of net inorganic N that was highly susceptible to leaching, compared to the more typical urine patch area of 0.2 m^2. At a paddock scale, when net inorganic N from the urine treatments is multiplied by an estimate of the quantity of urine patches per hectare in a single grazing, this equates to a reduction of 2.53 kg N/ha from a single autumn grazing. It is expected that increasing urine deposition area at multiple grazings would result in greater reductions in the annual NO_3^- leaching risk.

Over the two pasture harvests conducted in the trial, the pasture DM accumulation for the No-urine treatment produced an average of 3220 kg DM/ha. The two urine patch treatments achieved a similar level of pasture DM accumulation to that of the No-urine treatment. The lack of a pasture growth response from the added urine could have been influenced by the high clover content (35.9%) of the pasture, and in addition, there may have been adequate background soil mineral N levels, which together could have contributed to N not being growth limiting during the trial.

This research has demonstrated that increasing cow urine deposition area in autumn has potential to be an effective mitigation for decreasing N leaching losses from grazed dairy pastures. Further research is required to investigate the effects of increasing cow urine deposition area at multiple grazings, in order to determine the effect of this mitigation option on annual NO_3^- leaching and pasture production.

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Chapter 1. Introduction

In New Zealand the main agricultural land use is pastoral farming, with dairy farming being the predominant type of intensive pastoral farming (Di & Cameron, 2007). Dairy farming has continued to grow in this country, with the total dairy cattle population increasing from 5 million in 2002 to 6.6 million in 2016 (Statistics New Zealand, 2017). This growth in dairy cow numbers has resulted from both, the expansion and intensification of dairy farming, which has environmental implications. In particular, of prime concern is nitrate (NO_3^-) leaching losses from topsoil and the subsequent contamination of ground and surface waters (de Klein et al., 2010).

In grazed dairy pastures, the main source of NO_3^- leaching is from the urine-nitrogen (N) deposited by cows onto the pasture, because a high proportion of the N ingested by cows is excreted in urine. Typically, the urine-N is deposited at high concentrations in relatively small patches. Consequently, the NO_3^- that accumulates in the urine patches frequently exceeds the immediate plant requirements for growth, which reduces N use efficiency and the excess NO_3^- not taken up by plants is at risk of leaching when drainage occurs (Haynes & Williams, 1993).

It is becoming increasingly important to find efficient strategies to meet the dual challenge of increasing productivity and minimising the consequential environmental impacts, particularly as regional councils develop and implement plans that involve limiting farm N losses to water. For more than a decade, much research in New Zealand have focused on different potential mitigation strategies to reduce N leaching losses from pastoral farms, which has included the use of nitrification inhibitors, grazing management (e.g. restricted or duration-controlled grazing), cows diet manipulations and the use of different pasture species (de Klein et al., 2010; Selbie et al., 2015). However, there are still limited cost effective options for farmers to achieve significant N leaching reductions.

It has been shown that increasing the cow's urine deposition area could be an effective strategy to reduce the N leaching losses (Bryant et al., 2007). While this approach has the potential to be a low cost method for N leaching mitigation, its effectiveness has had limited evaluation until relatively recently (Bagley et al., 2016). This is, in part, due to no current method being commercially available to practically increase a cow's urine deposition area. However, in order to determine whether such a device has promise, further

research is required to determine potential benefits in terms of the potential to reduce N losses to water and also whether there is an effect on pasture production.

The objectives of this study were to evaluate the effect of increasing cow urine deposition area on the potential to reduce the risk of N leaching losses and increase pasture production on a Recent soil in the Manawatu Region, New Zealand. To achieve these objectives a field trial was established in the Manawatu Region, which quantified the effect of increasing urine patch size in autumn (1) on changes in soil mineral N in the soil profile over time, (2) on the potential for decreasing losses of soil mineral N below the effective root zone, and (3) on pasture accumulation.

Chapter 2. Literature Review

2.1 Introduction

The majority of New Zealand pastoral farms, including dairying, involve grazing livestock outside on pastures and/or fodder crops year-round, with little or no housing (Di & Cameron, 2007). A major adverse effect of these agricultural activities is the NO_3^- leaching, which affects surface and groundwater quality (Di & Cameron, 2002b). The main source of leached NO_3^- is the N excreted by the cows in the urine depositions (Decau et al., 2004).

This literature review presents an overview of the existing information on the N cycle in pasture systems, the contribution of urine depositions to NO_3^- leaching, as well as the characteristics and conditions that contribute to these losses. A summary of previous research on NO_3^- leaching mitigations is also presented.

2.2 Importance of Nitrogen in the agriculture

Nitrogen is one of the most abundant elements in the environment, and is also an essential nutrient for the growth of plants and animals (Follett, 2008). Nitrogen is often the most limiting factor in crop production, because even though it is naturally abundant, it is not all directly available to plants; due to most of it (99%) being in the form of molecular nitrogen (N_2) in the atmosphere (Blumenthal et al., 2008; Galloway et al., 2003; McLaren & Cameron, 1996). For N_2 to become available to plants, it must be biologically fixed via the symbiotic relationship between legumes and rhizobium bacteria or it needs to be manufactured by chemical processes used in fertiliser production (McLaren & Cameron, 1996). Consequently, in agriculture the use of legumes that promote biological N fixation and the application of N fertiliser, as well as the recycling of N through animal excreta and plant residues, are the main inputs of N for plants growth (Blumenthal et al., 2008).

The introduction of the use of N fertiliser has brought greater food security and assisted the rapid worldwide population growth experienced for more than half a century. But the increased use of N fertilisers has also lead to higher transfers of N to the aquatic environment. Therefore, there is a need for a transition of the current intensive agricultural systems into highly efficient resource use systems. This involves supplying the necessary

N for crop growth, but achieving higher agronomic N use efficiency, which will reduce losses to surface and ground waters (Spiertz, 2010).

2.3 Nitrogen cycle in pastoral systems

Soils contain between 0.1% and 0.6% of N in the top 15 cm, and is present in four major forms: (i) organic matter; (ii) soil organisms and microorganism; (iii) ammonium ions (NH_4^+) held by clay minerals and (iv) mineral-N forms in soil solution, including NH_4^+, NO_3^- and low concentrations of nitrite (NO_2^-) (Cameron et al., 2013). The transfer of N from one form to another within the soil/plant/animal/atmosphere N cycle is illustrated in Figure 2.1.

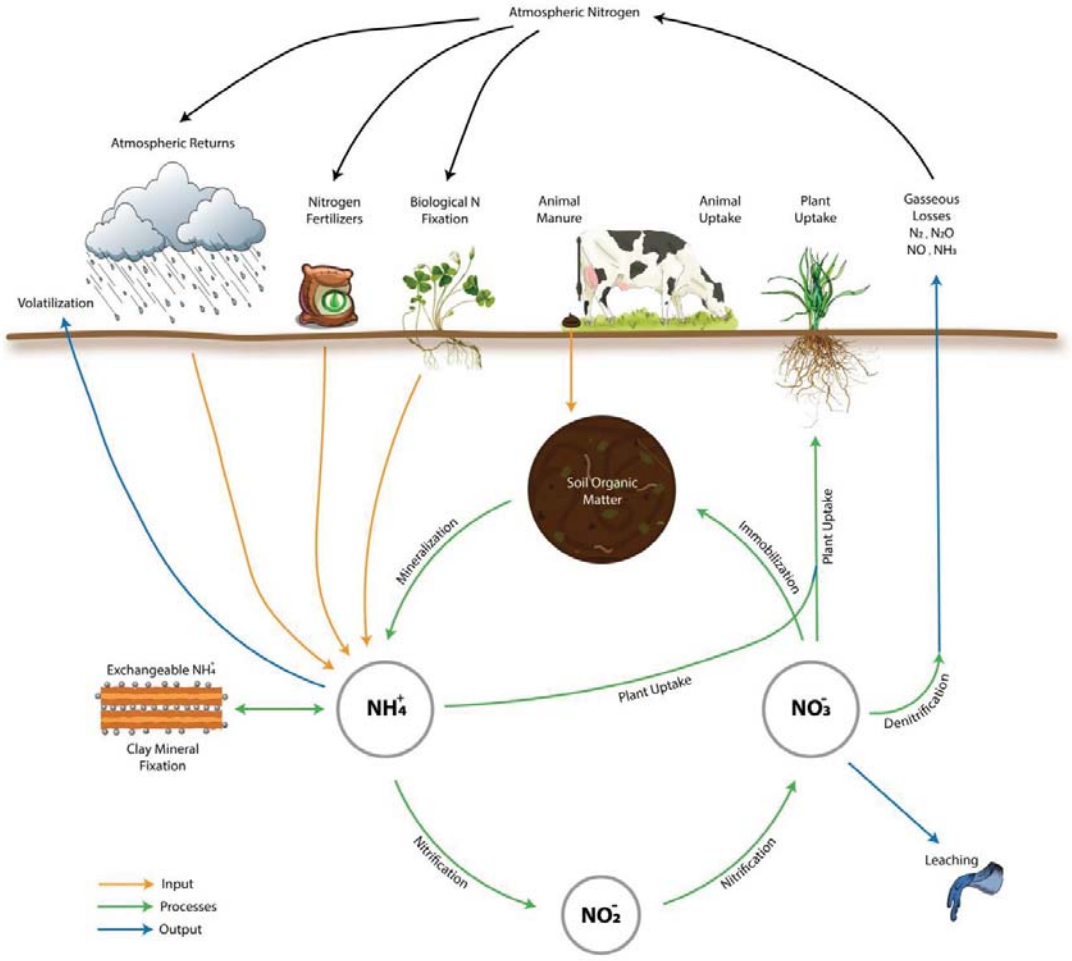

Figure 2.1. The Nitrogen Cycle in agricultural systems (Modified after Di and Cameron (2002b)).

The gains, losses, and transformations of N within the soil/plant system affect the availability of N to plants and the transfer of N to the environment. Therefore, in order to achieve a better nutrient management through more efficient N use, it is important to understand the processes that are part of the N cycle (Cameron et al., 2013).

2.3.1 Nitrogen inputs

In pastoral systems, the N inputs include the addition of fertilisers, biological nitrogen fixation (BNF), atmospheric N deposition, excreta from grazing animals (urine and dung), and effluents applied to land (Di & Cameron, 2002b; Follett, 2008; Ussiri & Lal, 2013a).

When N fertiliser use in pastures is increased this can result in a reduction of N inputs through BNF. The addition of N fertiliser increases the inorganic N in the soil, therefore, the legumes responsible of the BNF, tend to take up the available N instead of fixing the N_2 from the atmosphere because, as the latter has a higher energy demand (Ledgard & Steele, 1992; Parfitt et al., 2012). Additionally, the high soil inorganic N conditions promotes grass growth, which eventually dominates due to its competitive advantages over legumes (Ledgard, 2001).

In New Zealand, inputs of N deposition are small due to less intensive industrial systems, and being an island, the prevailing wind patterns tend to be blown the gaseous forms of N (e.g. ammonia, nitrous oxide) out to sea rather than deposited on land (Parfitt et al., 2006). Nonetheless, wet deposition from the rain in industrialised areas is estimated to be only 1-5 kg N/ha/yr (Parfitt et al., 2008).

The inputs through the effluents applied to land do not introduce high concentrations of N to the soil, compared to direct animal excreta depositions, because the effluent is diluted and evenly spread. Effluent is typically composed of approximately 10% dung and urine, diluted with wash-down water (Longhurst et al., 2000).

In grazed pastures, a substantial amount of N is returned as animal excretal depositions, in fact, from the N ingested by the animals in the pasture, between 5 and 35% of the N in pasture protein is converted into animal protein, and the remaining N is returned to the soil as dung and urine (Singh, 2006). The contribution of urine patches will be discussed in more detail in section 2.4.

2.3.2 Nitrogen transformations

The turnover on N through the different pools (plants, microorganisms, mineral soil, soil organic matter and the soil solution), or its loss from them, can be very fast. Therefore, and in order to improve the N use efficiency, and reduce losses, it is important to understand the transformation processes.

Mineralisation is the process in which the plant unavailable organic N forms are converted by microorganisms, into inorganic N forms (Ussiri & Lal, 2013b). The microorganisms breakdown proteins of the organic matter into amino acids, and then transform them into ammonia (NH_3) (ammonification), then the NH_3 released in this process is hydrolysed to NH_4^+. The NH_4^+ ions are further converted to NO_3^- to complete the mineralisation process (McLaren & Cameron, 1996). In addition to nitrification, the NH_4^+ ions can follow other different fates, including (i) plant uptake, (ii) retention into soil, (iii) loss through NH_3 volatilisation and (iv) utilisation by the microorganism (i.e. immobilisation) (Ussiri & Lal, 2013b). Exchangeable N occurs when NH_4^+ ions are adsorbed by cation exchange sites on the surfaces of clay and organic matter in the soil (McLaren & Cameron, 1996). The NH_4^+ ions are exchangeable, and when other cations are added to the soil solution, the NH_4^+ ions on the cation exchange sites are released back into solution (Singh, 2006).

The nitrification process, that converts NH_4^+ to NO_3^-, is mediated by specific nitrifying bacteria, and involves a two-step process; first the NH_4^+ is oxidised into NO_2^-, which is mainly carried out by microorganisms called Nitrosomonas; the second part of the reaction is the conversion of NO_2^- to NO_3^-, and depends on a genus of bacteria called Nitrobacter. The conversion rate of NO_2^- to NO_3^- is very fast and, therefore, there is negligible accumulation of NO_2^- in the soil (McLaren & Cameron, 1996; Singh, 2006). Nitrates formed through nitrification can follow various pathways, including (i) plant uptake, (ii) leaching losses, (iii) denitrification and (iv) immobilisation (Ussiri & Lal, 2013b).

Denitrification is the process in which the NO_3^- is reduced to N_2 gas, nitrous oxide (N_2O) or nitric oxide (NO) (Follett, 2008). This process results in the loss of plant available N and also in the release of N_2O, which is a potent greenhouse gas (Singh, 2006).

2.3.3 Nitrogen removed

Nitrogen removal from the soil-plant-animal system includes productive outputs, which are plant and animal tissues that are sold as products (crops harvested, milk, fibre or meat). But can also include unproductive losses, which have previously been described, including; gaseous losses, losses to water and immobilisation into soil organic matter.

Pasture plants can uptake N either as NO_3^- or NH_4^+, with pastures taking up between 200 to 700 kg N/ha/yr (Whitehead, 1995). From the pasture consumed by the grazing animals, only a small amount of the N ingested is converted into animal products, while the majority of the plant N consumed, between 60 – 90%, is returned to the soil pasture system in form of urine and dung (Di & Cameron, 2002b).

Ammonia volatilisation, which can represent a significant loss of N, is undesirable as the NH_3 can also threaten the environment, mainly because some volatilised NH_3 can returned to the earth surface through deposition, causing acidification and eutrophication in the natural ecosystems (Cameron et al., 2013). It is estimated that losses from urine patches generally represent between 15-25% of urine N (Haynes & Williams, 1993).

Leaching is referred to the loss of N through the soil profile as a result of the movement of drainage water (Nieder & Benbi, 2008). The main form of N leached is NO_3^-, due to its negative charge it is repelled by the cation exchange sites of the soil particles, therefore, it easily moves when water drains through the soil (McLaren & Cameron, 1996). Nonetheless, small proportions of NH_4^+ can also be lost by leaching (Nieder & Benbi, 2008). Likewise, Van Kessel et al. (2009) suggested that the losses of dissolved organic N (DON) through leaching can also contribute to the total N leached.

According to Cameron et al. (2013), the leaching of NO_3^- depends on (i) the concentration of NO_3^- present in the soil solution and (ii) the amount of drainage moving through the soil on a given period of time. The concentration of NO_3^- on the soil depends on the N inputs going into the system, nitrification rate, denitrification rate, net immobilisation rate and plant uptake. Meanwhile, the drainage occurs when the soil is at or near field capacity and when water inputs exceed evapotranspiration (Selbie, 2014). Therefore, in most areas of New Zealand, NO_3^- leaching occurs mainly in late autumn, winter and early spring. The leaching occurs whenever the amounts of mineral N present in

the soil is greater than the plants can assimilate, no matter the source of N (McLaren & Cameron, 1996).

Nitrate leaching is controlled by a number of factors such as season and climate, soil properties and land use.

(i) Season and climate: NO_3^- leaching is usually greatest when plant uptake of NO_3^- is low and the occurrence of drainage is high. Some extreme conditions can also cause higher rates of leaching. In very dry summers, NO_3^- tends to accumulate on the soil surface, due to the lack of water, the plant grow is low and N uptake is limited, therefore, the accumulated NO_3^- can leach when the drainage starts. Moreover, rewetting of the soil after a long period of drought can cause a rise of the mineralisation rate generating further NO_3^- to be leached (Cameron et al., 2013).

(ii) Soil properties: Generally, under the same climate conditions, NO_3^- leaching is greater in sandy soils than in clay soil, mainly because in clay soils the water holding capacity is higher. Additionally, in clay soils the potential for denitrification to occur is higher, which helps to reduce the amount of NO_3^- leaching (Cameron et al., 2013; Cuttle, 2008; Decau et al., 2003; Lantinga et al., 1987).

(iii) Land use systems: In general, N leaching increases with the intensification of the land use (McLaren & Cameron, 1996). In many regions of New Zealand, the land use making a major contribution to NO_3^- leaching is grazed dairy pastures, because it is the largest type of intensive farming. In these systems.

2.4 Nitrogen return as urine

Between 60-90% of the N ingested by the grazing animals is returned to the pasture in small concentrated areas of urine and dung patches (Cameron et al., 2013; Di & Cameron, 2002b). The percentage of the N returned to pasture depends on a range of factors including; animal type, production levels, N concentration in the herbage, and additional feed (Decau et al., 2003; Lantinga et al., 1987). The N returned in urine depositions is high compared to dung, with the concentrations of total N ranging from 3.4 to 5.0 g N/kg in dung, while in urine the concentrations range between 3.0 to 20.5 g N/L (Dijkstra et al., 2013; Petersen et al., 1998). In addition, the amount of readily mineralisable N is higher in urine because most of the N is in form of urea, which is

hydrolysed within a few days after the depositions, while in dung patches much of the N is bond in more complex forms of organic N (Wachendorf et al., 2005).

The N loading rate in a cow urine patch has been reported to be between 400 – 1366 kg N/ha (Cameron et al., 2013; Di & Cameron, 2002b; Pakro & Dillon, 1995). Selbie et al. (2015) calculated an average urine N loading rate of around 613 kg N/ha; the same authors indicate that Saarijärvi and Virkajärvi (2009) conducted the only study which measured urine concentration, volume and wetted area from dairy cows urinations at the same time and, therefore, they calculated a N loading rate of 514 kg N/ha. Furthermore, Pakro and Dillon (1995) documented the variations of N concentration in fresh urine over the year, reporting 653 kg N/ha for autumn urine depositions and 1366 kg N/ha for a spring urine depositions.

The high N loading rate in the urine, due to the urine being deposited to a relatively small area, means that the pasture cannot use all the available N, which can lead a surplus of NO_3^- that is susceptible to leaching losses when drainage occurs (Cameron et al., 2013; Di & Cameron, 2002b).

2.4.1 Urine patch area and number of depositions

It has been reported that the wetted area covered by a single urination can range from 0.16 - 0.49 m^2 (Haynes & Williams, 1993); with an average area of 0.24 m^2 (Selbie et al., 2015). Williams and Haynes (1994) found that urine can penetrate to a depth of 400 mm into the soil.

The area of pasture influenced by urine is more than twice the area actually wetted by the deposition, with a single urination being able to stimulate the pasture growth of an average area of 0.68 m^2 (Lantinga et al., 1987). Therefore, the area affected by urine patches can be divided into two parts: (i) the wetted area, which correspond to the area in which the urine is directed deposited, and (ii) the area immediately outside the wetted area, which is influenced by the urine deposition, because of the spread of plants roots on the edge of the wetted area, which are able to uptake the nearby urinary N (Moir et al., 2011). The combination of these two areas is called as the “effective area” of a urine patch (Selbie et al., 2015).

A study conducted by (Decau et al., 2003) showed the influence of urine patches on pasture growth by applying ^{15}N labelled urine to a 0.4 m^2 area (wetted area). Herbage was

collected a total area of 2 m^2, which was made up of separate samples from (i) the wetted area (0.4 m^2), (ii) an intermediate area of 0.63 m^2, and (iii) an outer area of 0.97 m^2. Over the experimental period, the authors found that the urinary N uptake by the pasture were < 0.5 g N/m^2 in the outer area; ~ 5 g N/m^2 in the intermediate area and; between 15 – 20 g N/m^2 in the wetted area. From these results, (Decau et al., 2003) concluded that the urinary-N uptake by the plants in the outside area of the urine patch was due to soil N diffusion and that the urinary N did not diffuse much beyond 20 cm from the edge of the patch.

Cows may urinate around 10-12 times per day (Cameron et al., 2013; Di & Cameron, 2002b; Lantinga et al., 1987), and in each urination the volumes can range from 0.30 to 7.83 L/event (Betteridge et al., 1986), with the average urination volume being 2.1 L/event (Betteridge et al., 1986; Selbie et al., 2015). The large variability in the volume of urine excreted is influenced by mineral and water intake (Farrell et al., 2016).

The paddock area covered by urine patches depends on the stocking rate; for instance, Pleasants et al. (2007) found that in one grazing event (24 h period), with a stocking rate of 180 cows/ha the urine patches will cover 8.62% of the paddock. Annually urine patches can cover around 20-30% of the grazed paddock (Cameron et al., 2013; Di & Cameron, 2002b). The total area covered by urine patches can vary significantly between seasons and between year. Generally in spring and summer higher coverages occur, while during autumn and winter the coverage is lower, because the number of deposition events are greater in summer and spring than in autumn and winter (Moir et al., 2011). The variations in the urine patch sizes and coverage between seasons are mainly because of the seasonal variation in animal feed crude protein content and water intake and changes in the number of livestock on farm. For instance, in autumn and winter, the feed and water intake are lower; additionally to this, at the end of the milking season in late autumn, the stocking number starts to decrease, resulting fewer depositions events and consequently less area covered. During winter, when cows are not being milked the grazing rotation is longer due to lower feed requirements and because a proportion of the herd may be wintered off of the farm. During spring, even though the temperatures can be similar to autumn, the feed intake as well as the stock number start to increase again, generating larger urine patches, more depositions events, as a result, more covered area.

The urine patch area and volume of soil affected is also influenced by the volume of urine deposited, as well as the soil characteristics such as slope, surface microtopography, moisture, porosity, vegetation cover and other soil physical conditions (e.g. infiltration rates) (Selbie et al., 2015). The variation in the area and soil volume affected by urine, in combination with soil heterogeneity and climatic conditions, creates a large variations in the potential for urinary N to be taken by the plants, as well as to be lost by leaching and/or volatilisation (Selbie et al., 2015).

Regarding the distribution of the urine patches within paddocks; Haynes and Williams (1993) indicate that the distribution is uneven and depends on animal behaviour. For example, livestock congregation sites, such as around water troughs, sheltered areas, ridges or hills, and feeding areas, can receive a greater rate of urine depositions compared to other areas. This is due to found that the distribution of excreta return being highly related to the time that the cows spend in an area (White et al., 2001).

2.4.2 Urine patches composition

The N in the urine can be presented in diverse forms, and the concentration is variable and ranges from 3.0 to 20.5 g N/l. Nonetheless, the predominant form of N is the urea, and its concentrations in the urine can varied between 2.1 and 19.2 g N/l, (Dijkstra et al., 2013), with average urea-N concentration of 6.9 g/L (Selbie et al., 2015). The urea concentration in the urine can be highly variable, depending on the cows diet, as well as water consumption. Urea concentrations, in urine ranges from 52.1% to 93.5% of total N, but is more typically between 70 – 90% (Di & Cameron, 2002b; Dijkstra et al., 2013).

Since most of the N in the urine patches is in form of urea, after the urine deposition, the urea content hydrolyses rapidly, leading to the transformations to NH_4^+ and NO_3^-, consequently these last components are available for plant uptake and/or leaching through the drainage (Dijkstra et al., 2013). Bristow et al. (1992) found that from the total N measured in cow urine, 69% was present as urea; while 7.3% was presented as allantoin, 5.8% as hippuric acid, 3.7 % as creatinine, 2.5% as creatine, 1.3% as uric acid, 0.5% as xanthine plus hypoxanthine, 1.3% as free amino acid N and 2.8% as ammonia. These non-urea forms of N are still labile and can contribute to the N mobility in the short term, although little is known about the transformation dynamics of these compounds in the urine patches.

The N concentrations of individual urinations events can show high variability within days, between days, between individual animals and between weather conditions (Betteridge et al., 1986; Hoogendoorn et al., 2010). For instance, Betteridge et al. (2013) measured N concentrations and volumes of individual urinations events and found that urinary N loads were typically higher during the night urinations than during the daytime. The urinations near the sunrise contained large N loads, but thereafter, the loads started to decrease. The higher N loads in the morning are likely to be due to cows consuming less water during the early hours of the morning.

In general, higher N diets increase the amount of N in the urine patches, but not necessarily higher urine N concentrations, mainly because water intake also influences concentration. Urea N content in the urine increases with high protein diets, but decreases with an increase in the supply of energy to the rumen microorganisms and to the host animal. Van Vuuren and Meijs (1987) found that if grazing cows are feeding with good quality supplements or with a relatively high energy and low protein forages, the efficiency of N utilisation can be improved, resulting in a reduction of the N deposited in the urine patches.

2.4.3 Fate of urine nitrogen

Once urine is deposited in the soil, significant changes of the soil conditions take places, such as pH, moisture, N and C concentrations, which influence the soil N transformations and the different pathways that the N can follow. When the urea content is rapidly hydrolysed to NH_4^+, the release of hydroxide ions (OH^-) raises the soil pH for up to 5 days after urine deposition. The high pH conditions favour the conversion of NH_4^+ to NH_3, which result in a portion of the N being lost as NH_3 gas via volatilisation. Over the subsequent 7-14 days the majority of NH_4^+ is converted to NO_3^-, which decreases the soil pH (Haynes & Williams, 1993; Holland & During, 1977; Williams & Haynes, 1994, 2000). Soil temperature is one of the main factors that influences nitrification rate. At temperatures between 7.5°C and 10°C nitrification is slow and is complete just after 60 days of the urine deposition, while with temperatures above 15°C nitrification can be completed within 30 days (Haynes & Williams, 1993).

Williams and Haynes (2000) reported that with time, the NO_3^- content of urine-treated soils decrease in the 0-15 cm depth; reaching control plot levels within 33 days in

treatments with short-term pastures (arable farm, rotation with crops), and 77 days under long-term pastures (permanent pasture). The decrease of soil NO_3^- is due to the range of transformations and/or losses previously mentioned, of those plant uptake and leaching losses are discussed in more details in the following sections.

2.4.3.1 Plant urinary N uptake and growth response

Urine patches influence pasture growth and nutrient content (Williams & Haynes, 1994). Di and Cameron (2002a) found that where urine was applied the amount of N offtake in pasture herbage was high, ranging from 421 – 587 kg N/ha/yr. While Di and Cameron (2007) reported that the application of urine at a rates of 0, 300, 700 and 1000 kg N/ha significantly increased herbage dry matter yield from 4.42 t/ha to 10.82, 13.90 and 19.74 t/ha respectively. Plant response to urine depositions is greatest when the pasture growth is less restricted by environmental conditions, such us low soil moisture during summer, or low temperatures during winter (Selbie et al., 2015). An experiment conducted from February to December (2009) determined that with 6 urine applications, from March to August (one application each month), at a rate of 800 kg N/ha, the pasture growth rate only increased after rainfall in April (Shepherd et al., 2010). At other times of the year either soil temperature or soil moisture were more limiting than N.

The pasture uptake of N and growth response also depends on the pasture composition (Haynes, 1980). In grass/clover pastures, the yield response to added N comes directly from the grass component, since clover is a poor competitor for available soil N (Haynes & Williams, 1993). Ball et al. (1979) found that in late spring-early summer, the applications of 0, 300 and 600 kg/ha of urinary N resulted in a proportion of clover in the pasture of 48, 19, and 12%, respectively, showing a suppression of clover from increasing rates of N. In addition, Saunders et al. (1982) reported that urine caused a marked depression in N fixation by clovers, particularly during the winter period.

2.4.3.2 Nitrate leaching

Losses of N leaching in grazed pasture occurs when urine-N rate exceeds the pasture's ability to use it, then surplus NO_3^- remains in the soil until drainage occur, which transports the NO_3^- down the soil profile (Buckthought et al., 2015; Cameron et al., 2013; Di & Cameron, 2002c). In New Zealand, NO_3^- leaching from dairy pastures is highly

variable, being influenced by soils characteristics, climatic conditions, irrigations use, pasture age and grazing management (Cameron et al., 2013; Di & Cameron, 2002a). According to Cichota et al. (2013), the amount of N that is leached from urine depositions is highly dependent on the interaction between the soil's capacity to retain the deposited N and the plant's ability to utilise it, at the same time, the weather is the major driver of these two conditions, influencing the plant growth and the consequent plant uptake, and the N leaching through the drainage. For example, for a Gley soil, with high rates of denitrification, the N losses to water modelled in the OVERSEER® Nutrient Budgets model can be lower than 15 kg N/ha/year. In contrast, for an irrigated stony Recent soil values can be higher than 60 kg N/ha/yr. More typically, N losses to water are in the range of 24 - 42 kg N/ha/yr (OVERSEER® Nutrient Budgets benchmark values for dairy farms). Nitrogen fertiliser use is another factor that can influences N losses to water, with higher rates of N fertiliser use increasing both direct loses from the fertiliser and by increasing urinary N losses. In a study that used N fertilisers inputs of 0, 100, 200 and 400 kg N/ha, resulted in mean annual leaching losses of NO_3^--N of 30, 34, 46 and 56 kg N/ha/year, respectively (Monaghan et al., 2000).

Decau et al. (2004) measured leaching from two levels of urine applications (525 and 825 kg N/ha) over a 0.4 m^2 area, in combination with three level of N fertiliser (0, 150 and 300 kg N/ha/year) applied in five dressings per year, and applied in three different seasons (spring, summer and autumn). This study found that the most important factor influencing N leaching was the amount of N deposited as urine. One additional kilogram of N added to the grass through the urine increased the N leaching three times more than 1 kg of N applied as fertiliser. This effect can be explained by the deposition characteristic of these two sources. The deposition of fertilisers is usually split in different dressings and is evenly distributed on the grass, in contrast, urine deposition is just on one given time and on a small area of the grass. In addition, Buckthought et al. (2015) showed that the potential of leaching from N fertiliser applied to urine patches is also low, and that avoiding N fertilisation over urine patches only reduced leaching losses by 2%.

Several studies have shown that the timing of urine deposition is an important factor determining the fate of the N deposited with urinations. In New Zealand, with higher drainage rates and lower pasture growth rates in winter result in this being the main period of NO_3^- leaching. However, the grazing events supplying urinary N that contributes to

these losses do not all occur at this time. For example, Di and Cameron (2002a) quantified that autumn urine applications contributed 38-58% of the annual leaching losses. Decau et al. (2003) also found that the autumn urine application contribution to the N leaching was greater than spring and summer applications. Moreover, Decau et al. (2004) found that urinary N contribution to total N leaching per season was 4.3, 12.9 and 21.4% for spring, summer and autumn, respectively. This can be explained by there being longer period for pasture to remove N from urine patches from the spring and summer grazing events, compared to the autumn grazing events, before the commencement of the winter drainage. Shepherd et al. (2010) identified high NO_3^- leaching risk from urine deposited between March and June, which correspond to autumn and early winter. In addition, Shepherd et al. (2011) registered the greatest losses from May urine applications, which correspond to late autumn, but also showed the grazing events as early in the season as late summer also contributed to leaching when drainage commenced.

2.5 Environmental impacts NO_3^- leaching

The main problem with NO_3^- leaching is the contamination of ground and surface waters, which represents a potential threat to human health and the wider environment (Di & Cameron, 2002b). High concentration of NO_3^- in drinking water can interfere with the transport of oxygen in the blood; particularly in infants, causing methemoglobinemia or blue-baby syndrome (Di & Cameron, 2002b). Additionally, the ingestion of NO_3^- in excess can cause respiratory infections, thyroid problems, birth defects, childhood diabetes and some cancers, such as colon, ovarian and stomach cancer (Bibi et al., 2016).

From the environmental point of view, leaching of NO_3^- into surface and ground water can disturb the aquatic ecosystems contributing to eutrophication, and high concentrations of NO_3^- can be too toxic for the survival of aquatic life. Eutrophication is an excessive plant and algae growth that can result in the alteration of the water quality (Boyd, 2015). Some of the adverse effects of the eutrophication are the depletion of dissolved oxygen in the water, greater sedimentation in the bed of the water body, due to the decomposition of dead algae, and higher turbidity resulting in less sunlight penetration.

2.6 Mitigation options for reducing N losses to water

The increased expansion and intensification of dairying in New Zealand over the last two decades, has resulted in a number of regional councils developing plans that include limits for N losses to water from intensive farms. Consequently, more research has focused on identifying and evaluating suitable mitigation options to reduce N leaching losses from pastoral farms, some of which are described in this section.

In order to maximise N efficiency to achieve lower losses of N to water, it is important to identify all the possible interventions at each step of the N cycle (Figure 2.2). For instance the intervention can be focused on: i) reducing N inputs, while maintaining production levels, ii) better N utilisation within the farm system, or iii) capturing or re-using N before it enters the different pathways (de Klein et al., 2010).

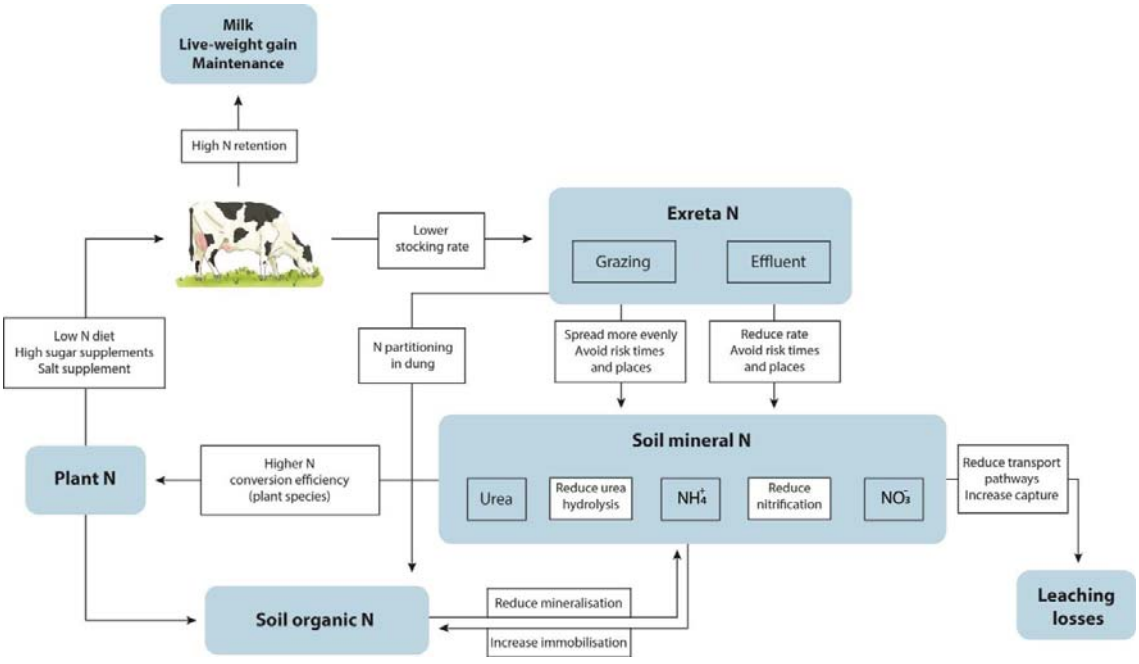

Figure 2.2. Intervention options in the N cycle to reduce N losses through leaching, for a grass/clover pastoral farm (Modified after de Klein et al. (2010)).

2.6.1 Duration – controlled grazing

One mitigation option to reduce the deposition of urinary N in concentrated patches is restricted grazing, which is a management system that limits the time that the cows spend grazing pasture. One specific type of restricted grazing, called duration-controlled (DC), involves two grazings of 4 hours per 24-hour period, while the remaining time the

cows spend being milked or in an animal shelter or stand-off facility where the animals receive supplementary feed (Christensen et al., 2012a). During the time the cows spend on stand-off facilities, the excreta can be collected as effluent, and later can be returned to the soils more evenly and at relatively low rates of N, which will help to reduce the risk of N leaching as well as maintain pasture production (Christensen et al., 2012b). Therefore, the DC grazing system is a strategy to improve N use efficiency by reducing the spatial variability in excreta nutrient return that is caused by the grazing animal. When Christensen et al. (2012b) used DC grazing the year-round, and compared it with a standard grazing (SG) of 7 hours day grazing and 12 hours night grazing, for three years, the DC grazing resulted in a reduction in NO_3^--N leaching of more than 50% compared with the SG.

When de Klein et al. (2006) restricted the autumn grazing of dairy cows to only 3 hours per grazing during March, April and May, for three years, the restricted autumn grazing reduced annual NO_3^- leaching losses by 41%, compared to the standard grazing system. This result also supports that autumn grazings are important contributors to annual N losses to water.

Despite the benefits related with restricted or DC grazing, it also has some disadvantages including the increase of gaseous losses from the animal shelters and the stored and recycled effluents; as well as increases in capital costs, in animal health costs and labour/energy requirements for the maintenance of the installations (de Klein & Legard, 2001).

2.6.2 Dietary manipulation

As mentioned in the section 2.4.2 (Urine patches composition), the N concentration in the urine is highly influenced by the N intake in the cow's diet. In general, high N diets result in high N concentrations in the urine patches. Dietary N requirements for dairy cattle are approximately 1.8% of DM for maintenance, 2.2% of DM for growing cattle and 3% of DM for young or lactating animals. However, with pastures, dietary N concentrations can exceed 3.5% of DM, particularly during spring, which results in a surplus. The surplus of N that is not used by the animal (converted into products or incorporated to the body during the growing period) is mostly excreted via the urine (Pacheco & Waghorn, 2008). Miller et al. (2001) showed that feeding dairy cows with high sugar ryegrass (HSG) led to

a more efficient use of feed N for milk production and, consequently, a reduction in urine N excretion by 26% compared with the control diet (standard variety of perennial ryegrass). Misselbrook et al. (2005) found that urine N concentration was 90% greater in high crude protein (CP) diets compared to low CP diets.

It has been shown that supplementing cows with salt (NaCl) reduced the N concentration in their urine by increasing the water intake of the animals. Ledgard et al. (2007) found that supplementing cows with salt at ranges of 0, 200 or 400 g/cow/day, the daily water intake averaged 19, 24 and 34 L/day respectively. As a result, the cows urine volume was significantly increased by around 2 and 3 fold, respectively, compared with the non-salt treatment. This lead to a reduction of urinary N concentration, with values of 9.6, 4.0, and 3.0 g N/L for the 0, 200 and 400 g/day of salt, respectively. Furthermore, Bryant et al. (2007) found that supplementing cows with salt lead to an 48% reduction of N leaching. While Dijkstra et al. (2013) mention that in the studies by Van Vuuren and Smits (1997) and Spek et al. (2012) salt was added to dairy cattle diet in order to increase the urine volume; which reduced urinary N concentrations from values of 10 g N/L of urine in treatments with not salt supplementation, to a values of 3.0 g N/L in treatments with higher levels of salt.

2.6.3 Nitrification inhibitors

Nitrification inhibitors are chemical compounds that reduce the conversion rate of NH_4^+ to NO_3^- (Di & Cameron, 2002b) and, in this way, contributes to reducing N losses from pastoral soils (N_2O gaseous emissions and NO_3^- leaching). In New Zealand, the most common nitrification inhibitor researched is dicyandiamide (DCD) and research into the use of DCD to mitigate the effects of urine patches in pastoral systems has been extensive (Selbie, 2014). DCD inhibits the first step of the nitrification process, the oxidation of NH_4^+ to NO_2^- (Cameron et al., 2014; Di & Cameron, 2002c). This inhibition retains N in the NH_4^+ form longer, which is able to be held on the soil's cation exchange sites. Therefore, it provides more opportunity to be taken up by plants, immobilised into soil organic matter, or fixed into clay mineral interlayers, instead of being leached (Cameron et al., 2014; Di & Cameron, 2002c).

It has been reported that applications of DCD significantly reduced the total NO_3^--N leaching losses from urine patches, resulting in reductions between 40-83% (Cameron et

al., 2014; Di & Cameron, 2002c, 2005, 2007). In addition, total N_2O emissions have been reduced by 40-82% with application of DCD to urine-treated soils (Cameron et al., 2014; Di & Cameron, 2002c) . Furthermore, the application of DCD to urine-treated soils have increased the herbage between 25-33% (Di & Cameron, 2002c, 2004, 2005, 2007).

On a paddock scale, Monaghan et al. (2009) studied the effects of DCD over a period of 4 years. During the trial, the paddock was grazed regularly at a stocking rate of 60 cows/ha/day. It was found that on annual basis, the application of DCD reduced the amount of N lost in the drainage by between 21-56%, depending on the year of study. Contrary to the benefits on reducing NO_3^--N leaching, no significant effect of annual or seasonal pasture production was found. In contrast, on a paddock scale study Kim et al. (2014) did not found a consistent reduction of NO_3^--N leaching from the application of DCD, but a 20% reduction was achieved in one of two years of the trial. This study did not found a significant influence of the DCD application on pasture accumulation. However, Kim et al. (2014) measured between 54-78% reduction in N_2O emissions.

Overall, the application of DCD is very effective on reducing N_2O emissions, but it requires more assessment to identify under which conditions the use of DCD is effective at reducing NO_3^- leaching and increasing pasture DM accumulation (Kim et al., 2014). However, currently DCD is not available for use. Traces of DCD have previously been measured in milk from farms using DCD and because currently there is no maximum permissible limit value set for food, no DCD traces in milk are allowed.

Chapter 3. Materials and Methods

3.1 Experimental design and trial establishment

3.1.1 Treatments

This study consisted of a field plot trial, comparing two different cow urine patch sizes and a control treatment without any urine application (No-urine treatment). The two urine patch sizes were a 0.2 m^2 urine patch (Urine (0.2 m^2) treatment), and a 1 m^2 urine patch (Urine (1m^2) treatment). It was decided to use these two urine patch areas based a similar previous study, which compared urine patch sizes on a volcanic soil in the Waikato region (Bagley et al., 2016). Published results from the Bagley et al. (2016) study were not available at the time of completing this thesis and, therefore, it was not possible to compare them with the results of this current study.

In both urine treatments, 2.1 L of real cow urine were applied, as this is considered to be an average urination volume for dairy cows (Betteridge et al., 2013; Selbie et al., 2015). Laboratory analysis of the urine was conducted to order to determine its N composition.

3.1.2 Experimental design

For the field trial, a Randomized Block experimental design was used, which consisted of six replicates (6 Blocks) of each of the five treatments. The original design allowed for two application dates of each of the two urine treatments, described in the previous section. However, due to the early occurrence of drainage (April), the second urine application date was not required. Each of the thirty plots used in the experimental design measured 2.5 m x 3 m (7.5 m^2). The experiment design layout is shown in Figure 3.1.

For the No-urine treatment, the whole plot area (7.5 m^2) was used for sampling; while with the Urine (0.2 m^2) and Urine (1 m^2) treatments, the patches were arranged to allow for multiple soil samplings and also to minimise the influence of edge effects between the patches (Figure 3.2). Therefore, two urine patches were used in each of the Urine (1 m^2) treatment plots and three urine patches were used in each Urine (0.2 m^2) treatment plots.

Figure 3.1. Trial design and layout.

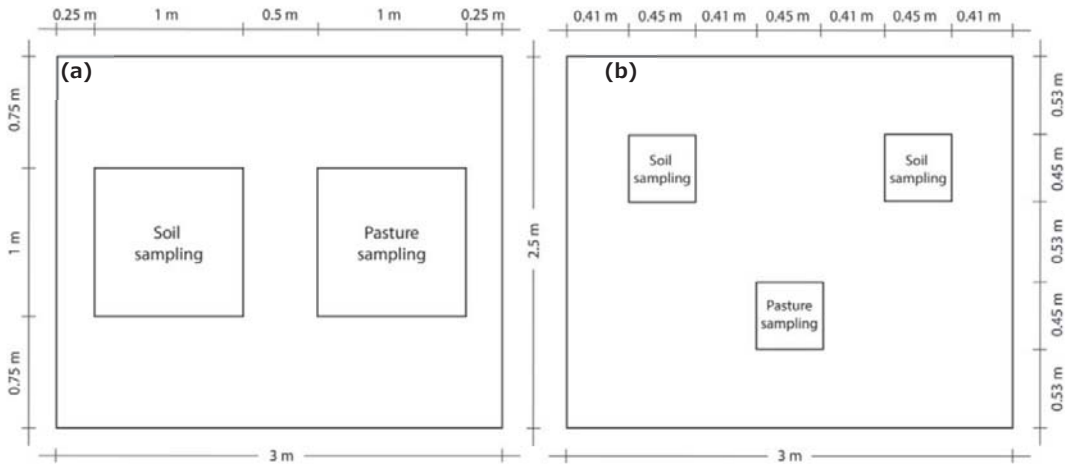

Figure 3.2. Treatments design for (a) Urine (1 m2) and (b) Urine (0.2 m2).

3.1.3 Trial site

The experiment was established in a paddock located next to the Massey University No. 1 Dairy Farm, Palmerston North. A map of the trial site is presented in Figure 3.3. The soil type at the trial site is the Manawatu silt loam, which is a Weathered Fluvial Recent Soil (NZ Classification) or Dystric Fluventic Eutroichrept (USDA Classification). The soil is formed from weakly weathered quartzo-feldspathic alluvial sediments, and has a silt loam texture in the top 0-65 cm and then grades to a sandy loam texture. The soil is well drained with moderate permeability throughout the profile. The pasture species composition consisted of perennial ryegrass (*Lolium perenne* L.), white clover (*Trifolium repens* L) and weed species. The paddock was not grazed for at least 6 months prior to the experiment commencing in order to minimise the influence of pre-existing urine patches.

3.1.4 Field trial establishment

The pre-trial site preparations and the field trial operations took place between the 1st of February and the 16th of May, 2017.

A. Pre-trial site preparation

The trial site preparations started on the 1st of February 2017, when the pasture was mown with a tractor mower. On the 3rd of February the treatments plots were measured out and marked with pegs (Figures 3.1 and 3.2). At this time, fertilisers where applied to all plots to ensure good base fertility. Urea and single superphosphate fertilisers were applied at rates that supplied 30 kg N/ha, 30 kg P/ha and 37 kg S/ha. The fertilisers were weight separately for each plot and applied by hand.

B. Urine collection and analysis

The dairy cow urine collections were made in a rotary farm dairy during milking times at Massey University's No. 4 Dairy Farm. A total of 61 L of urine were collected during three milking times (two during the afternoon milking time, and one from the morning milking), on the 1st and 2nd of March, 2017, and kept refrigerated at 4 ^{o}C for up to 2 days prior to the start of the trial. Before the application of the urine to the experimental plots, all the urine collected from the different milking times were mixed together.

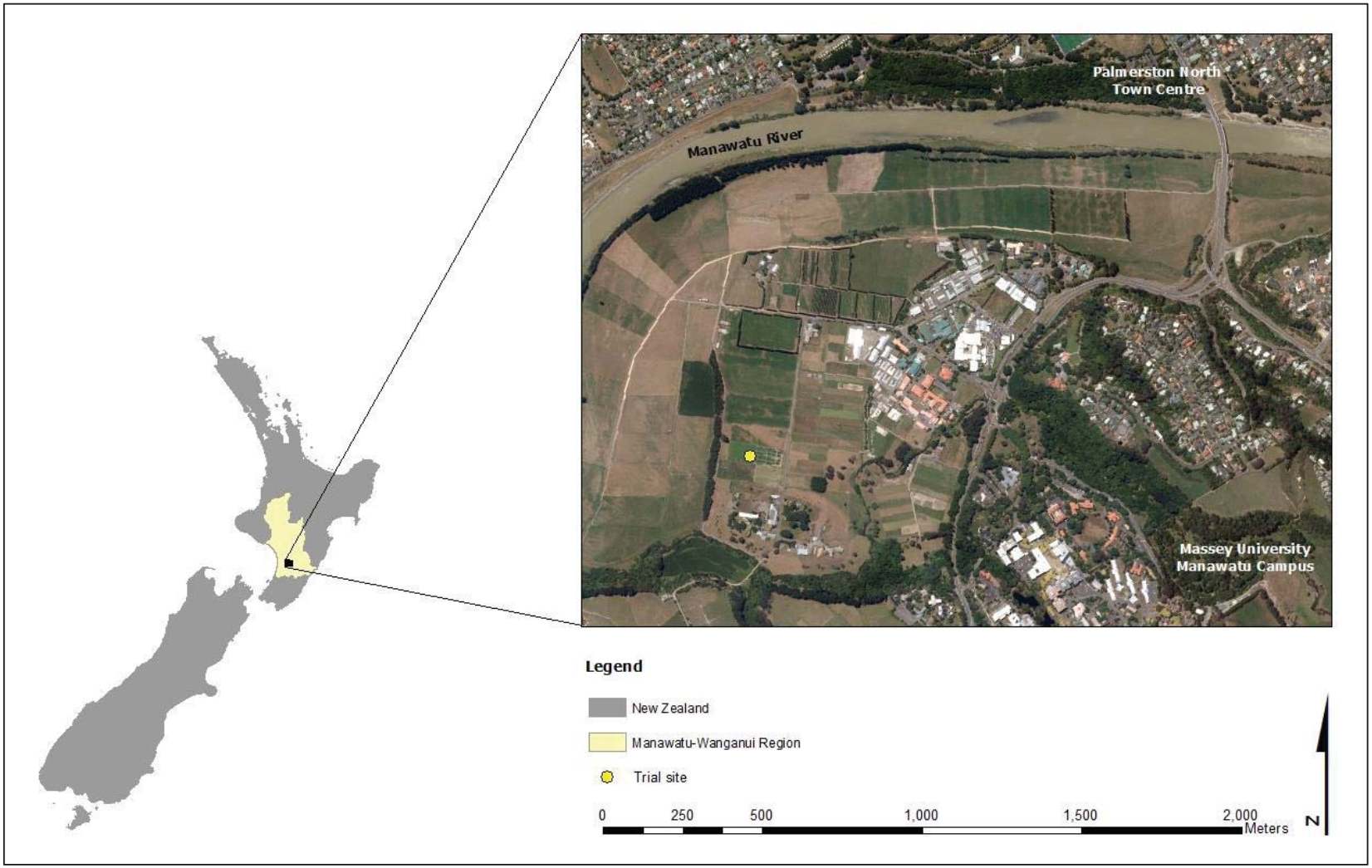

Figure 3.3. Trial site location.

Samples of urine from the individual milkings and the milkings combined were analysed for urea-N content and total N content (Figure 3.4). The urea-N content was determined using the method of Mulvaney and Bremner (1979); while a modified Kjeldahl method was used to determine the total N (Blakemore et al., 1987).

C. Urine application

On the 3rd of March, 2017, pasture at the trial site was mown with lawn mowers, the herbage was removed and three days later the urine treatments were applied. On the day of application, the urine was removed from refrigeration and given time to reach ambient temperature before application. A total of 2.1 L of urine was applied to each urine patch using watering cans (Figure 3.4). In order to confine the urine to the two urine patch treatment areas, urine was applied into square steel frames, which had areas of either 1 m^2 or 0.2 m^2. The 0.2 m^2 steel frame was partially knock into the soil surface to reduce movement of urine outside of the patch area during application.

Figure 3.4. Field trial establishment. (*a*) sampling of urine for further analysis, (*b*) 2.1 L of urine measured and then poured in to watering cans; (*c*) urine (1 m^2) treatment being irrigated with urine; (*d*) urine (0.2 m^2) treatment being irrigated with urine.

3.2 Sampling and analyses

For both urine patch treatments, one urine spot was used for pasture accumulation measurements, which was kept separate from the urine patches used for soil sampling. This was done to prevent the likelihood of the soil sampling impacting on subsequent pasture growth. For the Urine (1 m^2) treatment there was one urine patch for soil sampling and one for measuring pasture accumulation. While for the Urine (0.2 m^2) treatment there were three urine patches used; two for soil sampling and one for pasture accumulation.

3.2.1 Soil sample collection and preparation

At the beginning of the trial a composite soil sample (0 - 15 cm depth) was collected and sent to Hill Laboratories for standard soil fertility analysis. Over the duration of the trial, soil samples were collected three times for mineral N determination. The soil sampling dates where the 21st of March (1st sampling, 15 days after the urine application (DAUA)), 11th of April (2nd sampling, 36 DAUA) and 28th of April (3rd sampling, 53 DAUA). At the 1st and 2nd sampling times, three soil cores (2.5 cm internal diameter) were collected to a total depth of 60 cm from each treatment plot. The cores were divided into five different soil depths; 0 – 7.5, 7.5 – 15, 15 – 30, 30 – 45 and 45 – 60 cm, with the three cores from each depth being bulked together as a single sample from each treatment plot.

At the 3rd sampling, one soil core was collected to a depth of 120 cm from each treatment plot. The core was divided into nine different soil depth; 0 – 7.5, 7.5 – 15, 15 – 30, 30 – 45 and 45 – 60 cm using a corer with an internal diameter of 4.4 cm; while the remain four depth of 60 – 75, 75 – 90, 90 – 105 and 105 – 120 cm were collected using a corer with an internal diameter of 3.5 cm.

After sampling, soil samples were stored in a chiller at 4 °C until analysis. Within 1 – 2 days after collection, field moist soil samples were sieved through a 4 mm sieve, however, for samples that were too moist for this sieve size, a 8 mm sieve was used. Prepared soil samples were returned to the chiller until the analyses.

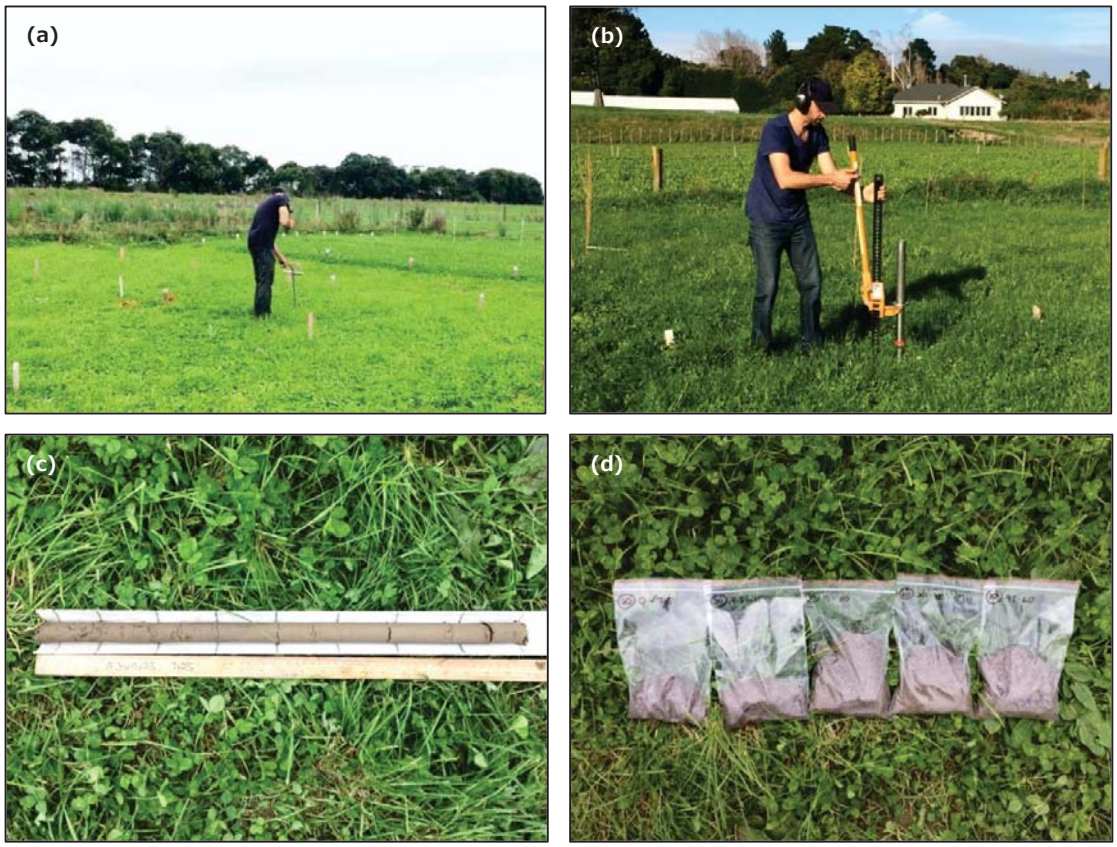

Figure 3.5. Soil sample collection. (*a*) 0-60 cm depth sampling; (*b*) 0-150 cm depth sampling; (*c*) division of the core in the different depths; (*d*) samples in bags according to the depths.

3.2.2 Soil analyses

All the samples collected during the trial were analysed for mineral N (NO_3^- and NH_4^+). The method used for analysing mineral N involved a 2 M KCl extraction. For the extraction, 3.5 g of sieved field moist soil was weighed into a centrifuge tube and 30 ml of 2 M KCl was added. Samples were shaken in an end-over-end shaker for 1 hour, and immediately after they were centrifuged at 5000 rpm for 5 minutes. Once the centrifugation was over, 10 ml of each extracting solution was transferred with a pipette into plastics tubes. Samples of extracting solution were stored at 4° C until analysed. The concentrations of NO_3^- and NH_4^+ in the extracting solution were measured using a Technicon II auto-analyser (Blakemore et al., 1987).

The moisture content of soil samples was determined by weighing approximately 50 g (actual weight of each sample recorded) of field moist sample and oven drying at 105 °C

for 24 hours. After drying, the samples were weighed again in order to determine soil moisture content.

The quantity of mineral N remaining in the soil from the urine patch treatments was calculated by subtracting the No-urine (control) treatment mineral N concentrations from the urine patch treatment concentrations and multiplying this concentration by the soil mass at each soil sampling depth. The mass of soil at each sampling depth was determined from the area of each urine patch, the depth of sample and bulk density of the soil at the different depths. The soil bulk density at different depths from the trial site was provided by P. Bishop (personal communication, May 10, 2017).

3.2.3 Pasture dry matter accumulation sampling

Over the duration of the trial, pasture samples were harvest on two occasions; 31st of March (25 DAUA) and the 16th of May (71 DAUA), for dry matter accumulation measurements. The pasture samples were harvested at a height of approximately 5 cm above ground level using a lawn mower. For the No-urine treatment and the Urine (1 m^2) treatment an area of 1.4 m^2 was harvested and for the Urine (0.2 m^2) treatment an area of 1 m^2 was harvested. The harvested areas were larger than the urine patch areas in order to include the edge effect on pasture growth from the urine patch treatments. The underside of the harvesting lawnmower and its catcher were wiped down to remove all of the sample between each sampling. Figure 3.6 shows some of the steps during the pasture sampling. In addition to mower harvests, pasture cuts were also collected from each of the No-urine treatments plots for pasture herbage botanical determination.

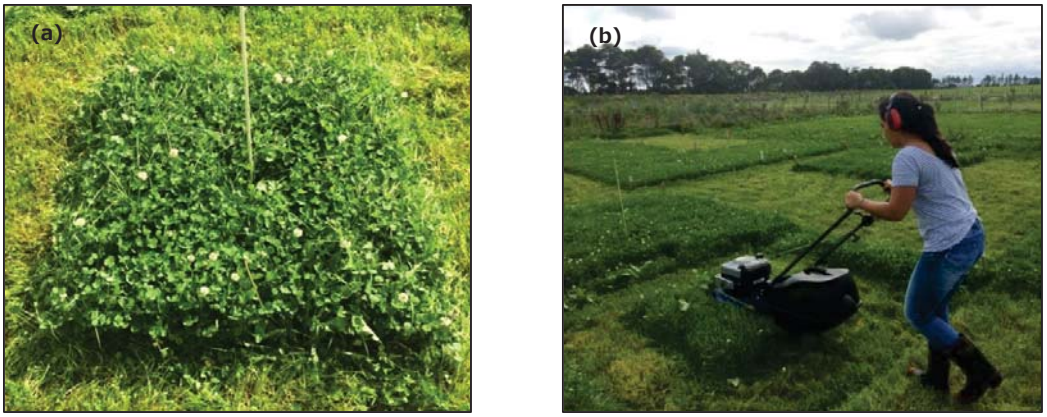

Figure 3.6. (*a*) urine patch after the mowing of outer surface; (*b*) pasture sampling.

From the lawnmower pastures harvests, the total dry matter (DM) accumulation was determined. The entire pasture sample harvested was weighed fresh, then a sub-sample was weighed fresh and then oven dried (45 °C) in paper bags. The dried sub-samples were weighed and used to determine DM content.

The herbage botanical samples collected from the No-urine treatment plots were separated into grass, clover and weeds. These samples were placed into paper bags and oven dried (45 °C) to determine dry weight.

3.3 Summary of trial activities

A rain gauge was installed at the trial site to record rainfall. Figure 3.11 presents a summary of the treatment application and sampling dates in relation to cumulative rainfall from the 1st of March, 2017.

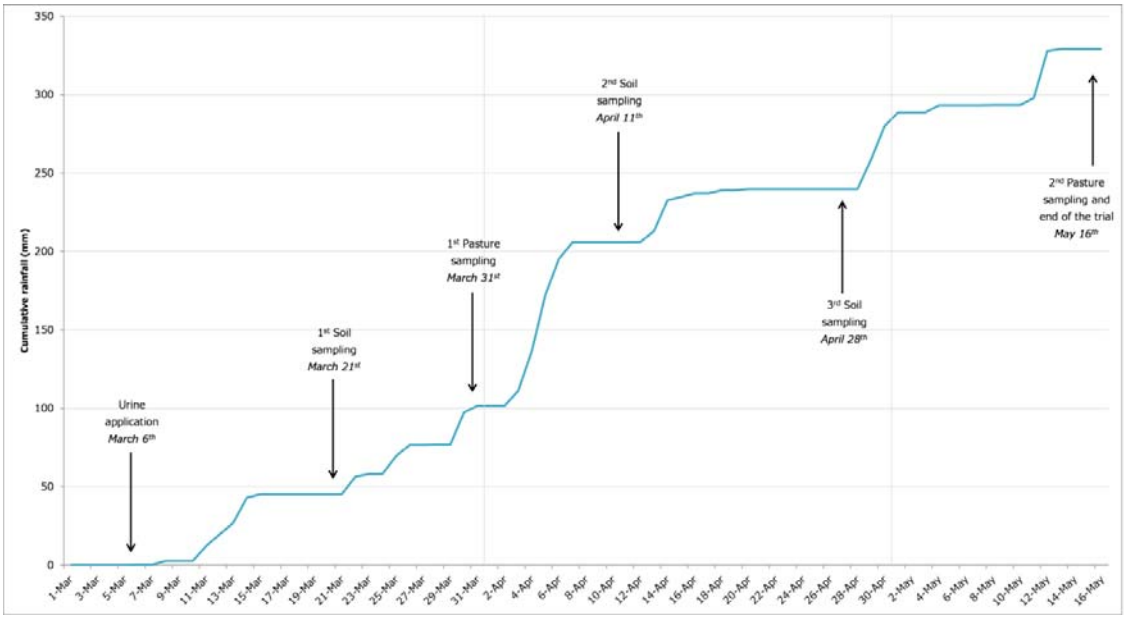

Figure 3.7. Field trial steps; urine application, soil sampling and pasture sampling dates in relation to the cumulative rain during the experiment.

3.4 Data analysis

Microsoft Excel 2010 was used to conduct the statistical analyses. Data from the field trial was analysed using an Analysis of Variance (ANOVA) test and the Tukey test was used to group treatment means, at the 5% significance level.

Chapter 4. Results and discussions

4.1 Introduction

The results from the field experiment are presented in the following sections. Key findings of the results are also discussed in order to evaluate the potential influence of increasing urine patch area on the quantity of mineral N movement to depth in soils and on short-term pasture growth responses.

4.2 Soil chemical characteristics

At the beginning of the trial, soil samples were collected and the chemical fertility was determined. In Table 4.1 a summary of the soil chemical characteristic of the soil at the trial site is presented.

Table 4.1. Soil chemical fertility of the trial site.

Soil test	Units	Level found	Medium/Optimum range
pH	pH units	5.5	5.8 – 6.2
Olsen P	mg/L	38	20 – 30
Sulphate sulphur	mg/kg	18	10 – 12
Potassium	me/100g	0.25	0.40 – 0.60
Calcium	me/100g	6.8	4.0 – 10.0
Magnesium	me/100g	1.40	0.80 – 1.60
Sodium	me/100g	0.11	0.20 – 0.50
Cation exchange capacity	me/100g	15	12 – 25
Total base saturation	%	57	50 – 85

Source: Hill Laboratories Analysis Report.

Most of the soil test values were either within or above the medium/optimum range guidelines, which shows a general high level of soil fertility. The only values that potentially could have had an influence on pasture yield were the pH and exchangeable K, which were below the medium/optimum ranges. This Recent alluvial soil has a naturally high reserve K capacity, therefore, it is unlikely that soil K availability would have been limiting pasture production in this study.

4.3 Rainfall and soil drainage conditions during the trial

The amount of rainfall for 2017, during the trial months in autumn (March - May), was compared with the average annual rainfall (2001 – 2014) in Figure 4.1. In 2017 the autumn rainfall was a total of 396 mm, which was more than double the average annual rainfall of 197 mm for this period. The monthly rainfall in April 2017 was particularly high, reaching a total of 178 mm. As a result of the higher than average autumn rainfall, drainage water movement in the soil is estimated to have started in early April (Figure 4.2).

To determine the occurrence of drainage, a soil water balance was developed for the trial site. Assuming that the soil water deficit on the day of urine application was 75 mm, the water balance estimated the drainage started on the 4th of April 2017 (29 DAUA), which is at least a month earlier than in a more typical year. Prior to the first soil sampling on the 22nd of March (16 DAUA) there was no estimated drainage. The second soil sampling on the 11th of April (36 DAUA), occurred 7 days after the start of drainage, by which timed 70 mm of cumulative drainage was estimated to have occurred. Between the second and third soil sampling (28th of April 2017, 53 DAUA) an additional 17 mm of drainage was estimated.

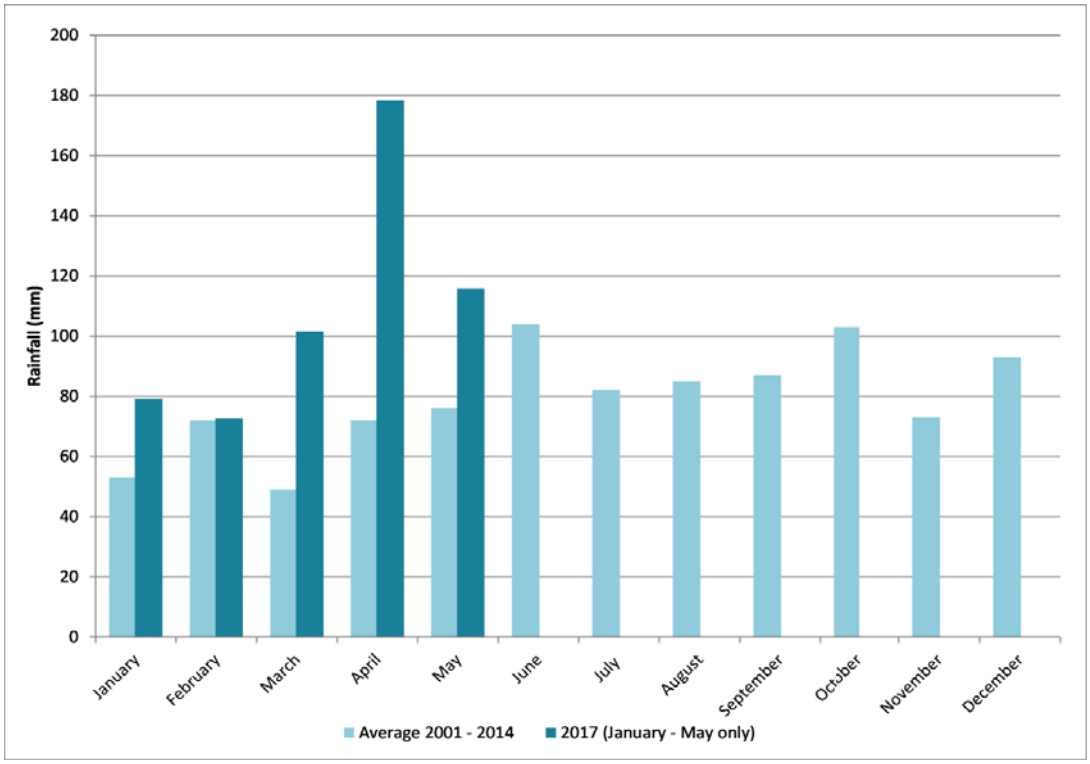

Figure 4.1. Registered rainfall for the experimental site during the trial compared with the average annual rainfall from 2001 – 2014.

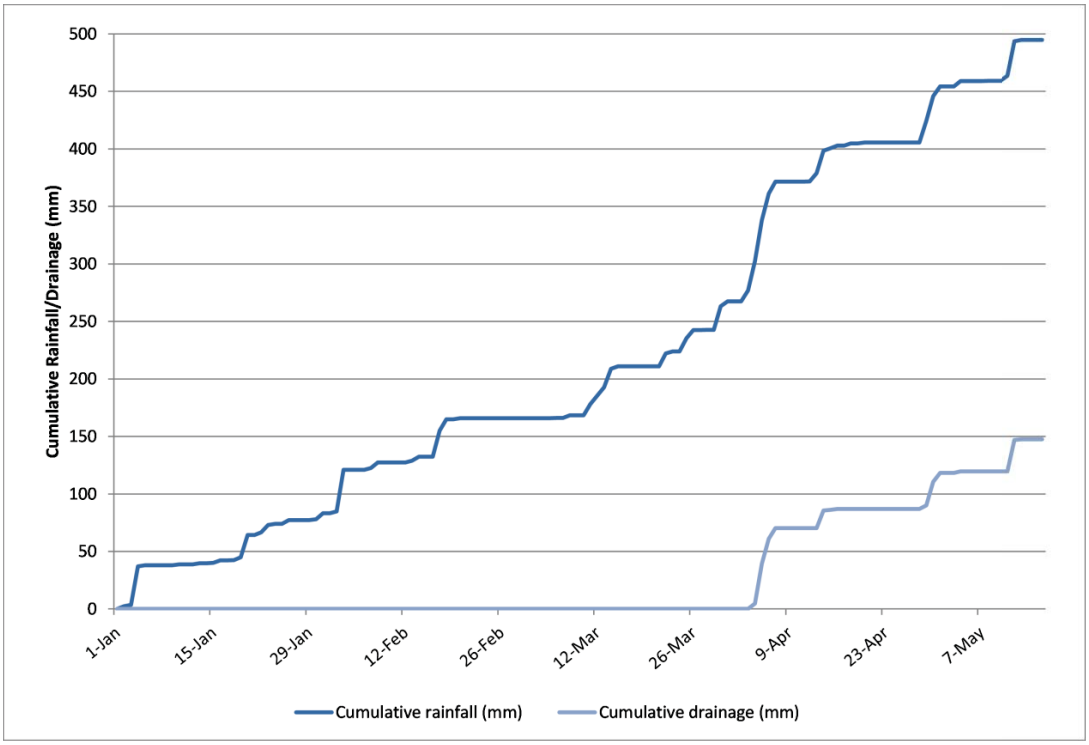

Figure 4.2. Cumulative rainfall and estimated cumulative drainage (water balance estimate) in the experimental site during the trial in 2017 (January-May).

4.4 Urine composition and N application rate

From the dairy cow urine- analysis, it was determined that it was composed of 2.9 g urea-N/L and 4.55 g total N/L. Therefore, 64% of the urinary N applied was urea-N. These results are near the lower end of the wide range measured by Dijkstra et al. (2013), who found urea-N concentrations in cow urine varied between 2.1 and 19.2 g N/l; and urea N percentage of total N ranged from 52.1 - 93.5%.

The urine collection was made at three different milking times, one during the morning and two during the afternoon. The urine from the morning milking had a urea-N content of 4.2 g N/L. Whereas the average for the two afternoon milking was of 2.4 g N/L for urea-N content. This diurnal pattern of the urine composition was also observed by Betteridge et al. (2013). As mentioned in the methodology section, in this current study the urine from the morning and afternoon milkings were mixed, resulting in a single composite sample used for both urine treatments.

The application rate of N applied with the urine to the urine treatments was directly related to the surface area it was applied to, as both treatments received the same volume

of urine per patch. For the Urine (0.2 m^2) treatment, in which urine was applied to an approximate area of a standard urine patch, the application rate was 478 kg N/ha, which is at the lower end of the reported total N loading rate range of a cow (400 – 1200 kg N/ha) (Cameron et al., 2013; Di & Cameron, 2002b). Nitrogen loading rate in urine can vary throughout the year due to differences in feed crude protein contents (Dijkstra et al., 2013; Misselbrook et al., 2005), which are typically lower in the late summer/early autumn period than in spring. The urine used for this study was collected in early autumn and the N application rate was similar to the typical autumn loading rate reported by Pakro and Dillon (1995) of 514 kg N/ha. For the Urine (1 m^2) treatment the application rate was 95.6 kg N/ha, due to the urine being spread over an area five times larger than the standard urine patch area treatment.

4.5 Effects of urine patch area on mineral nitrogen movement down the soil profile

4.5.1 Soil nitrate and ammonium concentrations

This section presents results on the concentrations of soil mineral N (NO_3^- and NH_4^+) at different soil depths. The NO_3^- concentrations down the soil profile, at the different sampling times are presented in Figure 4.3.

At the first soil sampling time (15 DAUA), the highest NO_3^- concentrations for the two urine patch treatments occurred in the 0-15 cm soil depth, being 22.7 µg NO_3^--N/g for the Urine (1 m^2) treatment and 71.9 µg NO_3^--N/g for the Urine (0.2 m^2) treatment, then both treatments followed a decreasing trend with soil depth. This compared to a concentration of 8.6 µg NO_3^--N/g measured for the No-urine treatment in the 0-15 cm soil depth. The soil NO_3^- concentrations of the Urine (1 m^2) treatment decreased to levels similar to that of the No-urine treatment at the 15-30 cm soil depth, while the Urine (0.2 m^2) treatment concentrations stayed higher than the No-urine treatment until the 45-60 cm depth. The soil water balance indicated that there had been no drainage prior to this sampling, which explains the higher NO_3^- concentrations at shallower soil depths. In spite of there being no estimated rain induced drainage, the Urine (0.2 m^2) treatment, which involved a 10.5 mm (2.1 L applied to 0.2 m^2) average application of urine, influenced NO_3^- concentrations to a soil depths up to 45 cm.

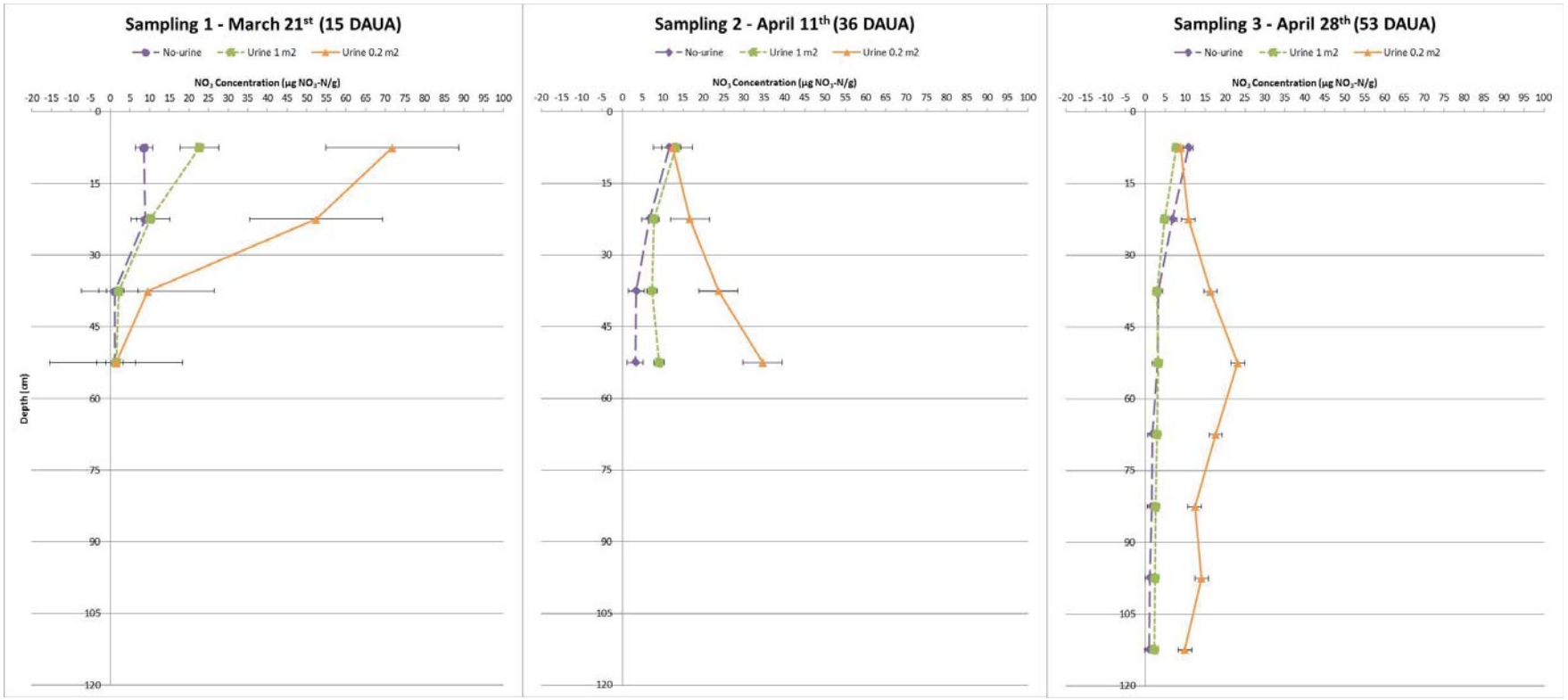

Figure 4.3. Average soil NO_3^--N concentrations at different soil depths for each treatment at the three sampling times (error bars represent standard error of the means).

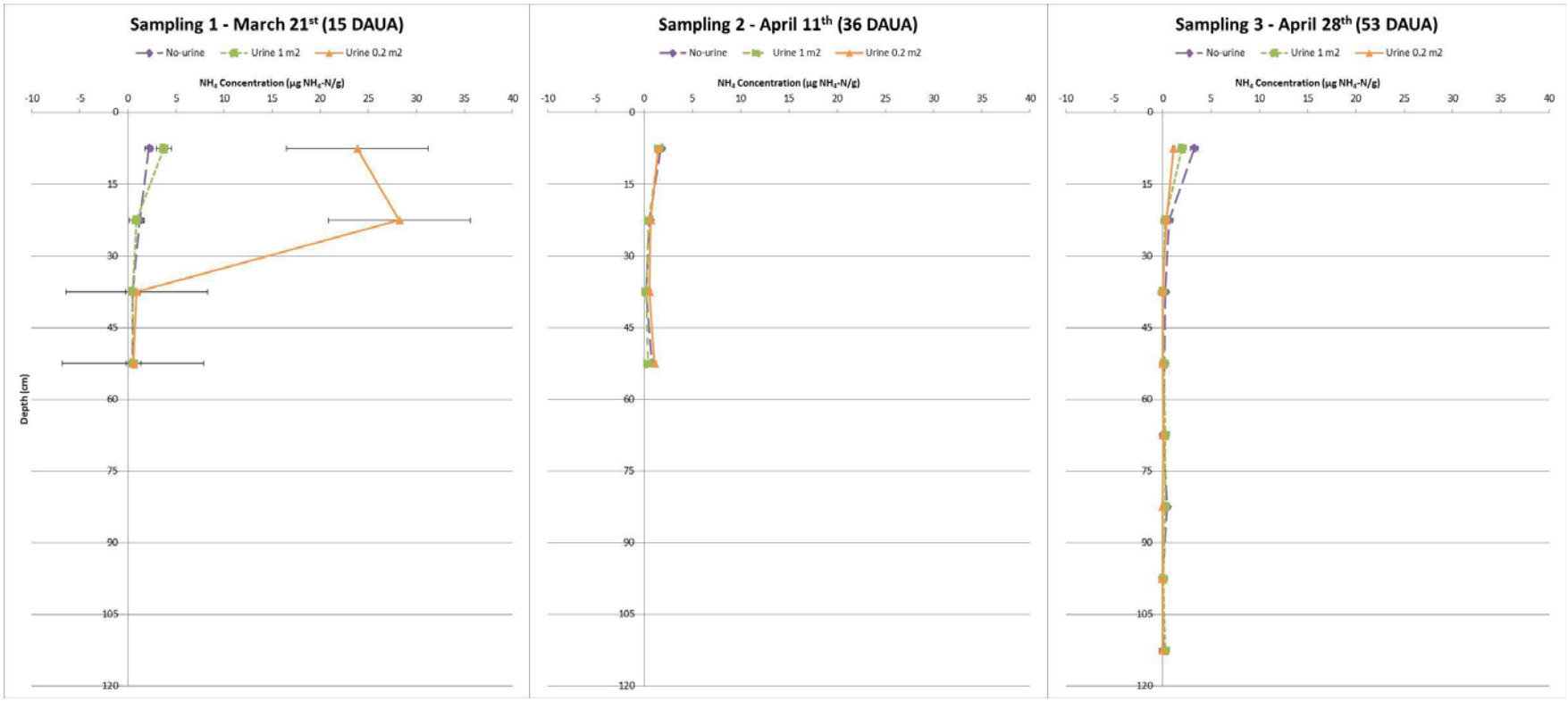

Figure 4.4. Average soil NH_4^+-N concentrations at different soil depths for each treatment at the three sampling times (error bars represent standard error of the means).

The soil NH_4^+ concentrations from the first sampling time, reached a value of 3.7 µg NH_4^+-N/g in the 0-15 cm soil depth of the Urine (1 m^2) treatment, which was only slightly higher than the value of 2.2 µg NH_4^+-N/g measured at the same depth in the No-urine treatment (Figure 4.4). In contrast, the Urine (0.2 m^2) treatment resulted in high NH_4^+ concentrations in both the 0-15 and 15-30 cm soil depths, being 23.9 and 28.2 µg NH_4^+-N/g respectively. The majority of the nitrification process typically occurs over a period of 1-2 weeks in aerobic soil conditions (Holland & During, 1977), and any remaining nitrification is typically completed within 4 weeks (Haynes & Williams, 1993). While it appears that almost all of the urinary N in the Urine (1 m^2) treatment had nitrified to NO_3^- at 15 DAUA, nitrification was not complete for the small urine patch treatment at this time.

At the second sampling time (36 DAUA), an opposite trend from the first sampling time was observed, with soil NO_3^- concentrations increasing with soil depth for the Urine (0.2 m^2) treatment. This treatment had a similar NO_3^- concentration to the other two treatments in the 0-15 cm depth, with a value of 12.5 µg NO_3^--N/g. Then the NO_3^- concentrations increased with soil depth, reaching a value of 34.6 µg NO_3^--N/g at the 45-60 cm depth. Between the first and second sampling times there was an estimated 70.4 mm of cumulative drainage (Figure 4.2), which would have caused the observed downward movement of NO_3^- for the Urine (0.2 m^2) treatment. Given that the higher concentration was measured at the deepest soil depth collected, it is likely that some unrecovered soil NO_3^--N had moved below the 60 cm depth, which was not sampled. Therefore, a third soil sampling (56 DAUA) was conducted to a depth of 120 cm in order to track the movement of NO_3^- below the 0-60 cm depth. While the Urine (1 m^2) treatment did not show an increase in NO_3^- concentrations with soil depth, its concentrations were higher than the No-urine treatment at the 30-45 and 45-60 cm depths. This showed that some urinary N from this treatment had also moved to depth, having a concentration of 9.1 µg NO_3^--N/g at the 45-60 cm depth, compared to 3.2 µg NO_3^--N/g in the No-urine treatment.

The NH_4^+ concentrations at the second sampling were low for all treatments, being less than 2 µg NH_4^+-N/g at all soil depths. By this time, 36 DAUA, it is expected that nitrification of the urinary N would have been completed, which was confirmed by the low NH_4^+ concentrations.

At the third soil sampling time (53 DAUA), the Urine (0.2 m^2) treatment presented higher NO_3^- concentrations compared to the other two treatments at all soil depths, except for the shallowest depth. The NO_3^- concentrations of the Urine (0.2 m^2) treatment peaked at 23.2 µg NO_3^--N/g at the 45-60 cm soil depth, then showed a general trend of decreasing to a concentration of 9.9 µg NO_3^--N/g at the lowest depth sampled (105-120 cm). The concentrations of NO_3^- for the Urine (1 m^2) treatment were similar at all soil depths to the No-urine treatment.

As with the second soil sampling, the NH_4^+ concentrations at the third sampling time were low for all treatments, with there being only small differences between the three treatments at the 0-15 cm depths, being 3.3, 2 and 1.1 µg NH_4^+-N/g for the No-urine, Urine (1 m^2) and Urine (0.2 m^2) treatments, respectively. At all other depths the concentrations in all treatments were low, being less than 1 µg NH_4^+-N/g.

The average soil NO_3^- concentrations in the 0-60 cm at the three sampling times, and in the 60-120 cm soil depth at the third sampling time were significantly different ($P<0.05$) between treatments. The average NH_4^+ concentrations in the 0-60 cm depth were significantly different ($P<0.05$) between treatment in the first and third soil sampling times (Table 4.2). The multiple comparison test showed that just in the first sampling the three treatments presented different NO_3^- concentrations, with the highest NO_3^- concentration being measured in Urine (0.2 m^2), followed by the Urine (1 m^2) and then the No-urine treatments has the lowest concentration. While in the second and third sampling, the Urine (0.2 m^2) treatment had significantly higher NO_3^- concentrations compared to the other two treatments. The NH_4^+ concentrations from the first sampling was higher for Urine (0.2 m^2), while Urine (1 m^2) and No-urine treatments were not significantly different.

At the third sampling time in the 60-120 cm soil depth, where the NO_3^- is less likely to be recover by pasture plants, the average NO_3^- concentrations for the Urine (1 m^2) treatment was 1.19 µg NO_3^--N/g higher than the No-urine treatment, while the Urine (0.2 m^2) treatment was 10.9 µg NO_3^--N/g higher than the No-urine treatment (Table 4.2). The net increase in concentrations at depth for the Urine (0.2 m^2) treatment was, therefore, more than 9 times greater than for the Urine (1 m^2). This was greater than the patch area difference, which was only five times higher for the Urine (1 m^2) treatment. The

implications of this difference on total urine-N movement in the soil is presented in the next section.

Table 4.2. Average soil NO3¯-N and NH4+-N concentrations in the different treatments and sampling times.

Treatment/ Sampling	Average NO_3^--N concentration (μ g NO_3^--N/g)			P value	Average NH_4^+-N concentration (μ g NH_4^+-N/g)			P value
	No urine	Urine 1 m^2	Urine 0.2 m^2		No urine	Urine 1 m^2	Urine 0.2 m^2	
1st Sampling (March 21st) 0 – 60 cm	6.342 *a*	12.486 *b*	49.929 *c*	<0.001	0.987 *a*	1.622 *a*	20.515 *b*	<0.001
2nd Sampling (April 11th) 0 – 60 cm	8.060 *a*	10.592 *a*	18.721 *b*	<0.001	1.128	0.959	1.126	NS
3rd Sampling (April 28th) 0 – 60 cm	4.317 *a*	3.687 *a*	16.747 *b*	<0.001	0.354 *a*	0.119 *b*	0.129 *b*	0.013
60 – 120 cm	1.232 *a*	2.424 *a*	12.127 *b*	<0.001	0.150	0.199	-0.026	NS

The differences between treatments are express as *a, b* or *c*. NS denotes treatment effect 'not significant'. Treatments without significant differences are labelled with the same letter.

4.5.2 Quantity of net inorganic N (nitrate and ammonium)

The quantity of net NO_3^- and NH_4^+ in the soil resulting from the application of urine in the Urine (1 m^2) and Urine (0.2 m^2) treatments patches were calculated by subtracting the background concentrations measured in the No-urine treatment. Where the concentrations of NO_3^- and NH_4^+ in the Urine (1 m^2) and Urine (0.2 m^2) treatments were lower than the No-urine treatment concentrations the quantities are represented as net negative values.

The average quantity of net NO_3^- measured in the Urine (0.2 m^2) treatment were 4.8, 2.8 and 3.8 g NO_3^--N/patch for the first (0-60 cm depth), second (0-60 cm depth) and third (0-120 cm depth) soil sampling respectively. When the urine was spread over a larger area in the Urine (1 m^2) treatment, the quantity of net NO_3^- reached values of 4.05, 2.4 and -0.014 g NO_3^--N/patch for each sampling, respectively. Statistically significant reductions ($P<0.05$) in the quantity of net NO_3^--N/patch due to urine spreading occurred at the third sampling but not at the previous two samplings. At the third sampling (0-120 cm depth),

the quantity of net NO_3^- in the Urine (0.2 m^2) treatment was 3.81 g NO_3^--N/patch higher than in the Urine (1 m^2) treatment. At none of the three sampling times the was quantity of net soil NH_4^+ (0-120 cm) presented significantly different (P>0.05) between the two urine treatments.

The net inorganic N was determined by the sum of net NO_3^- and net NH_4^+ measured in the urine treatment patches, as described in the previous section. As discussed in the previous section, most of the inorganic N in the soil profile was in the form of NO_3^- with there being minimal NH_4^+ being detected, apart for the Urine (0.2 m^2) treatment at the first sampling. Typically, inorganic N leached from urine patches is predominately as NO_3^-, which is due to NO_3^- not being held onto soil anion sorption sites, as is the case for phosphate and sulphate (Di & Cameron, 2002a, 2007; Shepherd et al., 2011). Whereas NH_4^+ is less prone to leaching as it can be retained on soil cation exchange sites.

The net inorganic N measured in the first soil sampling were generally higher in the shallowest soil depths and decreased in the deeper depths in both urine treatments (Figure 4.5). The Urine (1 m^2) treatment had 2.8 g net inorganic N/patch in the 0-15 cm soil depth, which decreased to 0.10 g net inorganic N/patch in the 45-60 cm soil depth. The Urine (0.2 m^2) treatment had 3.2 g net inorganic N/patch in the 0-15 cm, which in the 45-60 cm decreased to a value of 0.02 g net inorganic N/patch. The largest difference between the two urine treatments occurred in the 15-30 cm depth, at which the Urine (1 m^2) treatment had 1.17 g net inorganic N/patch compared to 3.20 g net inorganic N/patch in Urine (0.2 m^2).

At the second sampling time, the net inorganic N content in both treatments followed a similar general trend (Figure 4.5) of increasing with soil depth down to 60 cm. In the 0-15 cm soil depth, 0.20 and 0.07 g net inorganic N/patch were measured for the Urine (1 m^2) and Urine (0.2 m^2) treatments, respectively, which increased to 1.03 and 1.40 g net inorganic N/patch in the 45-60 cm soil depth, respectively. As previously discussed, this trend of increasing with soil depth down to 60 cm suggests that there is likely to be further net inorganic N from the urine treatments at soil depths below 60 cm, which were not sampled at this sampling time.

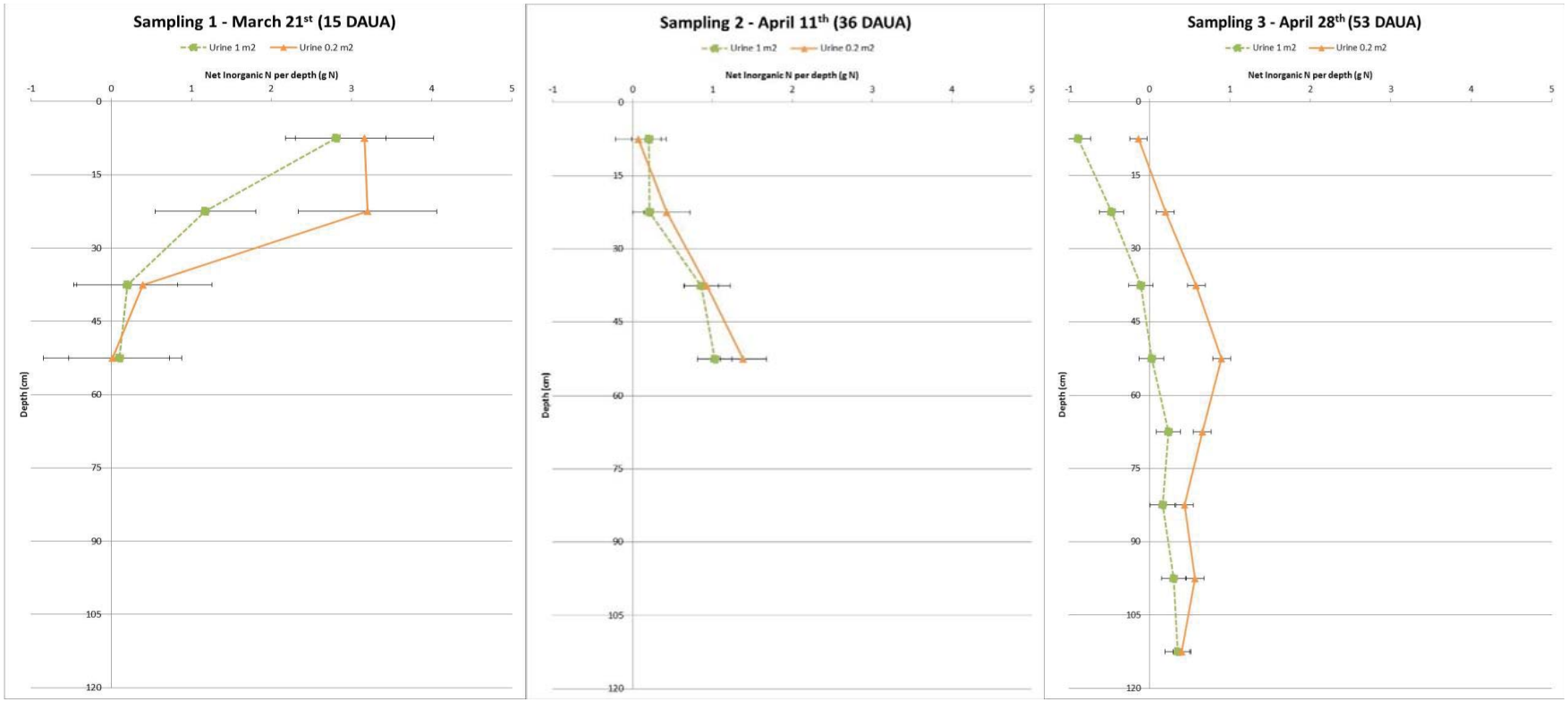

Figure 4.5. Net Inorganic N (g N/patch) through the soil profile in the 1st and 3rd soil sampling (error bars represent standard error of the means).

At the third soil sampling the two urine treatments showed similar trends in the quantities of net inorganic N with soil depth (Figure 4.5), however, the Urine (0.2 m^2) treatment was higher at all depths down to 120 cm. In the top three soil depths the concentrations of inorganic N for the Urine (1 m^2) treatment were lower than the No-urine treatment, which resulted in the Urine (1 m^2) having negative quantities of net inorganic N at these soil depths. At the deeper soil depths (45-120 cm) the quantity of net inorganic N, in each 15 cm soil depth interval, ranged from 0.03 to 0.35 g net inorganic N/patch for the Urine (1 m^2) treatment. In contrast, the Urine (0.2 m^2) treatment only had a negative quantity of net inorganic N in the 0-15 cm soil depth. For this treatment, there was a trend of increasing quantities of net inorganic N with soil depth down to peak of 0.90 g net inorganic N/patch in the 45-60 cm soil depth, and then showed a general trend of decreasing down to a value of 0.40 g net inorganic N/patch in the 105-120 cm. The Urine (1 m^2) treatment presented lower net inorganic N than Urine (0.2 m^2) in the whole soil profile.

4.5.3 Inorganic N leaching potential

In order to determine whether Urine (1 m^2) treatment has reduced the potential for inorganic N leaching, compare to the Urine (0.2 m^2) treatment, it is first necessary to identify the root zone for pastures below which there is minimal recovery of inorganic N. Ryegrasses can vary in their rooting depth, which can be influenced by factors such as climate and soil type. Maximum perennial ryegrass root depth recorded in the Canterbury region of New Zealand, on a silt loam soil was of 80 cm, however, 60% of root dry matter (DM) was measured in the 0-20 cm soil depth (Gibbs, 1986). In the Manawatu region, on deep, well-drained river silt soil, (Jacques) as cited in Wedderburn et al. (2010) observed soil ryegrass roots to a depth of 180 cm, but 73% of root DM was measured in the 0-30 cm soil depth, and only 5% below 90 cm. Whereas, in Wedderburn et al. (2010) study, in which the root growth of perennial ryegrass under both well-watered and drought conditions was analysed, the maximum observed root depth was 42.5 cm. Popay and Crush (2010) measured 96% of perennial ryegrass root DM in the 0-20 cm soil depth, with only 4% below the 20 cm, while 97-99% of the total root length density was between the 0-40 cm depth. Therefore, in the current study it has been assumed that after commencement of the drainage season, which in this study was estimated to be from the 4th April, any

inorganic N below the 0-45 cm soil depth is at a high risk of being susceptible to losses via leaching.

At the third soil sampling time, which was 24 days after the commencement of the drainage, the net inorganic N in the 45-120 cm soil depth was 1.08 g net inorganic N/patch for the Urine (1 m^2) treatment, compared to 2.97 g net inorganic N/patch for the Urine (0.2 m^2) treatment. This difference between treatments was statistically significant (P=0.007). Therefore, the Urine (1 m^2) treatment resulted in a 63.6% reduction in the quantity of net inorganic N at a high risk of being susceptible to leaching, compared to the standard urine patch area of 0.2 m^2. This result is similar to that achieved by nitrification inhibitors in some previous studies. For example, Menneer et al. (2008) evaluated the effect of the nitrification inhibitor dicyandiamide (DCD) applied to artificial cow urine (application rate of 596 kg N/ha) on pasture plots in May (autumn) or July (winter) on NO_3^- leaching. The autumn applied urine+DCD treatment achieved a 50% ($P < 0.05$) reduction in NO_3^- leaching at 55 DAUA, compared to the urine only treatment. However, subsequently greater amounts of NO_3^- were leached from the urine+DCD plots and by 159 DAUA there was only a 17 % reduction, which was no longer significant. Over time DCD had become ineffective in inhibiting nitrification, thus allowing the conversion of retained NH_4^+ to NO_3^- and its subsequent leaching from the root zone. This is where the spreading of urine as a mitigation has an advantage over nitrification inhibitors, as spreading urine does not result in accumulation of soil NH_4^+ that can subsequently nitrify and leach. Menneer et al. (2008) also showed that winter application of DCD was 62% more effective than the autumn application at reducing NO_3^- leaching losses by from the urine plots. These results suggest to DCD is more effective as a mitigation in winter, where its longevity is less critical, as there is less time between application and the cessation of the drainage season. Further research is required to assess whether spreading urine in winter is also effective at reducing NO_3^- leaching.

4.5.4 Urine nitrogen recovery

From the total urine-N applied at the beginning of the trial (9.56 g N/patch), at the first soil sampling, 4.25 g net inorganic N/patch was measured in the Urine (1 m^2) treatment and 6.77 g net inorganic N/patch in the Urine (0.2 m^2) treatment in the 0-60 cm soil depth. These quantities of N correspond to a urine N recovery of 44.8% for Urine (1

m^2) and 71% for Urine (0.2 m^2). The lower recovery for the Urine (1 m^2) treatment could be due to increases in plant uptake, ammonia volatilisation and/or immobilisation in the soil when the urine is spread over a larger area. The ammonia volatilisation losses could potentially increase with greater urine patches areas due to a larger area of surface soil having a high concentration of urea N from the urine application. These lower quantities of net inorganic N measured for the Urine (1 m^2) treatment, 15 DAUA, would have contributed to the lower quantities of inorganic N measured in the 45-120 cm soil depth at 53 DAUA.

In order to determine recovery of urine-N, at the third soil sampling, the quantities of net inorganic N for each soil depth that had positive values were combined to provide a value for each of the two urine treatments. The soil depths that had positive net inorganic N values were 45-120 cm for the Urine (1 m^2) treatment and 15-120 cm for the Urine (0.2 m^2) treatment. The 1.08 g net inorganic N measured for Urine (1 m^2) in the 45-120 cm depth was 11.3% of the total urine-N applied. Whereas, the 3.75 g net inorganic N measured for Urine (0.2 m^2) in the 15-120 cm depth was 39.2% of the total urine-N applied. If the entire 0-120 cm depth was used for this comparison (i.e. soil depths including negative values) the quantity of total urine-N still present in the soil profile would be estimated to be 0% for the Urine (1 m^2) treatment and 37.9% for the Urine (1 m^2) treatment.

4.6 Paddock scale risk of nitrogen susceptibility to leaching

In previous sections, the results for net inorganic N have been expressed on a per urine patch basis. However, it is also useful to estimate what these values would potentially be on a paddock scale, to better gauge the scale of the quantity of N that is susceptible to leaching losses. For this purpose, it was assumed that in a single grazing, cows will consume a total of 1500 kg DM/ha, with each cow ingesting 7.5 kg DM/cow/ha/grazing, therefore, this would feed 200 cows/ha/grazing. Considering that during the milking season, the morning grazing is ~7 hours and night grazing is ~13 hours, the average grazing time will be 10 hours/grazing. On average, dairy cows urinate 0.67 times per hour (Clark et al., 2010). Therefore, a 10 hours grazing will result in 6.7 urinations/cow. As a result, the total amount of urination during one single grazing will be 1340 urine patches/ha.

At the third soil sampling time (53 DAUA), the quantity of net inorganic N in the 45-120 cm soil depth (i.e. high risk of being susceptible to leaching loss), based on 1340 urine patches/ha/grazing, is 3.98 kg net inorganic N/ha for the Urine (0.2 m^2) treatment and 1.45 kg net inorganic N/ha for the Urine (1 m^2) treatment. Therefore, increasing the spread of urine from 0.2 to 1 m^2 decreased the quantity of inorganic N in the 45-120 cm soil depth by 2.53 kg net inorganic N/ha/grazing. While these values are low compared to the typical range of annual N losses to water from dairy farms (e.g. 19 - 34 kg N/ha/year (Monaghan et al., 2002)), they represent quantities of N at risk of leaching loss from a single grazing only. On dairy farms there are typically 9-10 grazings annually, with the late summer to early winter grazings (4-5 grazings) having the largest influence on annual N leaching losses in drainage (Shepherd et al., 2011). Therefore, it is likely that the use of spreading urine as a method of reducing N leaching losses will work for more than a single grazing. The losses estimated from the current study represent a grazing that occurs about 29 days before the commencement of the drainage. Although, the urine was applied in early autumn (6th March 2017), its timing in relation to the start of the drainage season is more typical of a late autumn grazing. It is therefore conceivable that at grazings where there was a longer period between the grazing event and the onset of drainage, increasing the urine patch area could be a greater impact on reducing N leaching losses. The better distribution on the shallower soil surfaces achieved by spreading the urine allowed a greater proportion of pasture plants to take up the available N, and in this way, reducing the N surplus and susceptible to be lost by leaching. Further research is required to investigate the effect of increasing urine spread area at multiple grazings, over the period from late summer to early winter, to determine the effect of this mitigation on reducing annual N leaching losses.

4.7 Effects of spreading urine on pasture production

The pasture composition during the trial was on average composed of 38% grasses, 35.9% of clover and 26.1% of weed. Caradus et al. (1996) estimated that in New Zealand the contribution of white clover to total pasture yield is about 20%. Clover amounts can vary widely depending on time of the season, climate, livestock grazing, soil fertility and pest pressure. A botanical survey of perennial ryegrass-clover dairy pasture conducted during autumn/winter reported for the Waikato and Bay of Plenty regions showed pasture composition was on average 59% ryegrass, 11% clover, 15% weeds and 15% other grasses

(Tozer et al., 2014). The higher clover content in the current study could influenced by the long duration (~6 month) that the pasture did not receive N input (livestock urine or fertiliser). Ball et al. (1979) found clover content up to 48% when the pasture did not receive any fertilisation.

At the first pasture harvest (25 DAUA), the pasture accumulation for the Urine (1 m^2) treatment was 200 g DM/1.4 m^2 quadrat, compared to 190 g DM/1.4 m^2 quadrat for the No-urine treatment. For the Urine (0.2 m^2) treatment the pasture production was of 137 g DM/1 m^2 quadrat, compared to 136 g DM/1 m^2 (equivalent) quadrat for the No-urine treatment. At the second pasture harvest (71 DAUA), the pasture accumulation for the Urine (1 m^2) treatment was 274 g DM/1.4 m^2 quadrat, compared to 261 g DM/1.4 m^2 quadrat for the No-urine treatment. For the Urine (0.2 m^2) treatment the pasture production was of 180 g DM/1 m^2 quadrat, compared to 187 g DM/1 m^2 (equivalent) quadrat for the No-urine treatment.

To determine the net pasture production per hectare, the pasture production measured in the No-urine treatment was subtracted from the pasture production measured in the urine treatments, and these values were multiplied by the quantity of urine patches per hectare in a single grazing determined in the previous section (1340 urine patches per hectare). The No-urine treatment yield was used determine the inter-urine patch areas.

Table 4.3. Pasture production from the different treatments, net increase in DM accumulation for the urine treatments and estimated DM accumulation for the different treatments.

Treatment	Harvest 1 (25 DAUA)	Harvest 2 (71 DAUA)	Total (Harvest 1 & 2)	Net increase for Urine treat.	Estimated DM accumulation (Harvests 1 & 2)
	g DM/ 1.4 m^2 quadrat	*g DM/ 1.4 m^2 quadrat*	*g DM/ 1.4 m^2 quadrat*	*Net g DM/ 1.4 m^2 quadrat*	*Kg DM/ha*
No-Urine	189	261	451		3220
Urine (1 m^2)	200	274	474	23.0	3251
	*g DM/ 1 m^2 quadrat**	*g DM/ 1 m^2 quadrat**	*g DM/ 1 m^2 quadrat**	*Net g DM/ 1 m^2 quadrat*	
No-Urine	136	187	322		3220
Urine (0.2 m^2)	137	180	317	-5.2	3213

* The value of the No-urine was obtained from 1.4 m^2 quadrant and was reduced to an estimated yield from an area equivalent to 1 m^2.
** The increase in DM accumulation was determined by multiplying the difference attributable to the urine patch by an estimated 1340 urine patches per hectare.

Over the two harvests, the pasture DM accumulation for the No-urine treatment produced an average of 3220 kg DM/ha. The two urine patch treatments achieved a similar level of pasture DM accumulation, being 3251 and 3213 kg DM/ha for the Urine (1 m^2)

and Urine (2 m^2) treatments respectively, which were not significantly different. The lack of a pasture growth response from the added urine could have been influenced by the high clover content of the pasture. In grass/clover pastures, the yield response to added N comes directly from the grass component (Haynes & Williams, 1993), because clover is less reliant on soil mineral N supply as they are capable of fixing atmospheric N. In addition, there may have been adequate background soil mineral N from the N fertilisation applied as a basal treatment a month before the trial started, and due to moist warm soil conditions contributed to N mineralisation from the soil organic matter. All these factors could have contributed to N not being growth limiting during the trial, which would explain why the pasture was not responsive added urine.

Chapter 5. Conclusions and future research

This study demonstrated that increasing cow urine deposition area in autumn reduced the quantity of urinary N susceptible to leaching loss. Increasing the urine patch area 5-fold, from 0.2 to 1 m^2, reduced net inorganic N in the 45-120 cm depth by 63.3%. This represents a large decrease in inorganic N leaching risk, and was achieved 53 days after urine application and 24 days after the estimated commencement of seasonal drainage. At this time, compared to No-urine treatment, only the Urine (0.2 m^2) had a substantial proportion (37.9%) of the N, attributable to the urine addition, remaining in the 0-120 cm depth. In this treatment, the pattern of soil mineral N concentration at the different soil depths, peaking at the 45-60 cm soil depth and decreasing to the 105-120, supports that the majority of urinary N remaining in the soil was present mostly within the 0-120 cm depth at this time.

Extrapolating the results to a paddock scale, showed that increasing the urine deposition area, reduced the amount of net inorganic N susceptible to leaching loss, from a single autumn grazing, by 63.6% (2.53 kg N/ha) on a whole paddock basis. If the mitigation option is applied to all grazings that have an influence the annual N leaching losses, then greater reductions would be expected.

The reduced accumulation of soil inorganic N, caused by increasing the size of the urine patch, is likely to be due to a range of factors, including increased plant uptake, ammonia volatilisation and/or N immobilisation in the soil. Future research is required to quantify the contribution that these factors make to reduced leaching losses and also to determine the effect that using this mitigation at multiple grazings has on reducing annual N leaching losses, especially over the period from late summer to early winter, which is considered the most influential time of the year.

Over the relatively short duration of the study, pasture did not show a significant response to urine addition, which is likely to be due to N not being the main pasture growth limiting factor at that time. Therefore, future research is required to assess the effects on increasing the urine deposition area at multiple grazings on annual pasture growth.

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