Comparing the spatial distribution of DCD and urinary nitrogen on well-drained and poorly-drained soils

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Abstract

Nitrogen (N) losses from urine patches can be significant contributors to greenhouse gas emissions and water quality issues. Nitrification inhibitors may reduce these losses by slowing down the transformation of urine-N to nitrate. Technologies exist that can detect urine patches and target inhibitor applications specifically to the patch area, thereby avoiding the need to apply the inhibitor over the entire paddock. However, the potential time delay between the grazing event and the inhibitor application, and the small volumes of inhibitor used could result in only partial interception of the urine by the inhibitor in the soil. This would limit the potential effectiveness of the inhibitor.

Two studies were undertaken to compare the movement of urine to the movement, and therefore potential interception, of the nitrification inhibitor dicyandiamide (DCD).
In the first study, patches of urine were created by pouring three different volumes of urine (1, 2 and 3 L) onto two soils of contrasting drainage at two different moisture levels.
In the second study, two volumes of DCD (the equivalent of 10 and 20 kg DCD/ha) were sprayed using a Spikey® spray unit onto urine (2 L volume) patches created within 80 cm diameter chambers in two soils of contrasting drainage at two different moisture levels.

The variation in urine-N concentration both within and between individual urine patches was substantial. Total urine N recovery averaged 38%. On average, 67% of the recovered N was recovered from the top 5 cm, 14% from 5-10 cm and 19% from 10-20 cm.

On average, 78% and 69% of the DCD applied at 30 mL and 60 mL, respectively was recovered from the soil. Of this, on average 67% was present in the 0-2 cm, 8% in 2-5 cm and 24% in 5-10 cm soil depths. DCD concentrations in the top 2 cm varied greatly and average concentrations of 15.5 and 11.4 mg DCD/kg soil were measured for 30 and 60 mL applications. There was little difference in DCD (1.45 mg DCD/kg soil) measured below 2 cm between application rates. Concentrations were significantly higher with a higher application rate at 0-2 cm on the Tokomaru soil but not on the Manawatū.
After five days, following 24 mm rainfall, DCD recovery remained the same but its distribution and concentrations among the soil depths changed indicating its downward movement. About half of the recovered DCD remained in the 0-2 cm soil, one-third accumulated in 2-5 cm depth and the remainder was in 5-10 cm depth.

The difference between urine and DCD distributions suggests that the DCD applications used in this experiment only intercepted 35-50% of the urine patch, without rainfall. With at least 24 mm of rainfall and 60 mL of DCD (13.8 kg DCD/ha) the DCD could be intercepting 80% of the urine-N. This will limit the effectiveness of DCD to reduce N leaching. It's impact on N_2O emissions is less certain.

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1. General Introduction

In New Zealand's outdoor, pasture-based systems, 95% of dairy excreta and almost 100% of non-dairy cattle, sheep, deer and other livestock excreta is deposited on pasture (Ministry for the Environment, 2019b). This means that significant quantities of N are being applied to the soil as relatively small, concentrated urine patches. The concentration of the N in the urine patch can typically range from 200-800 kg N ha^{-1} (Pakro & Dillon, 1995; Selbie et al., 2015). A study using ^{15}N, suggested that the N from these patches does not diffuse laterally much beyond 20 cm from the edge of the initial patch of urine (Decau et al., 2003). This makes it difficult for pasture to utilise all of the urinary N. Various studies have reported anywhere between 10 and 60% pasture recovery of urinary N (Clough et al., 1996; Ledgard et al., 1982; Moir et al., 2016; Whitehead & Bristow, 1990). The rest of the N is free to be leached, volatilised, denitrified or immobilised in the soil. For example, the incomplete reduction of nitrate-N results in the formation of nitrous oxide (N_2O), a potent greenhouse gas (Saggar et al., 2004). A given amount of urinary-N can produce 3-4 times the N_2O emissions than same amount of dung-N (Ministry for the Environment, 2019b; Simon et al., 2018). Dung excretion is also a source of N deposited onto soil, but it provides a much slower release and less concentrated source of N (Fischer et al., 2016; van Groenigen et al., 2005).

Agriculture is the largest contributor to New Zealand's greenhouse gas emissions, producing 49% of total emissions. It is estimated that about 22% of agricultural emissions is as N_2O, of which almost two-thirds comes from dung and urine, with additional indirect (i.e. volatilisation and leaching/runoff) N_2O emissions (Ministry for the Environment, 2019b). The global atmospheric concentration of N_2O has increased from 270 ± 7 ppbv in pre-industrial period to 328 ± 12 ppbv in 2018. Despite its low concentration and an atmospheric lifetime of ~120 years, N_2O is 265 times more potent than CO_2 (GWP_{100}) (IPCC, 2014) and a significant contributor to stratospheric ozone depletion (Myhre et al., 2013; Ravishankara et al., 2009).

The New Zealand Government has committed to reducing total net emissions by 30% of 2005 levels by 2030 (New Zealand Government, 2016), and carbon dioxide (CO_2) and N_2O to net zero by 2050 ("Climate Change Response Act," 2002). However, current mitigation technologies available to New Zealand farmers are not capable of achieving anywhere close to a net zero target by 2050, without significant offsetting (Biological Emissions Reference

Group, 2018; Reisinger et al., 2017). Therefore, new mitigation approaches and tools are required.

Another loss of N from agricultural soils is via nitrate (NO_3^-) leaching. This occurs when there is an accumulation of nitrate (NO_3^-) in the soil that coincides with a period of drainage. Because soils are positively charged, N in the form of NO_3^- is vulnerable to leaching (Di & Cameron, 2005). Nitrogen losses from agriculture to freshwater are resulting in elevated concentrations of N in New Zealand's waterways. This is having significant negative impacts on ecosystem health; NO_3^- can be toxic to aquatic fauna and can cause eutrophication. Nitrate levels are also above acceptable levels for human health in some groundwater sites (Ministry for the Environment & Stats NZ, 2019). Lysimeter trials from Silva et al. (1999) suggest that typical N leaching losses from cattle urine patches can range from 50-90% of total N leaching from a grazed paddock, depending on the amount of effluent and fertiliser applied. However, scaling these trials up to farm system level is difficult, as in practice, higher N fertiliser applications are usually accompanied by a higher stocking rate and, therefore, more urine patches.

Operative and proposed targets for N loss reductions will be very difficult for some farmers to achieve (Ministry for the Environment, 2019a; Parminter, 2018a, 2018b). Therefore, new mitigation approaches and tools are required. One potential tool to reduce N_2O emission and leaching losses of N from urine is the application of nitrification inhibitors. By slowing the conversion on ammonium to other forms of N, N_2O emissions and NO_3^-, leaching can be reduced. This has been demonstrated in a range of conditions, with a range of chemical inhibitors (Clough et al., 2007; 2008; Di & Cameron, 2002). Nitrification inhibitors have traditionally been applied across a whole paddock or field. However, new technologies may allow treatment of individual urine patches (Bates et al., 2015). This means that it is important to understand how these inhibitors move through soil when applied to a urine patch. The 3-dimensional (3D) movement of urine patches has not been extensively studied, however, one UK study found 2 L urine patches tend to mostly penetrate to 20 cm soil depth, with localised areas penetrating down to 40 cm (Williams & Haynes, 1994). A more recent NZ study of depth only, using 1-2 L urine volumes, found almost all urine N remaining in the top 15 cm (Giltrap, Jolly, et al., 2020). In contrast, inhibitors are only applied in small amounts, so it is not likely that they will travel as deep, perhaps only to 2.5 cm after 7 days (Bishop, 2010). This means that the inhibitor may only be intercepting a small portion of the urine-N. Therefore, techniques must be explored to improve this. Nitrification and denitrification can also occur before the 7

days measured by Bishop (2010) (Giltrap et al., 2010), so measuring inhibitor penetration closer to application time is also needed to address this gap in information.

This research study was part of an international research project funded under the Global Partnership in Livestock Emissions Research (GPLER) focussing on accurately measuring, mapping, and modelling the location, size and shape of urine patches to facilitate targeted N_2O emissions reduction by further developing Spikey® (a NZ-designed machine that detects and treats freshly-deposited urine patches) lead by Manaaki Whenua-Landcare Research with national (Massey University, Pastoral Robotics Limited and AgResearch) and international (Teagasc, Ireland and New South Wales Department of Primary Industries, Australia) collaboration.

The main objective of this research study was to map the distribution of urine-N in urine patches and quantify the proportion of urine patch intercepted by the application of a nitrification inhibitor, dicyandiamide (DCD).

To be more specific:

1. Determine the variation that occurs in the 3D spatial movement of dairy cow urine patches in different conditions (urine volume, soil moisture level and soil type)
2. Measure the depth penetrated by DCD when sprayed onto pasture under different conditions (urine volume, soil moisture level and soil type).

2. Background

2.1. Nitrogen

2.1.1. Introduction

Nitrogen (N) is an essential component of biological systems and food production. Historically, New Zealand farmers relied on clover species to input plant available N into their systems. However, over the past two decades, the use of N fertiliser has increased substantially, by 627% from 1990 to 2015 (Stats NZ, 2019). This increase has largely been driven by the growth of the dairy industry, growing from 2.4 million cows in 1990 to 4.9 million cows in 2017, contributing to a three-fold increase in milksolids production (Livestock Improvement Corporation & DairyNZ, 2018). However, N use efficiency (N output in product as a percentage of total N input) is usually low in agricultural systems, generally ranging from 10-65% in dairy systems across Europe, North America and Australasia, 21-42% in New Zealand (de Klein et al., 2016; Ledgard et al., 1998). This leaves a significant amount of N that can be lost from the soil to the surrounding environment. Not only is this an economic loss of valuable nutrient, it can also lead to significant environmental issues.

2.1.2. Nitrogen Cycle

Figure 1 shows a diagram of the nitrogen cycle. Plant available N can enter the soil-plant system on farms through biological fixation, dinitrogen (N_2) to clover plant N, as livestock urine (mainly urea, $CO(NH_2)_2$) or as a fertiliser. Fertilisers and soil amendments can apply N in different forms: complex organic forms, urea, ammonium (NH_4^+) or nitrate (NO_3^-), but over 80% of fertiliser N applied in NZ is as urea (Ministry for the Environment, 2019b).

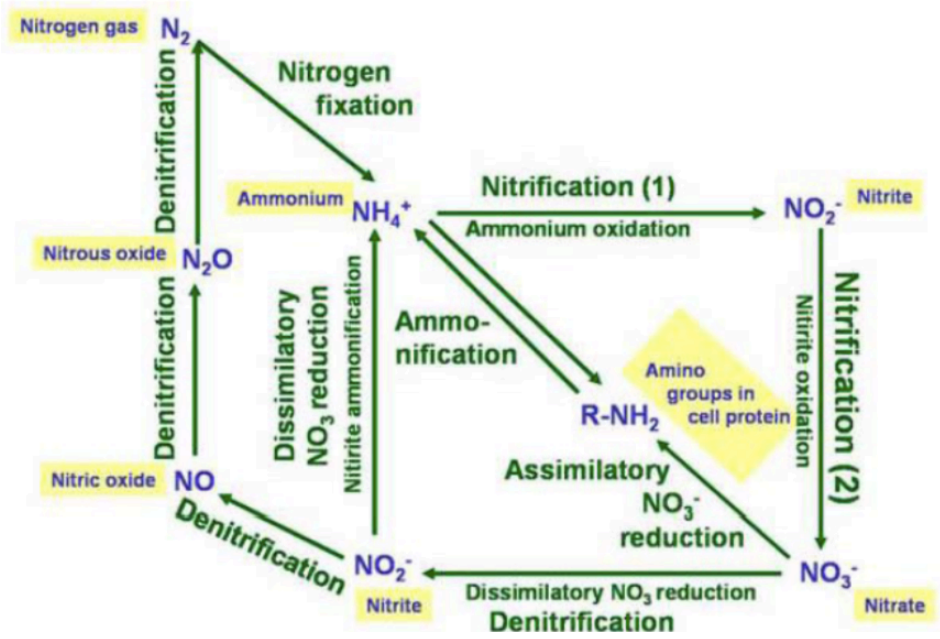

Figure 1 - Nitrogen cycle, pers comm Prof M Camps Aberstien

Urea and other more complex organic forms of N hydrolyse to form ammonium. This can deprotonate to form ammonia gas (NH_3), which can then be lost to the atmosphere through volatilisation. Various experiments have reported a range of values for NH_3 loss, on average 12.9% of urinary N applied in urine patches is lost as ammonia when measured at an individual urine patch level (range 1-38%). When measured at a whole-paddock level, using micrometeorological measurements, the average is lower, 8% (range 1-28%) (Selbie et al., 2015). Volatilisation losses can be difficult to determine due to methodological differences (Selbie et al., 2015). The picture is further complicated as emitted ammonia can also redeposit back to the paddock it was emitted from; 20-60% of ammonia emitted is redeposited within 2 m of the source (Ross & Jarvis, 2001). Ammonia can also redeposit into terrestrial and freshwater ecosystems, contributing to acidification and eutrophication in sensitive ecosystems. This is not a significant issue in New Zealand, but for other countries, particularly in the European Union, it can be a major challenge (Hicks et al., 2011; Hoogerbrugge et al., 2019; Remkes et al., 2019). Redeposited ammonium is then vulnerable to denitrification, producing nitrous oxide. An estimated 11% of New Zealand's N_2O emissions come from volatilisation (Ministry for the Environment, 2019b).

The N in NH_4^+ can be oxidised to form NO_3^-, via nitrite (NO_2^-), through the process of nitrification. There are several species of bacteria and archaea that are capable of facilitating nitrification, but the most common ones are *Nitrosomonas* and *Nitrobacter*. Nitrate is the main

form of N lost in drainage water via leaching. New Zealand's soils are predominantly negatively charged, therefore, while ammonium is attracted to soil surfaces, nitrate is repelled and becomes much more mobile and, thus, vulnerable to leaching to groundwater. On average 20% of the N in a urine patch is lost as nitrate (range 7-70%) (Selbie et al., 2015)

Denitrification is the reduction of nitrate to dinitrogen, however, incomplete denitrification can result in the formation and loss of nitrous oxide (N_2O) gas to the atmosphere. On average, 2.1% of urinary N applied in a urine patch is lost as ammonia, but this is highly variable ranging from 0-14%. New Zealand specific work reported emission factors ranging from 0.3%, on a well-drained stony soil, to 2.5% on a poorly-drained soil (de Klein, Barton, Sherlock et al., 2003). New Zealand's Greenhouse Gas Inventory uses an emission factor ($EF_{3(PRP- URINE)}$) of 1% (Ministry for the Environment, 2019b).

An estimated 12% of New Zealand's greenhouse gas emissions are nitrous oxide, 64% of which comes from dung and urine, plus additional indirect (i.e. volatilisation and leaching/runoff) nitrous oxide emissions (Ministry for the Environment, 2019b). The global atmospheric concentration of N_2O has increased from 270±7 ppbv in pre-industrial period to 328±12 ppbv in 2018. Despite its low concentration and an atmospheric lifetime of ~120 years, N_2O is 265 times more potent than CO_2 (GWP_{100}) (IPCC, 2014) and a significant contributor to stratospheric ozone depletion (Myhre et al., 2013; Ravishankara et al., 2009).

Nitrogen losses from agriculture to freshwater are resulting in elevated concentrations of N in New Zealand's waterways. This is having significant negative impacts on ecosystem health; at high levels, nitrate is toxic to aquatic fauna (Ministry for the Environment & Stats NZ, 2019). Even at lower concentrations, elevated N levels disrupt normal nutrient cycling, and promote the growth of algal blooms, which can smother habitat and deplete dissolved oxygen supply. In the range of 50-86% of river length in areas of pastoral agriculture in NZ did not meet the *Australian and New Zealand guidelines for fresh and marine water quality* Default Guideline Values for N over 2013-2017. At very high concentrations of NO_3^- in water there is potential impacts on human health as well. For example, 13% of groundwater testing sites breached the drinking water standard on at least one occasion over 2010-2014 (Ministry for the Environment & Stats NZ, 2019). Nitrogen lost to waterways is also vulnerable to undergo denitrification. An estimated 6% of New Zealand's nitrous oxide emissions come from leaching and runoff (Ministry for the Environment, 2019b).

2.2. Rooting dynamics

New Zealand's lowland pastures generally consist of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). Both are relatively short rooting plants. Up to 85% of pasture root mass can be found in the top 15cm of soil, with around half in the top 5cm (Evans, 1978; Weaver, 1950; Williams et al., 1989). This means that the capacity for uptake of soil N rapidly diminishes going down the soil profile. Some studies suggest that uptake is limited once it goes beyond 12cm (Williams et al., 1989). Other, deeper rooting pasture species have been suggested as a way to capture more N from the deeper soil depths. For example, tall fescue (*Festuca arundinacea*) mostly maintains its root density down to at least 45cm (Huang & Gao, 2000; Malcolm, Moir, et al, 2015). However, several studies have shown that deeper rooting plants do not necessarily result in more nitrogen capture by the plant, or lower nitrogen losses (Malcolm, Moir, et al., 2015; Moir et al., 2013; Pirhofer-Walzl et al., 2010). This highlights the importance of keeping and utilising urinary nitrogen in the top 15cm of soil.

2.3. Microbial spp.

Application of urine to soil also drives growth in the population of many soil microbes that use nitrogenous compounds in their metabolic pathways. Significant increases in the abundance of some NH_4^+ oxidising bacteria (AOB) have been observed upon the application of urine or ammonia, with populations increasing up to 10-fold and bacterial activity up to 170-fold (Di et al., 2014; Di et al., 2010; Di et al., 2009). This drives rapid nitrification of urinary N. Ammonia oxidising archaea (AOA) have also been found in soil. However, they do not appear to respond to urine is the same way as AOB, with either no effect, or a reduction in activity upon the application of urine, suggesting they favour low NH_4^+ environments, in contrast with AOB (Di et al., 2014; Di et al., 2010; Di et al., 2009). Likely because of this, AOB were found in greatest numbers in topsoil, while AOA were more common in one of the subsoils. It has been suggested that AOA do not use NH_4^+ oxidisation as their main source of energy, and as a result are not an important driver of NH_4^+ oxidation in agricultural soils (Jia & Conrad, 2009; Leininger et al., 2006). This spatial distribution of ammonia oxidisers/nitrifiers is important as it will change that rate of transformation of urine depending on how deep and how quickly urine travels through the soil profile. Inhibiting the activity of nitrifiers will slow the rate of nitrification.

2.4. Urine patch dynamics

In the Canterbury region, urine dynamics have been assessed using bromide (Br-) as a tracer, on Templeton silt loam soil (Typic Immature Pallic Soil). When cattle urine, with a 2 L "urine" volume, was poured from a height of 1 m and sampled after 20 min, the Br^- spread over a surface area of 0.38-0.42 m^2. 55-66% of the Br^- was recovered from the top 5 cm of soil. Most of the remaining Br^- was extracted from the 5-10 cm layer, although Br^- was detected down to a soil depth of 40 cm. The wetting front for the expected matrix flow of the Br- was calculated to be 17mm; since much of the Br- was recovered at depths below this, considerable preferential flow must have occurred (Whitehead & Bristow, 1990). Another study, again using a Br^- tracer, across ten different soils in the Southland, Canterbury and the Waikato regions found considerable variation in urine penetration of soil. On average, 67% of the Br^- was recovered from the top 10 cm (similar to Whitehead and Bristow (1990)), but this ranged from 95% on a Taupō Pumice, to 23% on a Waikato Ulitic soil (Monaghan et al., 1999). Modelling, accompanied by NH_4^+ measurements in two soils in the Manawatū (a sandy loam and a silt loam), produced similar results to Whitehead and Bristow (1990), 50-60% of urine-N remaining in the top 5cm, about 20-30% between 5-10 cm and 10-20% between 10-15 cm, for urine patches of 1-2 L (Giltrap, Jolly, et al., 2020). Urine volume has been shown to influence how deep the urine moves down the soil profile. When Whitehead and Bristow (1990) simulated using sheep urine with a volume of 0.2 L, the Br^- tracer was only detected down to 15 cm, in contrast to the 40 cm from the 2 L patch. However, the proportion of urine in the top 5 cm was the about the same, 55-66%. It should be noted that there was considerable variation between patches.

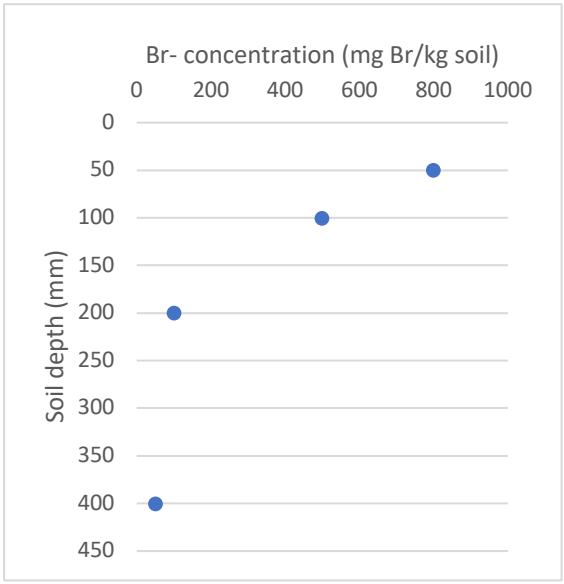

Figure 2 - Mean concentration of Br- in the soil profile following simulation of sheep and cattle urinations using KBr solution. Based on data from (Whitehead & Bristow, 1990)

There was a significant relationship between the proportion of Br- moving beyond 20 cm and the saturated hydraulic conductivity (K_s), but this relationship explained less than half of the variation seen. Correlations were improved when the K_s for deeper soil layers, beyond 20 cm, was used, while K_s at 0-5 cm was a particularly poor predictor of Br^- movement. It is not

possible to say whether this is because urine penetration is more strongly influenced by K_s in subsoil, or is just due to the high variability of K_s in topsoil due to higher biological activity (Monaghan et al., 1999). There was a poor correlation between urine patch surface area and depth penetration (with a consistent urine volume), although the relationship was significant for movement below 30cm only (Monaghan et al., 1999).

Clear relationships between soil moisture and urine movement below 20 cm were demonstrated on three of four soils, with a greater proportion of Br^- moving below 20 cm when the same soil type had a lower volumetric water content. However, this relationship did not appear on the fourth soil (a Gley soil) investigated. A clear relationship also failed to appear using the data from all of the urine patches in the study. This suggests that soil moisture does influence urine movement, at least below 20 cm, but the effects may be soil- or site-specific (Monaghan et al., 1999).

Whitehead and Bristow (1990) assessed the behaviour of urinary N itself. When sampled at 4 hours after application, at least half of the cattle urine N was still in the form of urea. However, after 2 days, it had all converted to ammonium. Modelling in Giltrap, Jolly, et al. (2020) showed similar conversion rates, although slightly more than half of the urine-urea was modelled as having been hydrolysed after 4 hours. In Whitehead and Bristow (1990), nitrate was not detected in significant quantities until the next sampling event, 14 days after application. After 29 days, most of the recovered N was as nitrate.

2.5. Nitrification inhibitors

Nitrification Inhibitors (NIs) are compounds that delay the bacterial oxidation of NH_4^+ to NO_3^- in the soil for a certain period, depressing the activity of nitrifiers, which can slow down the nitrification process. As a result there can be a decrease NO_3^- leaching, increase N assimilation and pasture yield, and a reduction in N_2O emissions. Dicyandiamide (DCD), 3,4-dimethylpyrazole phosphate (DMPP) and [2-chloro-6-(trichloromethyl) pyridine] (nitrapyrin) are the most frequently used commercial NIs in agriculture. These inhibitors have been demonstrated to slow the rate of nitrification in soil from fertiliser and urine-N, and reduce N losses (Clough et al., 2008; Wolt, 2004). DCD has been widely studied in New Zealand for and it has been shown to consistently reduce N_2O emissions (Cameron et al., 2014; de Klein et al., 2014; Kim et al., 2014; Ledgard et al., 2014) and nitrate leaching (Francis, 1995; Malcolm,

Caneron et al., 2015), and, in some studies, stimulating additional pasture growth (Carey et al., 2012). It was used in New Zealand until 2011 but was withdrawn from use on farms in due to the detection of traces of DCD in exported milk. Its use is currently restricted to research undertaken on small plot trials or lysimeters.

The mechanism by which NIs work varies across the different inhibitors. However, a common theme across inhibitors is interference with the bacteria and archaea responsible for driving nitrification, by denaturing a critical enzyme, or competing for the active site of an enzyme, or interfering with substrate uptake (Amberger, 1989; Bishop, 2010). When the oxidation of a substrate produces a highly reactive product, such as unsaturated epoxies, the inhibitor can covalently bind to the active site of AMO. This permanently deactivates it. However, it has no impact on the production of AMO, so once the inhibitor is consumed, the bacteria will recover rapidly (Bishop, 2010; McCarty, 1999; Subbarao et al., 2006). Another class of inhibitors targets the copper active site, by forming strong complexes that block the site. Many sulphur containing compounds containing C=S bonds can act as competitive inhibitors (Bishop, 2010; Subbarao et al., 2006).

The mode of action of DCD is currently uncertain. Suggestions have included Cu chelation, or the prevention of ammonia uptake or utilisation (Amberger, 1989; Lehtovirta-Morley et al., 2013; Ruser & Schulz, 2015). However, it has been demonstrated that it is a bacteriostatic inhibitor (supressing the biological activity of *Nitrosomonas* rather than killing them) (Subbarao et al., 2006). The inhibitor nitrapyrin (2-chloro-6-(trichloromethyl)-pyridine) acts by chelating the copper components of the cytochrome oxidase involved in ammonia oxidation (Powell & Prosser, 1986; Subbarao et al., 2006). A relatively new inhibitor, 3,4-dimethlypyrszole phosphate (DMPP), is suggested to have a similar mechanism, but this has not been proven (Fuertes-Mendizábal et al., 2019; Ruser & Schulz, 2015). Plants can also release biological NIs. The presence of ammonium ions in the rhizosphere triggers a H^+ flux across the root hairs. This increase the permeability of the root, allowing the release of these compounds (Bishop, 2010). There is some speculation that this may be one of the mechanisms by which plantain (*Plantago lanceolate*) reduces nitrate leaching (Carlton et al., 2018).

2.5.1. Application methods

The application of inhibitors largely depends on the inhibitor and how it interacts with plants and soil. DCD is a readily soluble powder and is commonly applied dissolved in water (Di & Cameron, 2002). It can also be applied as a granulated solid, coated on urea or as a stabilised fertiliser. Because it is highly soluble, it can migrate through the soil profile to some degree, allowing it to inhibit the nitrification enzymes where there is ammonium that they would otherwise nitrify. This contributes to its versatility for application. Initially trial work applied DCD as a solution, however, later a fine particle spray method was developed as this is more practical for use in a farm system (Clough et al., 2007). From 2007 to 2012, before it's withdrawal from the market, DCD was applied to 2.2 – 4.5% of the dairying area (Ministry for the Environment, 2014). DCD can also be fed directly to animals (Minet et al., 2016). To provide a comparison, nitrapyrin is another inhibitor, not commonly used in New Zealand, but used widely in the United States. It is strongly adsorbed by soil organic matter, which limits its movement from point of application. It is also prone to volatilisation. To mitigate these two issues, it is usually mixed with liquid fertiliser, such as anhydrous ammonia or UAN, and direct drilled (Bishop, 2010). A newer inhibitor, DMPP also has low mobility in soil but is still applied as a granulated solid (Bishop, 2010).

2.5.1.1. Targeted vs non-targeted application

Inhibitors are most effective when they are targeted to the sources of excess N. This can already be done with fertilisers, by using them as fertiliser additives. However, because urine is deposited often and in small areas by grazing livestock, in contrast to fertiliser, targeting an inhibitor to urine is not so simple. Ideally, the inhibitor would be applied only to the urine patch the moment urination occurs. One way to do this is to feed DCD directly to an animal as part of a total mixed ration. When the animal excretes the DCD in its urine and it will still retain its inhibitory properties. Minet et al. (2016) suggests an average rate of 30g DCD cow^{-1} day^{-1}, which produced the equivalent of 30kg DCD ha^{-1}. When homogenised to 30kg DCD ha^{-1}, it is just as effective as DCD applied to a urine patch after urination (Minet et al., 2018). There are several issues with this method, however. The first is that ingested DCD is not partitioned solely to urine, some DCD will also be excreted in milk (Welten et al., 2016). There is currently no food safety standard for DCD, and any contamination of food by DCD is not permitted. Unless a food safety standard is created and can be met, this method is not suitable for targeted DCD application. The other issue is that it requires farmers to be feeding some kind of

supplementary feed throughout much of the year, which is not the case on some of New Zealand's pasture-based farming systems.

An alternative approach is to apply an inhibitor to just the urine patch shortly after urination has occurred. This requires a method of detecting the location and size of the urine patch and then applying the inhibitor on the detected area. There are several possible methods for detecting urine patch areas, including thermographic imaging (Dodd et al., 2015; Jolly et al., 2019), visible and/or ultraviolet imaging or LiDAR (usually with a drone) (Jolly et al., 2019; Maire et al., 2018; Roten et al., 2017; Walklate et al., 2002), and conductivity measurements (Bates et al., 2015; Dodd et al., 2015; Jolly et al., 2019). Spikey® is a technology developed by Pastoral Robotics Ltd, which is capable of detecting urine patches by measuring soil conductivity. It then sprays the detected area with an inhibitor and/or growth promotor. The currently available versions are designed to be towed over a paddock after stock have grazed it and a robotic version is under development (Bates et al., 2015). This addresses several issues with the inhibitor being applied to non-urine areas. Because this enables less inhibitor to be applied, and the inhibitor that is applied will be consumed more quickly in high N urine patches (Kim et al., 2012), the inhibitor it is less likely to leach and less will be consumed by cattle. It also reduces the quantity of inhibitor needed, which would make more expensive types of inhibitor more cost effective to use.

2.5.2. Effects on N-cycle, leaching, N_2O emissions and plant uptake

A range of NIs have been trialled and shown to reduce emissions and nitrate leaching to various degrees, in various farming systems. Because of DCD's compatibility with New Zealand's pasture based agri-systems (i.e. relative cost and mobility), it is the only nitrification inhibitor to have been extensively used in NZ. Because it is only NI to have been extensively trialled, it is the only NI to be included in models such as the national Greenhouse Gas Inventory and Overseer. Di and Cameron (2002, 2003, 2006); Di, Cameron, and Sherlock (2007) have conducted several trials with DCD, looking at how it affects N_2O emissions. There results suggest that DCD, when applied to urine at 7.5-15 kg/ha, can reduce the emissions factor of urine by 56-87% (Clough et al., 2007). Further studies from Di et al. and Smith et al. in 2007 and 2008 reported reductions of 54-78%. From all of these studies, Clough et al. (2008) proposed from this data that New Zealand takes this into account in its national Greenhouse Gas Inventory and reduce the emissions factor for both fertiliser (EF_1) and pasture applied urine

($EF_{3(PRP-URINE)}$) by 67% when DCD is used within 10 days after a urination event (Ministry for the Environment, 2019b). Nitropyrin and DMPP are not commonly used in New Zealand, but are used in other countries for crop-based systems. They have been shown to be, on average, similarly effective at reducing N_2O emissions when applied to/with fertiliser, but this varies with land class and soil type (Akiyama et al., 2010; Di & Cameron, 2012; Ruser & Schulz, 2015). Smaller masses of inhibitor are required with these two inhibitors, only 0.5-1.5 kg ha^{-1}, compared to DCD (Bishop, 2010).

Di and Cameron (2002, 2003, 2004b, 2005, 2007) have also studied at how DCD application to pasture soils affect N leaching. Their trials have shown reductions from 13-77% (average 60%) when 5-15 kg DCD/ha are applied to urine patches. Based on the review of Clough et al. (2008), the Inventory calculates a reduction in the fraction on N leached ($Frac_{LEACH}$) of 53% (Ministry for the Environment, 2019b). The effect of nitrapyin on leaching losses has mainly been studied on cropping systems, mainly corn, with reductions from 0-40% (mean = 16%) reported in the Midwestern USA. Nitrapyrin was found to generally be more effective at reducing N_2O emissions than reducing N leaching (Wolt, 2004). DMPP has been shown to be similarly effective as DCD at reducing NO_3^- leaching in NZ pastures when applied at 1 kg/ha (Di & Cameron, 2012).

Di and Cameron's studies have also measured plant responses to DCD. In theory, if N losses are slowed, then this should give the pasture more opportunity to take it up, thus, leading to a pasture growth response. Di and Cameron (2002, 2004b, 2006) found responses that ranged from 0-49%. Clough et al. (2007) proposed assuming a conservative increase in dry matter production of 10-15%. This takes into account differing responses from urine and non-urine affected areas of the pasture.

The effect of nitrapyrin on grain yield can vary widely, from a 20% reduction in yield to a 61% increase, with 75% of observations showing an increase in yield (Wolt, 2004). Pasture yield increases of 0-31% have been reported following the addition of DMPP to urea fertiliser in Australia (Dougherty et al., 2016; Koci & Nelson, 2016; Rowlings et al., 2016).

2.5.3. Factors influencing inhibitor efficiency

2.5.3.1. Canopy interception

The pasture canopy can intercept DCD sprayed onto it, preventing a proportion of the inhibitor reaching the soil. This interception can range from 4 to 40%. Taller and/or denser pasture intercepts more DCD, due to a larger leaf area index on which DCD can be captured and retained. Rainfall following application can wash the DCD off the pasture and onto the soil, with higher rainfall being more effective (Kim et al., 2012). However, at the same time, rainfall can also wash urine deeper into the soil. Because nitrapyrin is usually direct drilled or incorporated into soil (in a cropping system), canopy interception in not usually an issue. Because DMPP is usually applied as a granulated solid with N fertiliser, canopy interception is also not usually an issue.

2.5.3.2. Interaction with soil particles and soil organic matter

DCD is a neutral molecule, so tends not to interact with soil exchange site. This can be seen with its ability to leach, with some studies reporting up to 58% of the volume of the applied DCD leaching beyond the root zone (Menneer et al. 2008). However, some studies have shown that other properties of soil affect the effectiveness of DCD. As mentioned earlier, while DCD has no overall change, it can interact with soil organic matter when the amide groups ($-NH_2$) of DCD bond to the carboxyl groups (-COOH) on organic matter. DCD also tends to bind more strongly to Allophanic soils (Singh et al., 2008; Zhang et al., 2004). This means that DCD may be less effective on soils with high organic matter (especially peats) and/or allophane. Singh et al. (2008) demonstrated that DCD was more effective at reducing N_2O emissions in the lower carbon (C) Tokomaru silt loam (Pallic soil; 88% reduction in emissions) that the higher C Egmont brown loam (Allophanic soil; 44% reduction in emissions). This was an incubation study so didn't show DCD mobility, but binding to soil surfaces will reduce the mobility of DCD. Nitrapyrin and DMPP bind much more strongly to soil surfaces (Sahrawat et al., 1987). Urine can decrease DCD sorption, but this effect can vary across soil types. This means that DCD may be more mobile on a urine patch, than it would be on urine free soil.

The inhibitory effectiveness of nitrapyrin has been shown to greatly decrease with increasing OM content, with little to no inhibition at all observed after 14 days in soil with 5% OM (Hendrickson & Keeney, 1979). DMPP has been shown to bind to clay surfaces, reducing its effectiveness as an inhibitor (Barth et al., 2001). Binding to soil OM has also been

demonstrated, although this relationship was not as strong as that with the soil clay fraction (Barth, 2007). The greater sorption of DMPP and nitrapyrin to soil reduces their movement through the soil profile. This means that, to maximise effectiveness, these inhibitors need to be applied with the source of N needing to be inhibited and, ideally, incorporated or injected into the soil, otherwise they are unlikely to move down the soil profile with any ammonium. This is not currently feasible when treating urine patches.

2.5.3.3. Movement in soil

As discussed above, DCD can move relatively freely through soil. However, because only small amounts of DCD are required to significantly reduce nitrification, application rates are relatively low, which can limit how far the DCD can penetrate into the soil profile. Bishop (2010) applied DCD to Manawatū silt loam and Dannevirke loam (Weathered Fluvial Recent Soil and Typic Allophanic Brown Soil respectively). Most of the DCD was recovered after 7 days in the top 1.5cm of soil, with no DCD reaching 4.5cm. However, (Di & Cameron, 2005) state that at least 5kg of DCD per ha down to 10cm (5μgDCD g^{-1} dry soil) is required for the DCD to effectively inhibit nitrification (Di & Cameron, 2005). Based on the DCD distribution in the soil profiles measured by Bishop (2010), then DCD is unlikely to effectively inhibit nitrification in the entire urine patch for at least the first 7 days.

The inhibitor DMPP has somewhat limited mobility in soil, and after a ten-day incubation of DMPP-fertiliser granules in soil, more than 80% of the DMPP remained within 5 mm of the granules. Higher temperatures and higher soil moisture content did increase the mobility of the DMPP, but only by small amounts (Azam et al., 2001). Immobile inhibitors can be problematic if what they are inhibiting is more mobile than they are. After ten days incubation of DMPP-fertiliser granules, NH_4^+ had begun to separate from the DMPP, with increasing NH_4^+-N to DMPP ratios and up to 13% of the recovered NH_4^+ beyond 25 mm with less than 3% of the recovered DMPP (Azam et al., 2001). As time goes on, the DMPP is able to inhibit less of the N it is applied with. This issue is more acute when treating urine patches, as the inhibitor is being applied after the urine has already been applied to the soil, usually with a delay of hours to days, already resulting in a potential separation of urine-N and inhibitor.

In theory, DCD can move further down the soil profile by applying higher volumes of inhibitor solution. Modelled quantities of DCD applied at 21, 42 and 63 kg DCD ha^{-1} suggest that it is

possible to get increased concentrations of DCD, over the 5kg DCD ha^{-1} threshold, at lower depths with higher application rates. However, these differences were not significant until at least 16 days after DCD application (Bishop, 2010). Also, while there was some improved penetration of DCD, most of the extra DCD simply filled the top 4 cm of soil (Bishop, 2010). No experimental work was carried out to confirm the effect of higher application solution volumes.

Rainfall also moves DCD through soil. DCD has been shown to reach 15 cm following 40 mm of simulated rainfall (typical of a UK storm). The distribution profile of the DCD matched that of urinary ammonium applied at the same time to simulate a sheep urine patch, down to 15cm. The same experiment was done using DMPP, with generally similar distribution profiles compared with DCD (Marsden et al., 2016).

Given enough time and rainfall NIs can leach beyond the root zone, into groundwater. A survey of large stream/river sites in Southland, shortly before DCD was taken off the market, found no detectable DCD. However, low levels were detected in small Waikato streams. This suggests that dilution in the larger streams reduced the concentration of DCD to below the detectable limit, but DCD leaching could still be of concern in small agricultural streams (Matthaei et al., 2014). Aside from the environmental concerns, it shows that DCD is mobile enough to move through the soil profile. However, questions remain as to how quickly this occurs, given that nitrification can begin almost immediately. Even though previously mentioned studies suggest DMPP is less mobile than DCD, it has been shown to leach with very small amounts (max 0.061 mg L^{-1}) detected in drainage water from lysimeters monitored over several seasons (Fettweis et al., 2001).

2.5.4. Inhibitor half-life

DCD breaks down in soil, via guanylic urea, guanidine and urea, to carbon dioxide and ammonium. Because DCD is bacteriostatic, this means that nitrification can resume relatively quickly after DCD degrades. Analysis for several studies from different countries suggest the half-life $t_{½} = 168e^{-0.084T}$ where T is temperature (°C) (Kelliher et al., 2008). This degradation means that the ability of DCD to inhibit nitrification effectively decreases over time and decreases faster at higher temperatures. As an example, this means that in July, when the mean soil temperature at Ruakura, Waikato, is 8°C the half-life of DCD would be 86 days. In

comparison, in January at 19°C (Chappell, 2014) then the half-life would be 34 days. DMPP also degrades in soil, with degradation being faster at higher temperatures. However, DMPP degradation is not as temperature dependent as DCD (Guardia et al., 2018; Menéndez et al., 2012). However, conflicting results report the half-life of DMPP as being both shorter (Guardia et al., 2018) and longer (Weiske et al. 2001) than DCD. Nitrapyrin also degrades in soil, also more quickly at higher temperatures. At 25°C, this inhibitor's half-life has ranged from 5 to 42 days in a number of soils (Wolt, 2000). For comparison, DCD would be expected to have a 21 day half-life at this temperature (Kelliher et al., 2008).

The effect of temperature on the longevity of an inhibitor has implications for how effective it will be at different times of the year. DCD and DMPP will be most effective in the winter when it is coldest, and less effective in the summer. This has been shown in several studies: Zaman, Saggar, Blennerhassett, and Singh (2009) found that DCD reduced N_2O emissions by 52% in autumn, but only 16% in the summer. The high risk time for nitrate leaching tends to be the late summer-early winter period (Shepherd et al., 2015), and nitrous oxide emissions the time from autumn through spring. Therefore, DCD is most effective at the higher risk periods. DCD will also be more effective in colder climates like Southland, compared to climates like Northland.

2.6. Conclusion

Nitrogen is an important component of agricultural systems. However, losses of N to the atmosphere and to water are causing significant issues for the climate and aquatic ecosystems, both in NZ and around the world. Nitrification inhibitors have been demonstrated to be able to significantly reduce nitrification in soils when applied to a paddock, which in turn reduces N_2O emissions and NO_3^- leaching. However, as application technology evolves, further research is needed to understand how these inhibitors behave when applied to individual urine patches. Studies in NZ and overseas have shown that urine can easily penetrate down to 10cm deep. However, experimental and modelling work suggest that mobile inhibitors like DCD do not reach beyond 5cm deep for some time, and largely remains in the top 1.5cm. This disparity means that a significant portion of the urine patch may not be effectively treated. Further research is need to better understand the interception of urine-N in urine patches by inhibitors, like DCD, and to determine whether changes in application method, such increasing the application volume, can be used to increase their effectiveness.

3. Measuring urine patch distribution

3.1. Issue

Urine patches usually deliver a higher nitrogen (N) load than the pasture is capable to taking up (Selbie et al., 2015). Part of this is due to the distribution of roots down the soil profile, with up to 85% of pasture root mass can be found in the top 15cm of soil, with around half in the top 5cm (Evans, 1978; Weaver, 1950; Williams et al., 1989). To understand how much urine-N is vulnerable to leaching beyond this active root zone, it is important to understand how urine-N moves laterally and down the soil profile following urine deposition by livestock grazing the pasture.

Nitrogen transformation urease and nitrification inhibitors are a potential tool to slow down urine-N transformations and reduce N losses from urine patches. However, the proportion of urine-N captured by inhibitors applied post urination is not well quantified. To quantify the proportion of urine-N captured it is necessary to first understand the distribution of urine-N within a urine patch.

A field study was undertaken to explore the 3D distribution of urinary N in individual urine patches on two contrasting soils.

3.2. Background

Urine is made up of many compounds, including a range that contain N. On average, 73% of urinary N is as urea (range 60-90%), but other compounds include allatonin (2-11%), hippuric acid (3-8%), creatinine (2-5%), creatine (1-4%), and ammonia (0-9%) (Bristow et al., 1992; Selbie et al., 2015). Because most of the urinary N is in the form of urea, measuring urea and its products mineral N (ammonium, nitrite and nitrate) will be the most representative measure of urine-N.

The mineral N measurements to detect urine-N distribution in a urine patch do include background mineral N present in the soil. Therefore, to differentiate between urinary N and background N resulting from previous urine patches, or fertiliser applications or mineralisation of organic matter it is essential to estimate a background levels of mineral N, and establish a threshold for urine-N. There are limitations of this approach as it requires a clear difference between background mineral-N and urine mineral-N. To overcome these limitations bromide (Br^-) can be used as a tracer. It is easy to extract and detect in the lab, it behaves same as other

ions in urine such as chloride (Cl^-), and is not usually present in soil in significant amounts (Williams, 1988).

However, while Br^- is a good tracer for showing urine patch distribution, it is not a proxy for N movement. It can move more rapidly through the soil profile than nitrate (Field et al., 1985). This is probably since conversion of urea to ammonium precedes conversion to nitrate, and positively charged NH_4^+ is better retained by soil surfaces. Also, Br^- does not represent the rate of nitrification, i.e. the ratio of ammonium to nitrate. Since N is of specific interest in this study, Br^- is not a suitable alternative to direct measurement of mineral N.

Another alternative is to use ^{15}N labelled urea as ^{15}N is not naturally occurring, so any ^{15}N detected will have come from the applied urine. ^{15}N translocation and transformations in the soil will be as N. However, ^{15}N has the disadvantage of being more expensive and time consuming to measure. In this case, due to the number of urine patches being assessed and number of soil samples required, ^{15}N is not suitable for this study. Therefore, despite the potential issues with distinguishing the edge of the urine patch, simple mineral N measurements is the most appropriate method.

3.3. Methodology

3.3.1. Site descriptions

Site 1

The Massey site was located on Dairy 4, 500 m south of Massey University's Manawatū campus, on the southern outskirts of Palmerston North (see Figure 4). The site is on Tokomaru silt loam, a Argillic-fragic Perch-gley Pallic soil, and is poorly drained. Dairy 4 is a working dairy farm.

Site 2

The Ruakura site was located at AgResearch's Ruakura research site, on the eastern edge of Hamilton (see Figure 3). The site is on Horotiu sandy loam, a Typic Orthic Allophanic Soil, and is well drained. AgResearch's Ruakura farm is a working farm with dairy grazing and beef finishing cattle.

Figure 4 - Dairy 4 field trial site at Massey University – Manawatū. © 2019 Google. Insert: Manawatū-Whanganui region

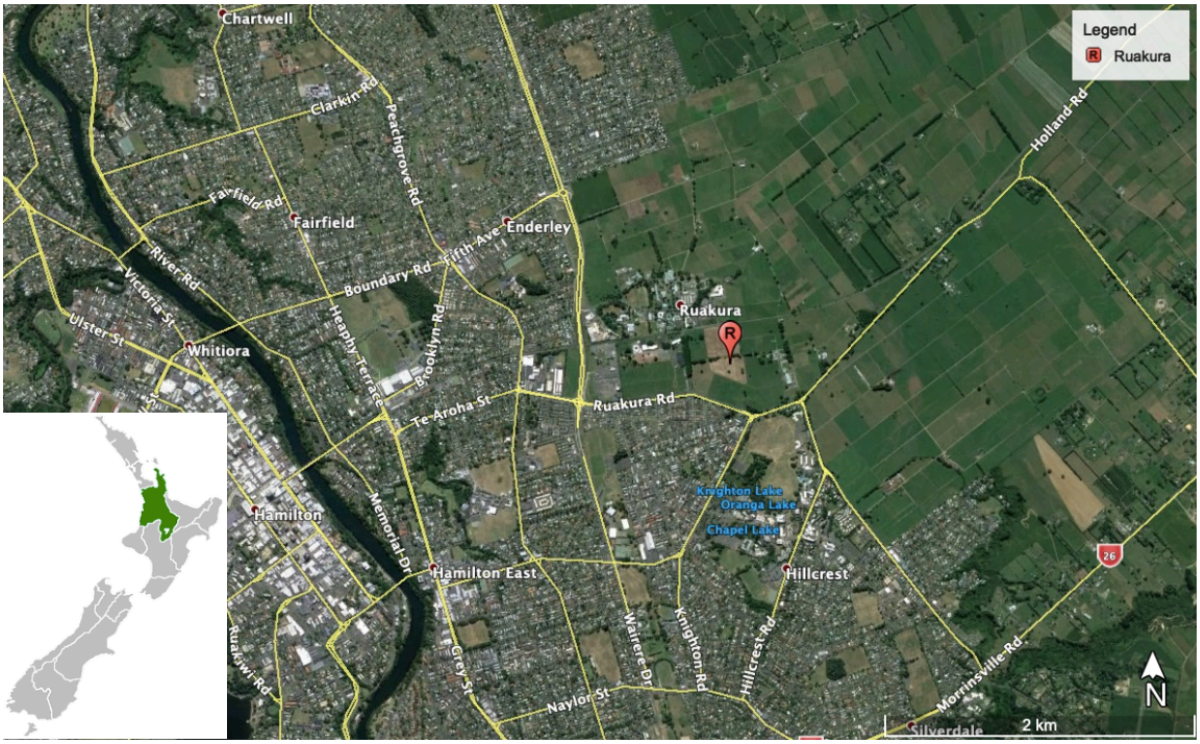

Figure 3 – Ruakura field trial site at AgResearch, Ruakura, Hamilton. © Google 2019. Insert: Waikato region

Table 1 - Site details and physicochemical properties (0–100 mm depth) of the soils studied

	Site 2 Well-drained soil	Site 1 Poorly drained soil
Location	AgResearch Ruakura	Massey University Dairy 4
Soil name	Horotiu	Tokomaru
Soil texture	Sandy loam	Silt loam
Soil $pH_{(H2O)}$	6.5	5.88
Sand (%)	60	8
Silt (%)	30	68
Clay (%)	10	24
Bulk density (g cm^{-3})	0.9	1.24
Total Porosity (%)	50	51
Total C (%)	3.63	3.75
Total N (%)	0.38	0.35
CEC (cmol(+) kg^{-1})	17.5	14.2

3.3.2. Field procedure

Both sites did not have animals grazing on them, nor N fertiliser application, for at least three months prior to the experiments. This provides a "blank slate", free from existing urine patches that could confuse measurements from deliberately placed urine patches. Within a week of the study commencing, pasture was cut to approximately 5cm, to simulate a post-grazing residual.

Two different soil moistures were created on each site, by irrigating the "wet" half of the site and/or covering the "dry" half when rain was expected, as weather conditions required. The aim was to have the "wet" half at field capacity, soil depth 0-7.5 cm (i.e. WFPS = 65-75% depending on soil type), and the other half drier than field capacity (i.e. WFPS = 45-60% depending on soil type.

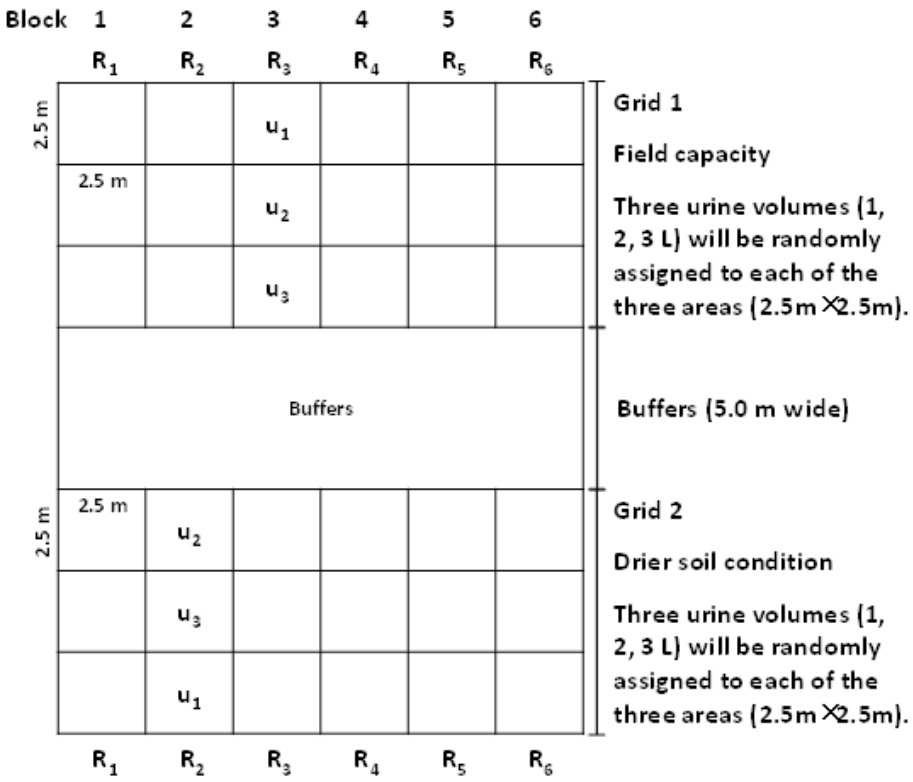

Figure 5 – Layout of the trial sites

The synthetic urine applied was prepared by dissolving: 13.65 g urea, 3.4 g glycine, 16.31 g potassium bicarbonate, 1.61 g potassium sulfate and 5.68 g potassium chloride L^{-1} in deionised water (Ledgard et al., 2014). While real urine is recommended for quantitative nitrous oxide emission trials and the like (de Klein et al., 2003), the components of the artificial urine should be sufficient for this kind of experiment. Urine was applied at three different volumes: 1, 2 and 3 L. These were chosen as the average urination volume is around 2.1 L in dairy cattle, with 1 and 3 L being within the measured ranges (Selbie et al., 2015). The urine was warmed to about 40°C to enable the use of a thermographic camera; this is similar to the body temperature of a cow, 38.5°C (Regan & Richardson, 1938; Vasconcelos et al., 2006).

Urine was poured from a height of 1.2 m, the average height of a cow, in one continuous stream to mimic real urination.

30 seconds following urine application, a thermographic image was taken of the urine patch. This was to provide an alternative measurement of urine patch area to compare with the mineral N data if needed.

One urine patch for each treatment was randomly selected for mineral N analysis. There was no replication due to time and resource constraints. 20 cm deep soil cores were taken across

each urine patch in a grid, sampling every 10 cm. Based on predicted urine patch distribution area (Williams and Haynes, 1994), 3 L urine patch was sampled in a 80 x 80 cm grid, the 2 L in 70 x 70 cm, and the 1L 60 x 60 cm. Each soil core was sectioned by depth: 0-5 cm, 5-10 cm and 10-20 cm. Soils were kept refrigerated at 4°C while waiting for extraction

3.3.3. Laboratory procedure

Soils were extracted by sieving field moist through a 4mm sieve. 3-4 g of soil was weighed into tubes and shaken end-over-end with 20 mL 2 M KCl for 1 hour. The tubes were then centrifuged at 2000 rpm for 10 min, and 10 mL of supernatant pipetted into 10 mL vials. These vials were frozen until analysis.

Extracts were analysed for nitrate/nitrite and ammonium using an autoanalyzer with the methods described in Blackmore, Searle, and Daly (1987).

3.3.4. Determining background N concentration

The boundary of the urine patch was determined based on the inflection point of concentrations measured when ordered from lowest to highest for each patch and depth, with some discretion used with non-contiguous values. Values below the inflection point were deemed background-N, and above, urine-N. The average of the concentrations deemed background were subtracted from the values deemed urine.

Alternatively, an area with no urine applied could have been sampled as a control, but this was not done to reduce the number of cores needed.

3.4. Results and discussion

3.4.1. N concentration and recovery

3.4.1.1. Determining background N concentrations

Determining background N concentrations require differentiation between urine-N, and background soil N. Figure 6 shows the NH_4^+-N concentration distribution for the three patches measured on the dry Horitiu soil. There are reasonably low background NH_4^+ levels, and then obvious inflection points around 38 g NH_4^+-N/kg soil where NH_4^+ concentrations increase, indicating presence of urine-NH_4^+. This approach provides fairly accurate determination of the threshold for urine patch N.

In contrast, the inflection point was less clear for the three patches measured on the drier Tokomaru soil (Figure 4), making it slightly difficult to differentiate between background and

urine-N. In this study background N concentrations averaged 4 g NH_4^+-N/kg soil on the Horotiu soil, and 7 g NH_4^+/NO_3^--N on the Tokomaru soil.

Figure 8 then shows how this information was used to remove the background N from a 3D cross-section of a urine patch.

These results indicate that it is possible to distinguish between urine-N and background soil-N without using ^{15}N provided the soil samples are collected the same day urine was poured. Figure 8 shows an example of a urine patch where this was done.

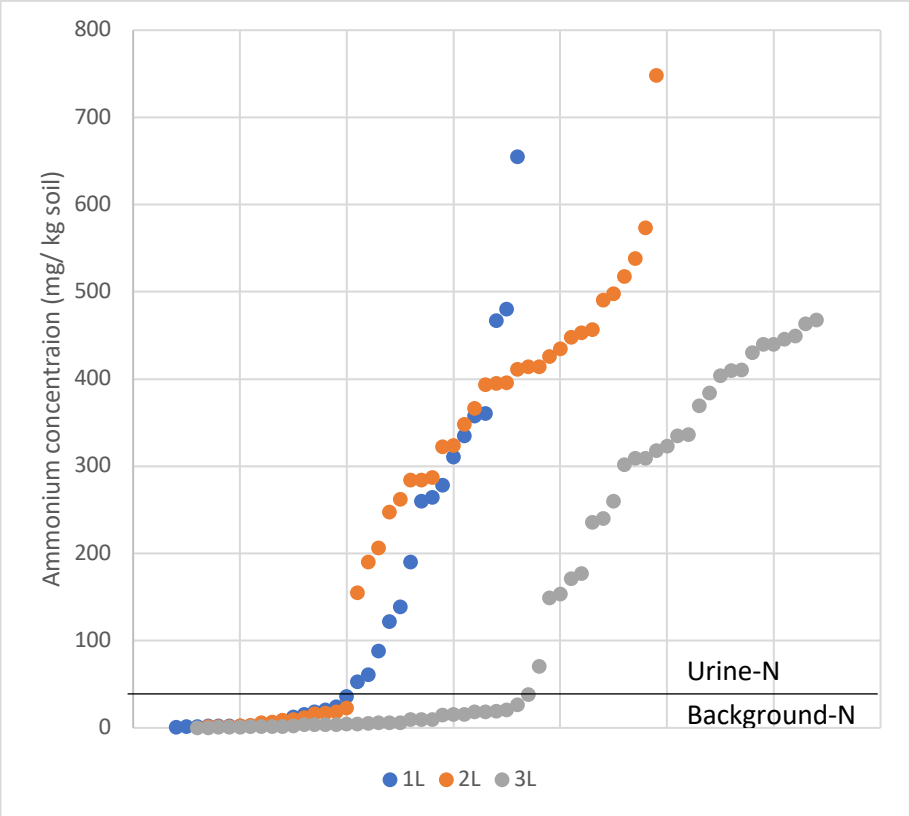

Figure 6 – measured concentrations of NH_4^+-N in the top 5 cm of the drier Horitiu soil, ordered by concentration

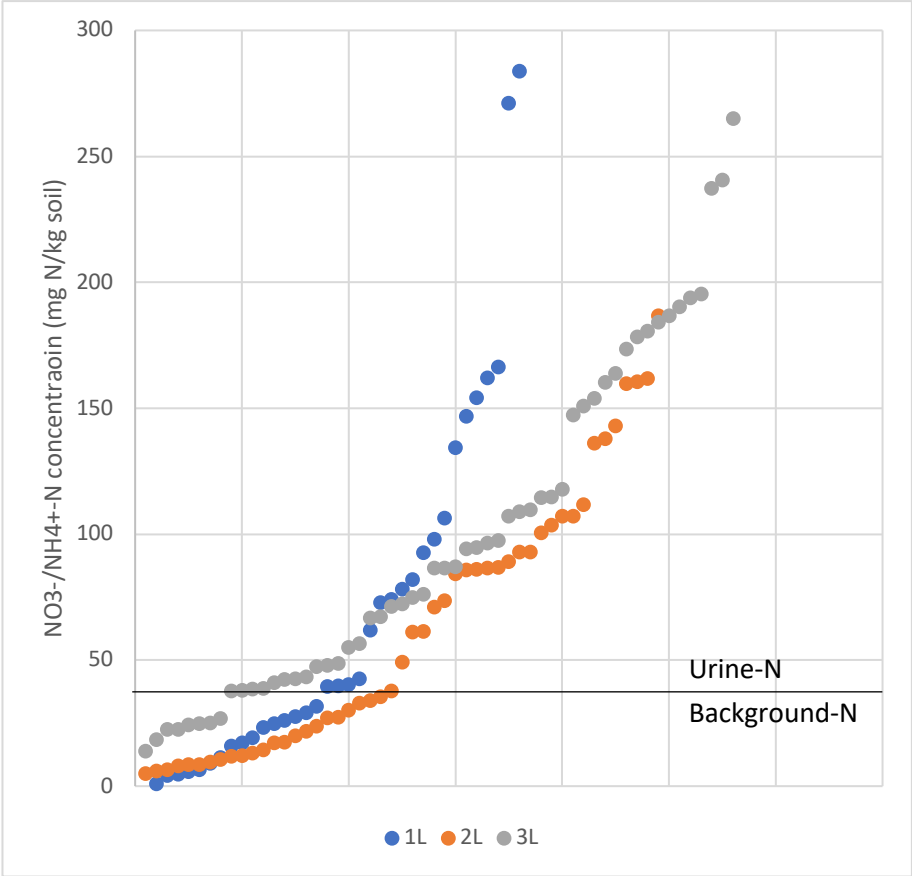

Figure 7 – measured concentration of mineral N in the top 5 cm of the drier Tokomaru soil, ordered by concentration

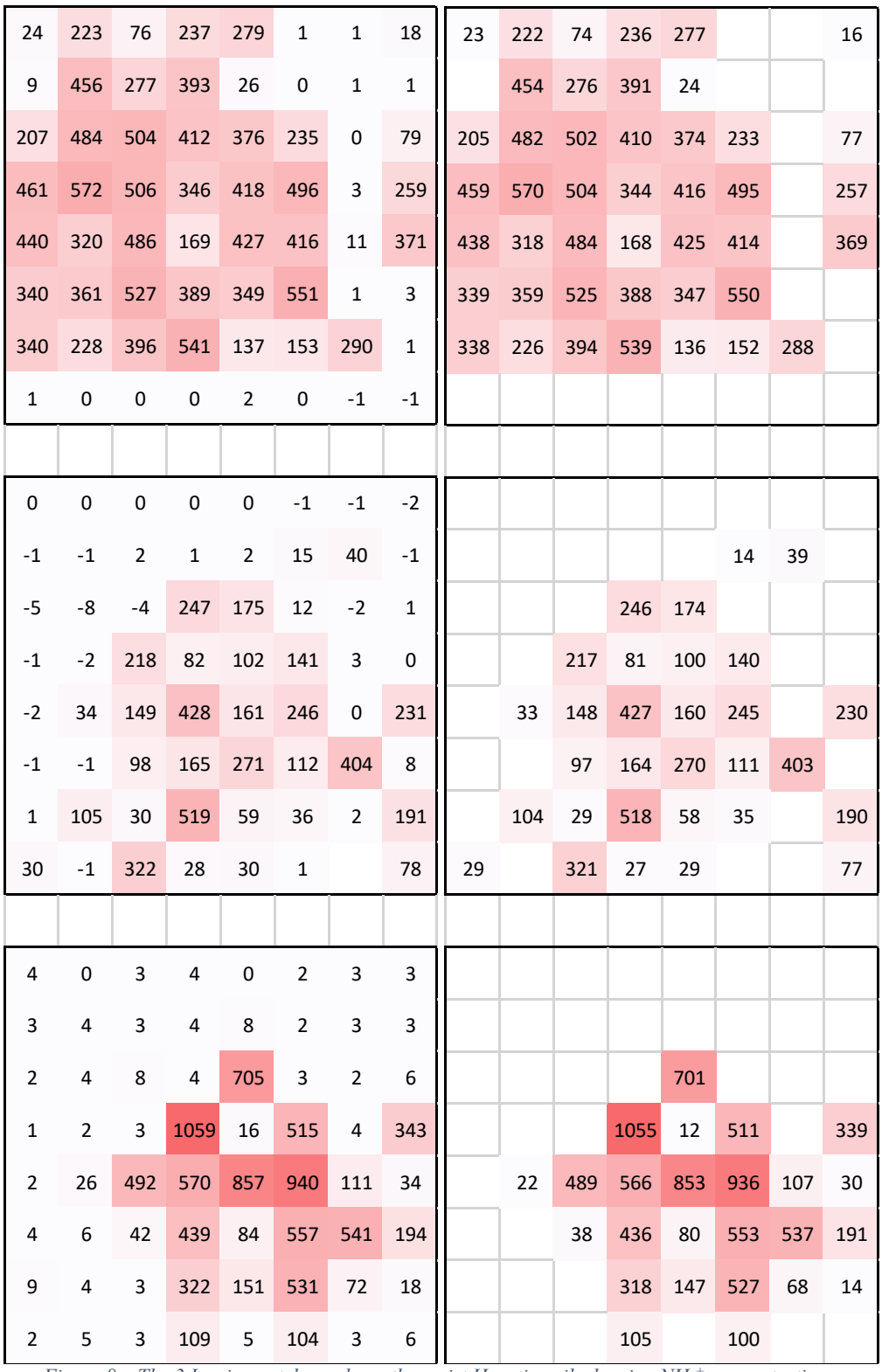

Figure 8 – The 3 L urine patch, made on the moist Horotiu soil, showing NH_4^+ concentrations

3.4.1.2. Urine-N concentrations measured across urine patches

N concentrations measured in the top 5 cm ranged considerably within each urine patch, often from 50 to 500 g urine-N/kg soil from the Horotiu samples and 40 to 150 g/kg from the Tokomaru samples (see Figure 9 & Figure 10). Concentrations in the top 5 cm did not show any discernible trend with increasing urine volume. There appeared to be somewhat higher N

concentrations measured below 5 cm with increasing urine volume, with median concentrations measured in the Tokomaru soil at 5-10 cm increasing from 25 g N/kg soil to 40g/kg, and increasing from around 70 g/kg on the moist Horotiu to 125 g/kg. However, this trend did not appear on the dry Horotiu patches and was less consistent with measurements taken at 10-20 cm. Without replication it is not possible to make any statements with statistical confidence.

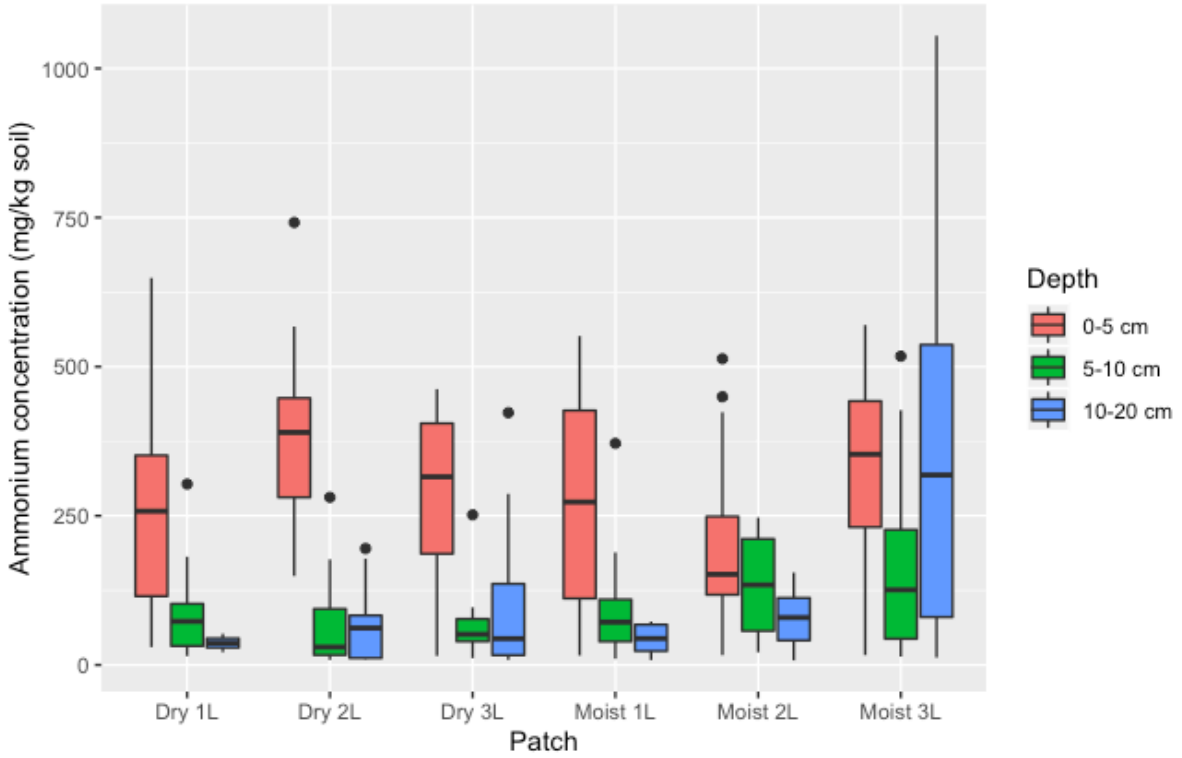

Figure 9 – NH_4^+ concentrations measured across the urine patches created on Horitiu sandy loam

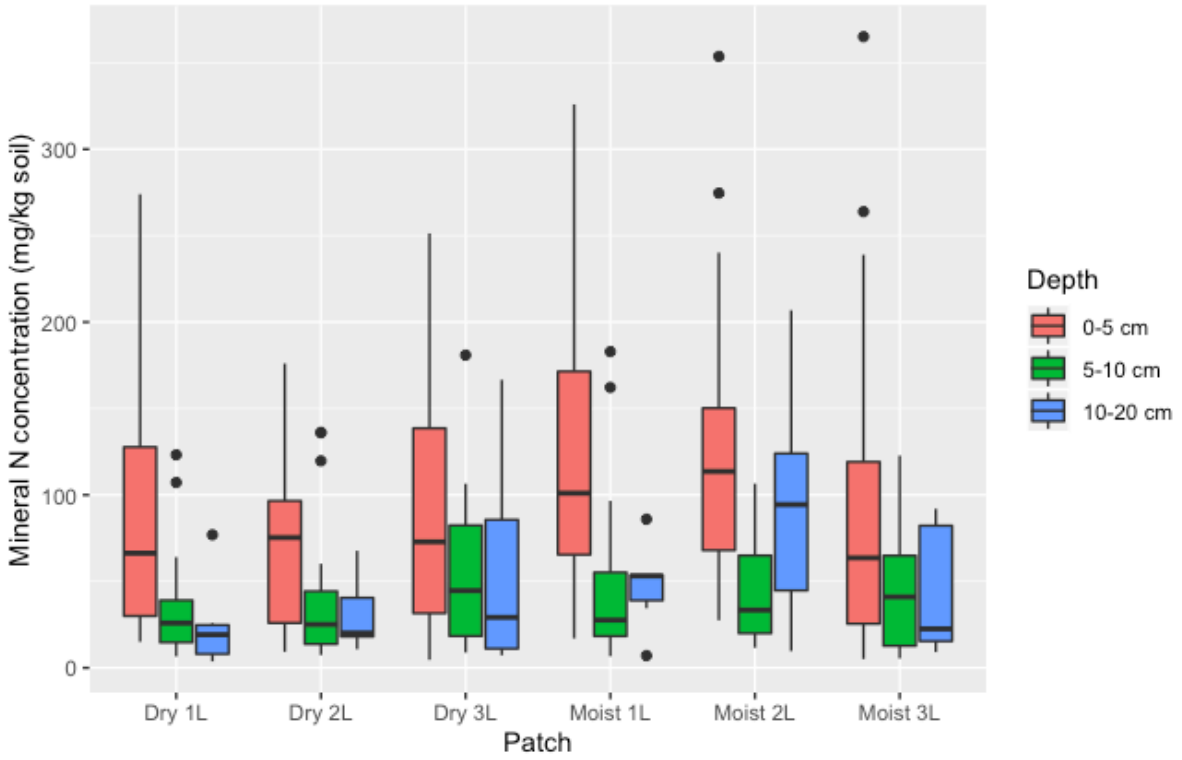

Figure 10 – Mineral N concentrations measured across the urine patches created on Tokomaru silt loam

3.4.1.3. Urine-N recovery

Recovery was around or below 50% for most urine patches. The exception to this was the moist, 3 L, Horotiu patch, which had unusually high NH4+ levels in the 10-20 cm patch (see Figure 11).

This generally low recovery can in part be explained by incomplete sampling of the whole urine patch. Because the urine patches were generally larger than expected, and the choice of where to sample was not as accurate as it could have been, no single sampling grid managed to capture an entire urine patch, although some were closer than others.

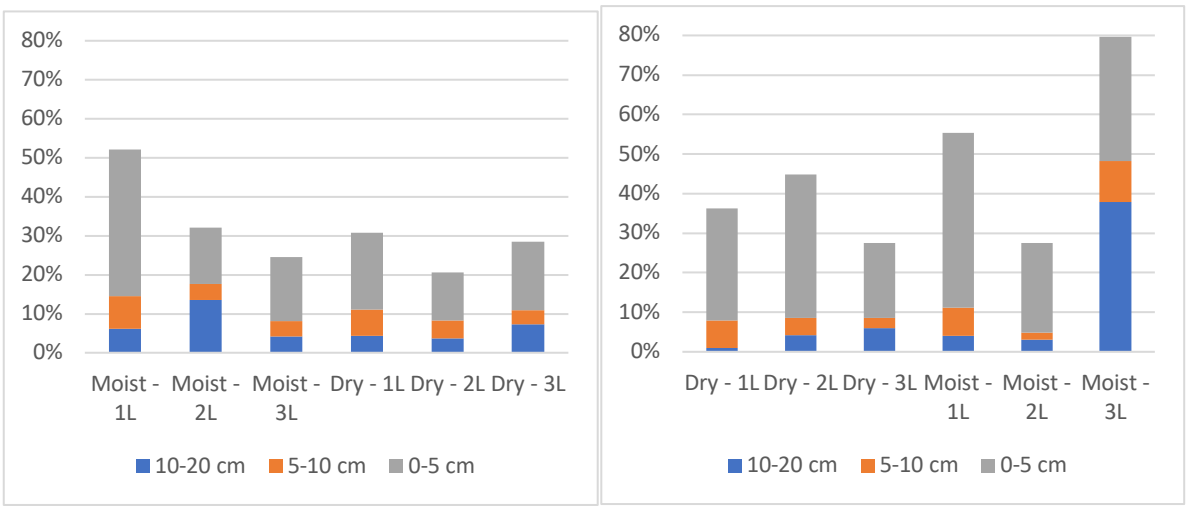

Figure 11 – Urine recovery and depth distribution for each urine patch measured. Left: Tokomaru. Right: Horotiu.

3.4.1.4. Urine N depth distribution

Urine-N was measured down to below 10cm in all urine patches. However, with two exceptions, the majority of it was recovered from the top 5 cm, averaging 67% but often up to 80%. 14% on average was recovered from 5-10 cm, and 19% from 10-20 cm (Figure 12). Again, this was variable between patches. Generally, more N was recovered from 10-20 cm with increase urine volume. The distributions measured were broadly similar to Giltrap, Jolly, et al. (2020); Monaghan et al. (1999); Williams and Haynes (1994); the "urine" (Br-) in Williams and Haynes (1994) seemed to penetrate slightly further down the soil profile than these urine patches, with 55-66% recovery in the top 5 cm, but this is potentially just due to soil type, certainly the sizeable variation in urine patch distribution shown across the range on soils in Monaghan et al. (1999) suggest that soil physical properties can have strong influence on urine patch behaviour.

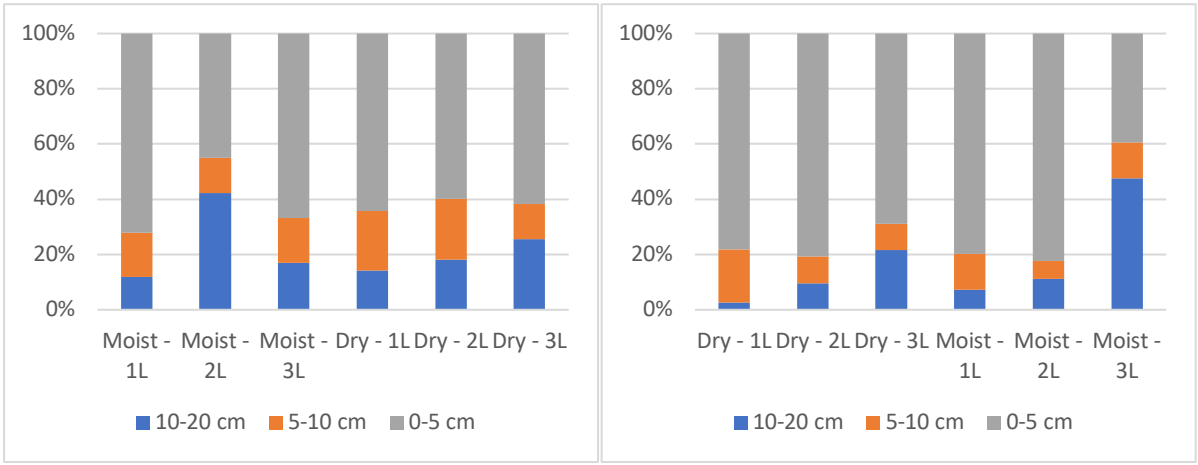

Figure 12 - depth distribution of recovered urine-N for each urine patch

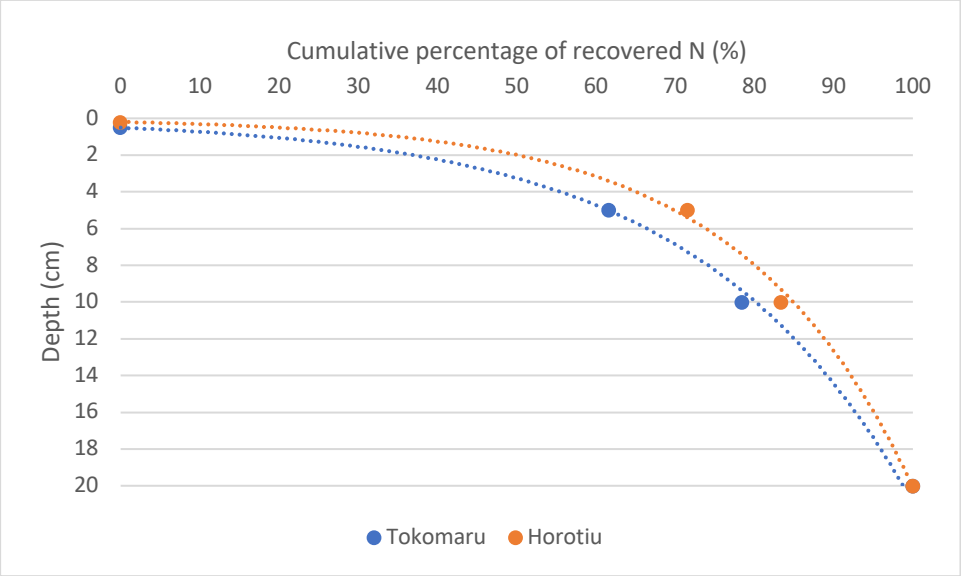

Figure 13 – Average cumulative distribution of recovered urine N, for the Tokomaru and Horotiu urine patches

3.4.1.5. N transformation

The delay in taking soil samples from the Massey site highlights that significant nitrification can occur after four days, with the proportion of NO_3^--N (including a small amount of background N) increased from 17% to 42%. The nitrification in this soil is more rapid than that reported by Williams and Haynes (1994), who only recorded similar levels of NO_3^- after 14 days.

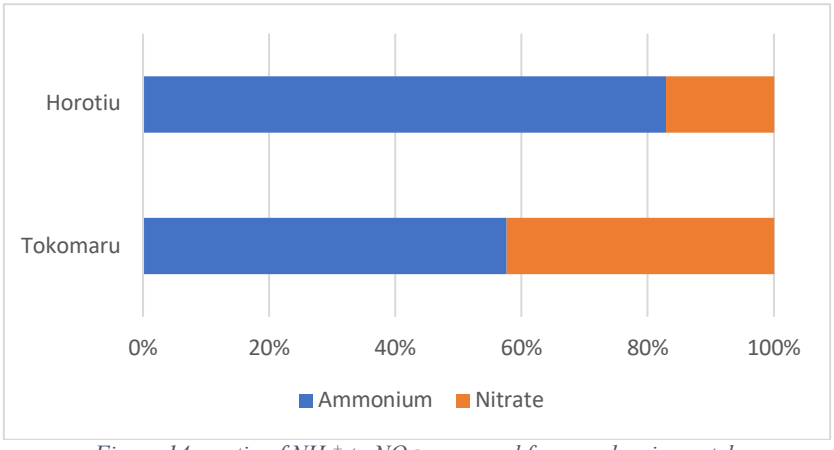

Figure 14 – ratio of NH_4^+ to NO_3^- measured from each urine patch

3.4.2. Urine patch area

Urine patch areas measured ranged from 0.17 m^2 to 0.64 m^2 (Table 2). These were calculated by assuming a single soil core represented the 100 cm^2 around it and adding the number of cores where urine-N was measured together. Generally, higher volumes of urine resulted in

larger urea patch areas, but not always. Urine patches on the dry soils were generally smaller than on the moist soils. There was considerable variation between urine patch areas and shapes.

Table 2 – Measured urine patch areas (m^2)

	Moist – 1L	Moist – 2L	Moist – 3L	Dry – 1L	Dry – 2L	Dry – 3L
Tokomaru	0.3	0.24	0.64	0.24	0.35	0.63
Horotiu	0.25	0.37	0.44	0.17	0.29	0.3

These generally agree with the urine patch area measured by Spikey-R and the thermographic camera. Areas on the Tokomaru soil ranged from 0.3 to 0.35 m^2 for 1 L, 0.4 to 0.5 m^2 for 2 L and 0.55 to 0.8 m^2 for 3 L. On the Horotiu soil they were smaller, generally ranging from 0.2 to 0.3 m^2 for 1 L, 0.25 to 0.4 m^2 for 2 L and 0.4 to 0.5 m^2 for 3 L (Jolly et al., 2019). These are also broadly within the ranges reported in Selbie et al. (2015); Williams and Haynes (1994) for comparable urine volumes. Some variation between studies is to be expected, as urine patch areas can vary considerably between soils, depending on the physical properties of the soil (Jolly et al., 2020, in preparation)

Some patches, however, are below the ranges measured with Spikey-R and the thermographic camera (Jolly et al., 2019). This was due to an unforeseen problem in that often not all of the urine patch was captured by the sampling grid.

To ensure that the entire urine would be captured in if this were to be repeated in the future, the most obvious solution would be expanding the area sampled by at least 20 cm each side, but this significantly increases the analytical workload, for this experiment from 1800 samples to 2900 approx. It may be possible to limit the necessary increase to only an extra 10 cm for length and width by utilising a thermographic camera to help guide where to start sampling, and how large the sampling area needs to be. This was utilised on the Ruakura site after encountering this problem at Massey but wasn't fool proof. More detailed reference point marking of the site may help with positioning; although this was not possible on our trials as the other measuring tools, particularly Spikey-R, needed to do their measurements "blind" to avoid any bias in the processing of their data. Practice beforehand with the thermographic camera may also be helpful.

3.4.3. Replication

Considering the number of samples to be collected and analysed it practically not possible to have replications. This does limit the statistical assessment given the significant variation is urine patch shape and surface area measured using the thermographic camera, Spiky-R and the RPAS imagery (Jolly et al., 2019, 2020). Reducing the number of treatments, such as only two

urine volumes or only one soil moisture and conducting separate experiments at different soil moistures is an alternative in future research. A lower resolution grid is another option but by compromising the quality of data for the accuracy of surface area.

3.4.4. Improvements to the method and next steps

As discussed above, the sampling grid needs to get bigger, and the positioning of the grid over the urine patch needs to become more accurate to ensure that the entire urine patch is captured. However, this will significantly increase the work required for an experiment that still wouldn't be capable of measuring any treatment effect. Adding replicates to the experiment would require a level of resourcing that may be too great.

Instead, is it possible to utilise remote sensing technologies in conjunction with physical soil sampling to allow for more cost-effective replication, without sacrificing data quality? A sensing tools used to measure urine patch areas such as. a drone mounted camera, that measures pasture response (described in Jolly et al., 2019) is probably not suitable as it measures the effective urine patch, rather than the actual wetted area, and is not capable of measuring a patch until after several days. But both Spikey-R (which detects urine by measuring the capacitance of soil) and a thermographic camera (which measures the temperature difference between freshly applied urine and the surrounding soil) (also described in Jolly et al., 2019) have been demonstrated as effective tools to measure a urine patch area in a range of conditions (Forrestal et al., 2020; Jolly et al., 2019, 2020; Mehra et al., 2020). The thermographic camera is a much cheaper and easier tool to use than Spikey-R, but it did struggle on the Ruakura site in Jolly et al. (2019) as the sun warmed the ground, and on an Irish soil in Forrestal et al. (2020), possibly due to the soil and/or weather conditions. The most appropriate tool may be site specific.

The surface area data could then be used to determine where to take cores from to determine the depth distribution of the urine. The samples within each patch could be aggregated together by depth to reduce the analytical workload. You would lose some detail of the spatial variation in within a patch, but this experiment has already demonstrated that the information gap is more in treatment effect of urine volume and soil moisture. The grid resolution (number of cores per m^2) could also be reduced, as measuring the edge of the urine patch is no longer as important if it can be done with other tools. It might increase the risk of missing some atypically high urine-N concentrations, especially at lover depths but this may be an acceptable trade-off.

3.5. Conclusions

- The variation between individual urine patches in shape, area and depth distribution was substantial. Area ranged from 0.25-0.64 m^2, and recovery from the top 5 cm ranged from 31% to 80%.
- The variation in measured N concentration in the top 5 cm within individual urine patches was also substantial (interquartile range typically 100 mg N/kg soil).
- Total urine N recovery was low, averaging 38%. This can be explained in part by incomplete capture of the whole urine patch area in the sampling grids.
- On average, 67% of the recovered N was recovered from the top 5cm, 14% from 5-10 cm and 19% from 10-20 cm
- The method used is adequate to measure and map urine patch area and depth distribution, provided the improvements to the method discussed above are used. However, it is a very time and resource intensive process, and the suggested improvements would make it even more so. Instead, it may be possible to make substantial gains in the number of urine patches measured, with minor sacrifices in data quality by utilising a hybrid measurement-remote sensing method.

4. DCD Field Experiment

4.1. Introduction

DCD has been shown to effectively inhibit nitrification, reducing nitrous oxide and nitrate leaching losses (Clough et al., 2007; Clough et al., 2008). However, to limit application costs, DCD is often applied at very small volumes. This may limit its ability to adequately intercept the majority of dairy cow urine patch nitrogen.

DCD was withdrawn from the market in 2013 due to milk contamination and is no longer available for commercial use. However, DCD has potential to return to the market once milk safety standard is developed for the Codex Alimentarius. In addition, the development of new application technologies (e.g. Spikey®), that target only urine patches with DCD, reduce the amount of DCD applied to pastures, which would reduce the potential for cow ingestion and the level of milk contamination.

A field study was undertaken to measure the spatial distribution of dicyandiamide (DCD) applied to urine patches using the Spikey® sprayer. DCD was applied to a well-drained soil and a poorly-drained soil, at two application rates and two soil moistures. Soil was then sampled and extracted and measured in the lab.

4.2. Methodology

4.2.1. Laboratory extraction experiment

A small experiment was also undertaken before the trial to determine the proportion of DCD that could be extracted using the method described above.

Field moist samples of Tokomaru silt loam soil taken from 0-10cm deep were sieved and mixed thoroughly. Three samples of soil were also oven dried at 104°C for soil moisture measurements (Blackmore et al., 1987). A 1mL aliquot of DCD at the following rates; 0, 31.25, 62.5, 125 and 250 mg/L, were added to separate 5 g samples of soil (the equivalent of 0-50 mg DCD/g soil). Three replicates of each treatment were made, making 15 individual samples. An additional 15 more soil samples were made without DCD added. The samples were allowed to sit for four hours then shaken and filtered using the same method detailed in 4.2.4. A 1 mL aliquot of the same five concentrations of DCD were then added to the supernatants, reach rate replicated three times, providing a total of 15 samples, which previously had not received DCD.

All samples were then acidified, centrifuged and analysed using the same method detailed in 4.2.4.

4.2.2. Site descriptions

Site 1

Site 1 is located on Massey University's near Collinson Road, 500 m south of Massey University's Manawatū campus, on the southern outskirts of Palmerston North (Figure 15). The site is on Tokomaru silt loam soil, an Argillic-fragic Perch-gley Pallic soil, which is poorly drained. Dairy 4 is a commercial seasonal supply dairy farm.

Site 3

Site 3 is located next to the University's Dairy 1 near Poultry Farm Road, 750 m northeast of Massey University's Manawatū campus (Figure 15). The site is on Manawatū silt loam soil, a Weathered Fluvial Recent Soil, which is well drained.

Figure 15 – Field trial sites 1 and 3 near Massey University – Manawatū. © 2019 Google. Insert: Manawatū-Whanganui region of New Zealand.

Table 3 - site details and physicochemical properties (0–100 mm depth) of the pasture soils studied

	Site 3 Well-drained soil	Site 1 Poorly drained soil
Location	Massey University Dairy 1	Massey University Dairy 4
Soil name	Manawatū	Tokomaru
GPS coordinates	40°39'65" S, 175°66'17" E	40°22'S, 175°39'E
Sward species	*Lolium perenne* L. *Trifolium repens* L.	*Lolium perenne* L. *Trifolium repens* L.
Soil texture	Silt loam	Silt loam
Soil $pH_{(H2O)}$	6.05	5.88
Sand (%)	35	8
Silt (%)	45	68
Clay (%)	20	24
Bulk density (g cm^{-3})	1.22	1.24
Total Porosity (%)	58	51
OM (%)	4.38	6.49
Total C (%)	2.53	3.75
Total N (%)	0.28	0.35
CEC (cmol(+) kg^{-1})	ND	14.2

4.2.3. Field work

Leading up to the experiment, two different soil moistures were created on each site by covering up half of the experimental areas to prevent rain from entering the soil, thus, building up a bigger soil moisture deficit. Alternative approaches could have been wetting up the soil half of each site or doing half of the experiment at a later date when the soil moisture content was different naturally. However, because of the time of year, time constraints and the rainfall patterns of Palmerston North, the chosen method was considered the most practical. The covers were made by tying plastic sheets over large pasture cages, leaving the sides facing away from

the prevailing wind direction open to allow airflow (see Figure 16). In practice, the covers did not appear to work well at Site 2, for some reason, and only a very small difference is soil moisture was measured.

Figure 16 - The rain cover used at site 3. A similar structure was used at Site 1.

Both sites have not had animals grazing on them for at least several months prior to the experiment. Each site was mown down to 5cm above the soil surface on the day of the trial, to simulate a post-grazing residual. Two litres of artificial urine were poured onto each plot from a height of 1.2 m in one continuous stream to mimic a real urination. The urine itself was based on the same recipe as the previous experiment: 13.65 g L^{-1} urea, 3.4 g L^{-1} glycine, 16.31 g L^{-1} potassium bicarbonate, 1.61 g L^{-1} potassium sulfate and 5.68 g L^{-1} potassium chloride. This provides a total N concentration of 5.92 g N/L, which is within the range of concentrations for real cow urine. While real urine is recommended for quantitative nitrous oxide emission trials (de Klein et al., 2003), the components of the artificial urine are adequate for of the parameters measured in this experiment. The urine was also heated to 40°C to allow the use of the thermographic camera. A thermographic image was taken of each patch immediately after it had been poured on to the soil. An 80 cm diameter chamber was used to provide a reference scale for the thermographic camera, and the patches were also marked out with markers.

Approximately 24 hours after urine application, DCD was sprayed onto the urine patches at a target rate of 10 kg DCD/ha/application, using a Spikey® sprayer mounted on a tripod with a skirt to block the wind (Figure 17). The rate of 10 kg DCD ha^{-1} was the most commonly recommended and used amount (Clough et al., 2008). Half of the patches received one application of 10 kg DCD/ha and the other half received two applications of 10 kg DCD/ha (20 kg DCD/ha in total). The intention was to see if twice as much DCD and solution volume would move DCD further down the soil profile. Diluting the DCD to apply twice as much volume, but the same mass of DCD was considered, but it was decided that two applications at the same concentration would show the same effect proportionally but reduce the chance of DCD being diluted below the detection limit.

Figure 17 – Spikey® spray unit

Soil samples were collected from Site 1 between 4-7 hours after DCD application and from Site 3 between 16-18 hours after DCD application. A total of 17 cores of soil were taken from each urine patch were taken in two transects, 10 cm apart, sectioned into 0-2 cm, 2-5 cm and 5-10 cm depths (Figure 18). Three cores per soil moisture were also taken from outside the treated areas for control (background) samples; no DCD is expected to be detected here. At this time, herbage was also sampled from each patch, cut as close to ground level as possible, to measure an DCD that was still present on the pasture foliage.

Table 4 - Layout of site at Dairy 4. Plot number (number of squirts). The same setup was used on Dairy 1.

1 (1)	2 (1)	3 (2)		7 (2)	8 (1)	9 (2)
6 (2)	5 (1)	4 (2)		12 (1)	11 (2)	10 (1)
‘Drier’				‘Wetter’		

A second round of sampling was conducted five after the first sampling, following significant rainfall (24 mm was recorded at Site 3). This sampling consisted only of four cores per urine/DCD patch, one per quarter of the patch, chosen randomly from each quarter. The cores were sectioned into the same depths as the previous round of sampling and then the four samples were aggregated by depth. This was not intended to provide the same level of spatial detail as the first round of sampling, time constraints prevented this, but it was intended to give some indication of the extent rainfall (or irrigation) moves DCD further down the soil profile.

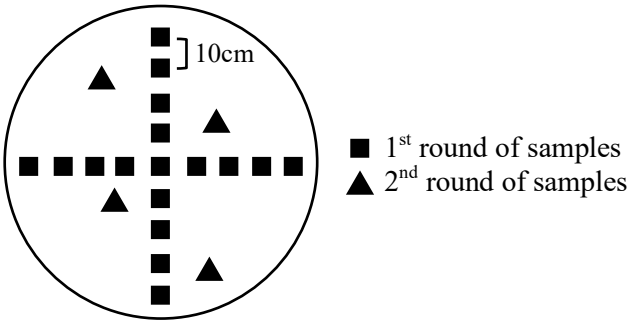

Figure 18 - Diagram of DCD patch soil sampling, with transects for the 1st round of sampling and more random coring for the second

4.2.4. Lab work

DCD was extracted with deionised water, using 10 g field moist soil at a 1:2 soil to solution ratio, and with a 1 h shaking time. The solution was centrifuged for 5 min and filtered with 41 Wattman filter paper. Each herbage sample was shaken with 1 L of water. A 10 ml aliquot of each extract was acidified with 0.25 ml 2 M H_2SO_4, then spun on the megafuge at 5000 rpm for 2 hours. 1.5 ml was pipetted into vials and the DCD concentration in the solution was analysed using an HPX-87H cation-H guard column with a 0.025 M H_2SO_4 mobile phase, a flow rate of 0.5 ml min^{-1} and a 210 nm UV detector on a Waters 2695 High Performance Liquid Chromatograph (HPLC). (Di & Cameron, 2004a; Schwarzer & Haselwandter, 1996). Herbage samples were analysed using a longer, 30 cm Amenex HPX-87H column due to the much higher concentrations of the samples. A 10 g of soil was also oven dried at 104°C for soil moisture correction. There was not enough soil in the 0-2 cm samples to allow for both DCD extraction and soil moisture measurement, so any remaining soil from these samples was combined with the corresponding 2-5 cm sample for a combined soil moisture measurement. The 0-2 cm soil made up no more than two fifths of the composite soil moisture sample, to ensure the proportions were correct. This was not an issue in the second round of sampling as cores were aggregated by depth giving more soil is each sample, so separate soil moistures were measured.

4.2.5. Statistical methods

The individual core measurements were averaged for each patch by depth for analysis, except where it is explicitly stated otherwise. The soil moisture impact was analysed with a linear model. The remaining treatments were analysed with ANOVA and Tukey's honest significant difference test.

4.2.6. Estimating DCD effectiveness

DCD effectiveness can be estimated using Equation 1, where U_{max}/U is percent inhibition and K (mmol g^{-1}) is a soil specific inhibitor constant On Manawatū silt loam, K has previously been measured as 0.0990 (Bishop, 2010).

Equation 1 – Estimating DCD effectiveness (Bishop, 2010)

$$\frac{Umax}{U} = 1 + \frac{[DCD]}{K}$$

The half-life of DCD can be estimated using

Equation 2, where T is the soil temperature (Kelliher et al., 2008). Temperature data for Palmerston North was taken from Chappell (2013).

Equation 2 – DCD half-life (Kelliher et al., 2008)

$$t_{\frac{1}{2}} = 168e^{-0.084T}$$

4.2.7. Spray unit efficiency

Following the field trial, a small experiment was carried out to check how much of the volume that the Spikey® spray unit was delivering actually reaches the ground. This was done by spraying 30mL of water onto a crumpled plastic sheet and weighing the sheet for the mass of water captured. Five repeats were conducted for both the for 30 mL and 60 mL application rates.

4.3. Results and discussion

4.3.1. Laboratory extraction experiment

The average amount of DCD extracted from the soil averaged 85% of the amount added and was largely consistent across the DCD concentrations used (Table 5). Extraction of the DCD added to the supernatant averaged 100%, suggesting that 85% extraction efficiency is entirely due to DCD sorption onto the soil. This is similar (albeit at the lower end) to the work conducted by B. Welten, Kear, Dexter, and Judge (2012) across a range of soils from the Waikato region. While Organic soils and high organic matter Allophanic and Pumice soils had

low extraction efficiencies (60-80%), other mineral soils, including a Pallic and two Recent soils ranged from 85-95%. As the organic matter contents are similar, this suggests that extraction efficiency of the Manawatū silt loam, the soil at Site 3, will be within a similar range as that of the Tokomaru silt loam, although it was not assessed in the current study.

Table 5 - DCD recoveries of known DCD added in the lab ± standard deviation

DCD Concentration Added (mg/L)	DCD Added to Soil	DCD Added to Supernatant
0	no DCD detected	no DCD detected
32.25	86% ± 1.0	99% ± 1.1
62.5	86% ± 0.8	100% ± 0.2
125	86% ± 0.6	99% ± 0.4
250	83% ± 0.4	100% ± 0.3
Average	**85%**	**100%**

4.3.2. Spray unit efficiency

On average, 78% of the 30mL application was recovered on the plastic sheet. Recovery was lower for the 60 mL application, which was only 69%. This would mean that the unit was effectively delivering 7.8 and 13.8 kg DCD/ha were reaching the ground, when applications of 10 and 20 kg DCD/ha were made, respectively. These recoveries were somewhat variable, with a standard deviation around 4-5%. Some liquid was seen adhered to the base of the spray skirt and a fine mist also came out of the top of the spray skirt. These two losses would contribute to not all of the applied DCD reaching the ground.

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4.3.3. Urine Patches

The thermal images provided a good idea of the size and shape of the urine patches applied. They generally covered around half of the 0.5 m^2 ring, although the size and shape of individual urine patches varied considerably. The thermal camera performed well at Site 1, but distinguishing the edge of the urine patch was much harder at Site 3. At Site 3 urine was applied later in the day, when the surface soil was warmer and the temperature gradient between the urine and soil was not as contrasting.

4.3.4. First soil sampling from field sites

Soil moisture measurements, taken just prior to urine application, showed that the two level of soil moisture were 29.9 and 34.2% at Site 1 and 27.4 and 28.8%, at Site 3, for the ‘drier’ and ‘wetter’ soil treatments, respectively. The covering the ‘drier’ plots resulted in only a small soil moisture content difference at Site 3. It is unknown why the difference wasn’t greater as was the case at Site 1.

Average recovery of DCD was 82 and 64% at Site 1 and 84% and 48% at Site 3, for the 30 and 60 ml application rates, respectively. Much of these discrepancies in recovery can be explained by the inefficiencies in the spray unit, especially for the 60 ml rate. All subsequent recoveries shown are corrected for how much DCD is estimated to have reached the pasture and soil, based on the results shown in section 4.3.2.

Soil moisture

Soil moisture did not appear to have any effect on DCD concentration or distribution in the soil ($p>0.1$). Figure 19 shows how recovery varies at each depth at different soil moistures.

There is potential for more moist soils to force the DCD solution deeper, this does occur with urine patches, soil moisture can impact the shape and spread of a urine patch (Giltrap, Jolly, et al., 2020). However, the volume of liquid applied may be too small for this to matter, or the range of soil moistures created not great enough. Urine influences DCD adsorption onto soil (Marsden et al., 2016), so the size and shape of a urine patch could also matter. However, this effect appears not to have been significant. For subsequent analyses the different soil moisture treatments are combined together.

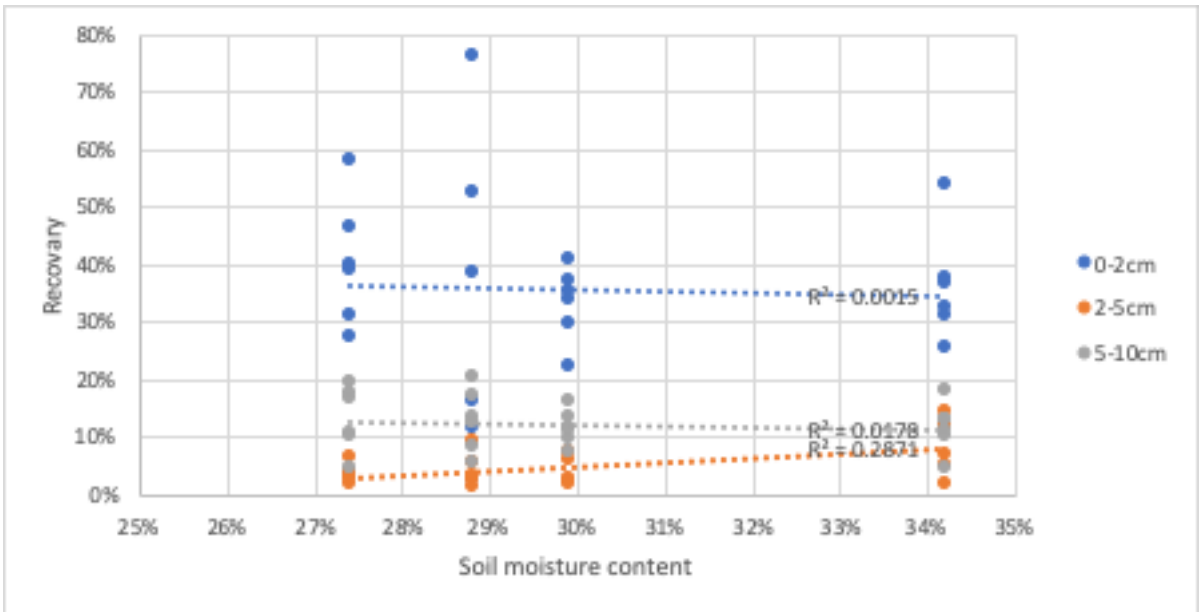

Figure 19 – DCD recovery vs soil moisture content, at each depth

The recovery of DCD in soil and pasture samples was close to 100% at Site 1 and for the 30 ml rate of DCD at Site 3 (Table 6). However, the recovery of DCD at for the 60 ml rate at Site

3 averaged 70%. This lower value was due to both lower recoveries for the herbage and soil. Across the two sites, pasture recovery averaged 41%, which highlights that potential for a high proportion of the DCD that reaches the ground does not immediately enter the soil, which further delays the movement of this DCD into the soil, until subsequent rainfall has occurred, and ability for it to intercept urine N in the soil.

On average, across the two sites, 53% of the DCD estimated to have reached the ground was recovered from the soil; 36% from the 0-2 cm, 5% from the 2-5 cm and 12% from the 5-10 cm soil depths (Table 7). It interesting to note that, for some reason, significantly more DCD and higher concentrations was recovered from the 5-10 cm depth compared to the 2-5 cm depth. It should be noted that 2-5 cm represents 3 cm of soil, while 5-10 cm represents 5 cm, partially explaining this difference. When the DCD concentrations between the two soil depths are compared the difference is not so large (see Figure 20). It is possible that the soil corer was carrying soil from the upper depths downwards, however, it was not possible to establish this.

These results are largely consistent with the modelling in Bishop (2010), although his model showed a higher proportion of DCD below 2 cm, than was measured in the current study. This is possibly because the Bishop (2010) study modelled using a time-step of 7 days. In contrast, modelling in Giltrap, Portegys, et al. (2020) could not get DCD to move beyond 2 cm in the timeframe of this experiment of 1 day. This could be because the model is not accounting for all of the potential DCD transport pathways.

Table 6 - Percentage of DCD estimated to have reached ground that was recovered in soil and herbage. Letters showing Tukey HSD

	Site 1		**Site 3**	
	30 mL	**60 mL**	**30 mL**	**60 mL**
Herbage	50% a	44% *ab*	37% *abc*	33% *abcd*
0-2 cm	36% *abc*	33% *abcd*	49% *a*	26% *bcde*
2-5 cm	7% *e*	5% *e*	5% *e*	2% *e*
5-10 cm	13% *de*	10% *e*	17% cde	8% *e*
Sum	**106% *x***	**92% *x***	**109% *x***	**70% *y***

Table 7 - % of total DCD recovered from soil. Letters showing Tukey HSD

	Site 1		Site 3	
	30 mL	**60 mL**	**30 mL**	**60 mL**
0-2 cm	64% *a*	71% *a*	67% *a*	68% *a*
2-5 cm	12% *bcd*	10% *cd*	7% *d*	7% *d*
5-10 cm	24% *b*	20% *bc*	25% *b*	25% *b*

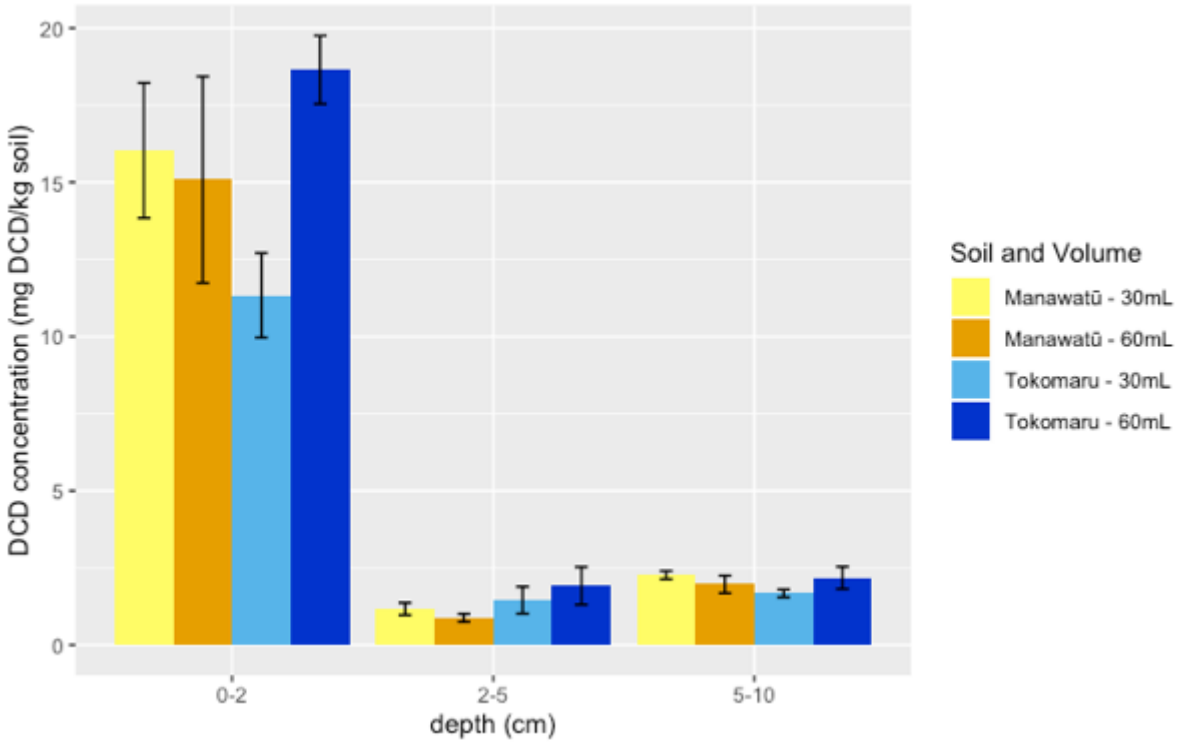

Figure 20 - Average measured concentrations of DCD at each depth for each application volume and soil type, pre-rainfall.

The average concentration was 13.2 mg DCD/kg soil, 1.0 mg DCD/kg soil and 1.8 mg DCD/kg soil in the 0-2, 2-5 and 5-10 cm soil depth, respectively. DCD application rates of 5 kg/ha have been shown to be ineffective at reducing N_2O emissions in the field, which equates to 5 g DCD/kg soil down to 10 cm (Bishop, 2010; Di & Cameron, 2005). Lower concentrations of DCD have been shown to be effective in laboratory studies on soils with low organic matter (<0.5%) (McCarty & Bremner, 1989), so concentrations below 2 mg DCD/kg soil may not be ineffective on all soils. However, the soils on the two sites of this study did not have organic matter levels this low (Bishop, 2010; Hoogendoorn et al., 2017).

For the Manawatū silt loam soil (Site 3), K has been measured at 0.0990. This means the potential inhibitory effect can be estimated for a range of concentrations. Based on this information, the concentrations measured below 2 cm are unlikely to be inhibiting nitrification by more than 25%. It should be noted that DCD concentrations need to be high enough initially, so that as the DCD degrades, there still remains enough to continue to effectively inhibit nitrification. Therefore, 25% is the maximum inhibition, and effectiveness will drop in the future.

Table 8 - Percent inhibition of DCD on Manawatū silt loam with a range of DCD concentrations, estimated with data from (Bishop, 2010)

[DCD]	1.0	2.0	3.0	4.0	5.0	6.0

Inhibition (%)	11.1	21.2	31.3	41.4	51.5	61.6

Having said this, Table 9 shows the range of half-lives of DCD expected at the sites used, and suggests that DCD is only likely to degrade quickly enough for this to be a concern in the months around summer. The persistence of DCD below 2 cm is also not necessarily a concern if rainfall or irrigation can wash DCD deeper into the soil (see section 4.3.5).

The K value for each soil in the Bishop (2010) study varied considerably, even between similar soils. This changes the potential effectiveness of DCD considerably. Therefore, the inhibition values in Table 8 are not suitable for estimating DCD effectiveness in Tokomaru silt loam.

Table 9 - Half-life of DCD at a range of temperatures typical of Palmerston North during when DCD could be expected to be applied. Temperatures used are the mean air-temperature (representing the soil surface) and the mean soil temperature at 10cm deep at 9am, for March and July (Chappell, 2013). Half-lives calculated using The half-life of DCD can be estimated using

Equation 2, where T is the soil temperature (Kelliher et al., 2008). Temperature data for Palmerston North was taken from Chappell (2013).

Equation 2

	Surface		10 cm	
	Temperature (°C)	Half-life (days)	Temperature (°C)	Half-life (days)
March	17.5	38	16.1	43
July	9.5	75	7.1	93

Application rate

As mentioned earlier, recovery was lower with the 60 mL application at Site 3 but not on the at Site 1. The application rate did not change the distribution of DCD down the soil profile ($p>0.1$). Doubling the application rate did increase the concentration of DCD measured in the soil, but only in top 2cm in the Tokomaru soil with 11.3 and 18.7 mg DCD/kg soil measured in the 30 mL and 60 mL applications respectively. This suggests that the higher application rate just results in more DCD accumulating in the top 2 cm, rather than moving it deeper into the soil.

Soil/Site

Canopy interception was lower at Site 3. A possible explanation for this was the canopy closure for the two pastures, the pasture of Site 1 appeared quite uniform with generally good canopy closure, while the pasture on Site 3 was less uniform, with more clumps of dead material and

bare soil. This may be due to the mowing regime preceding the trial; the Dairy 4 pasture had been mown regularly over the past months for other GPLER experiments which would encourage tillering of the ryegrass, resulting in more dense and uniform pasture (McKenzie et al., 1999), whereas the Dairy 1 site had been left unmown and ungrazed (see Figure 16 for some idea of pasture length) until a few weeks before the trial, and was only mown twice before the experiment. However, without weighing pasture samples from each site it is impossible to say for sure. Should this experiment be repeated, pasture samples should be weighted. No significant difference in depth distribution was seen between soils. Comparing the measured concentrations between soils in not necessarily useful as the amount of DCD measured per kg of soil may be different simply due to the difference in bulk densities between the soils. The difference in canopy interception will also influence the difference in concentrations, even though this will not be due to the soil type itself.

Accounting for the unusual Site 3 – 60 mL values

Concentrations in the top 0-2 cm for the 60 mL treatment are around double the 30 mL for at Site 1 soil and on the 'drier' soil treatment at Site 3. However, on the 'wetter' soil treatment at Site 3, the measured concentrations from the 60 mL application are less than half the concentrations measured from the 30 mL application. There is no clear explanation for the lower recovery for this treatment, other than one of the three replicate plots having a very high pasture interception of 68%, which could have contributed to lover recovery. However, pasture interception did not account of all of the lower DCD recovery measured.

4.3.4.1. Spatial variation of measured DCD concentrations

The concentration of individual DCD measurements (not averaged across each patch) in the top 2 cm of soil varied greatly across the surface of each individual urine patch. Figure 21 shows an example of a DCD-urine patch. For a 30 mL DCD application, the quartiles of concentrations were 3.7 and 13.6 mg DCD/kg soil. For a 60 mL DCD application the quartiles were 4.6 and 20.1 mg DCD/kg soil. "Maximum" concentrations (defined as within 1.5*IQR of the upper quartile) were measured at 28.6 and 43.4 mg DCD/kg soil for 30 mL and 60 mL DCD applications, respectively. The maximum recorded outlier was 82.3 mg DCD/kg soil (Figure 22). This is likely due to the non-uniformness of the pasture canopy.

0-2 cm								
				4				
				20				
				44				
				28				
46	19	19	23	22	16	8	2	1
				10				
				15				
				34				
				37				

2-5 cm								
				2				
				3				
				1				
				3				
7	1	1	5	0	10	0	0	0
				4				
				1				
				3				
				5				

5-10 cm								
				2				
				2				
				3				
				1				
3	1	4	1	2	0	2	2	3
				1				
				1				
				2				
				8				

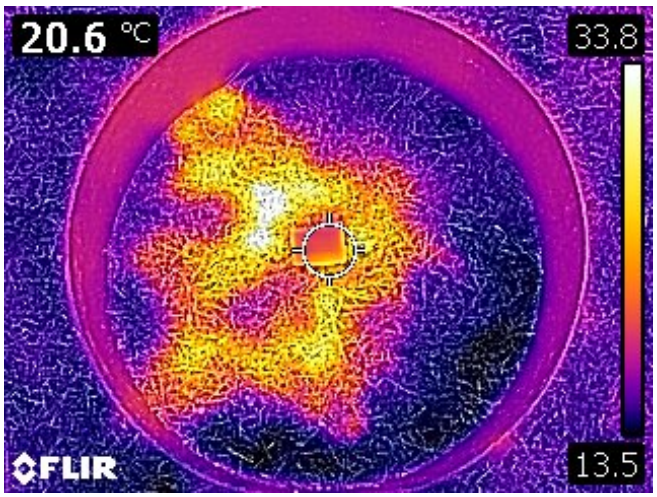

Figure 21 - An example of a DCD/urine patch. Left: measured concentrations of DCD (mg DCD/kg soil) for each depth. Top-right: thermal image of the urine patch immediately after application. Bottom-right: visible image of the urine patch, immediately after application. See appendix for all patches

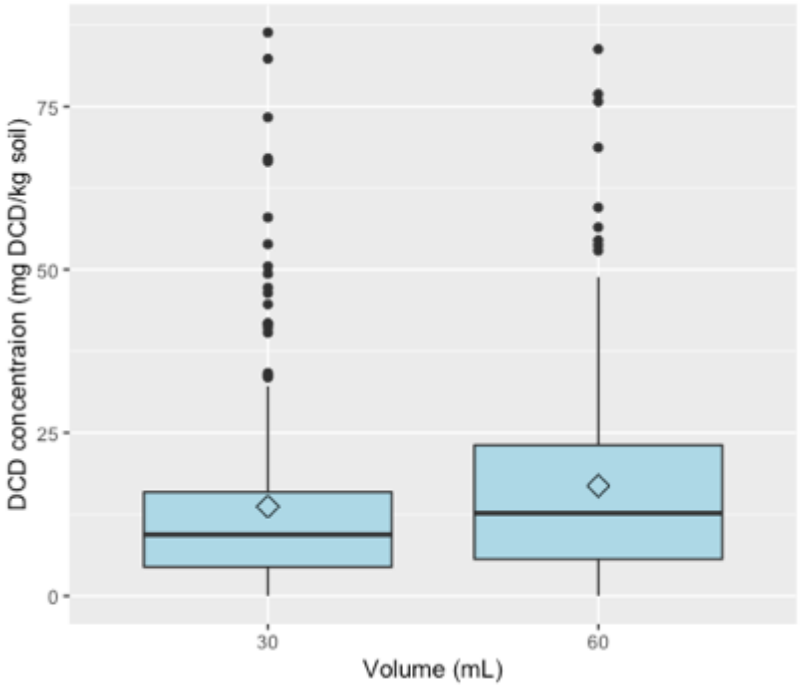

Figure 22 - DCD conc showing spatial variation across a urine patch at 0-2 cm. Diamond shows mean

Most of the DCD concentrations below 2 cm were clustered around 0.3-2.5 mg/kg soil, quite low concentrations and unlikely to be effectively inhibiting nitrification (Bishop, 2010; McCarty & Bremner, 1989). However, there were several outliers above 5 mg/kg. The positive skew was much more pronounced at these depths when compared with the 0-2 cm concentrations (see Figure 23). This suggests that generally small amounts of DCD were moving down the soil profile below 5 cm, but occasionally, around 1 soil core in 20, a much larger amount of DCD moved down the soil profile, probably through a biopore, such as a worm channel, resulting in the group of unusually high DCD concentrations measured.

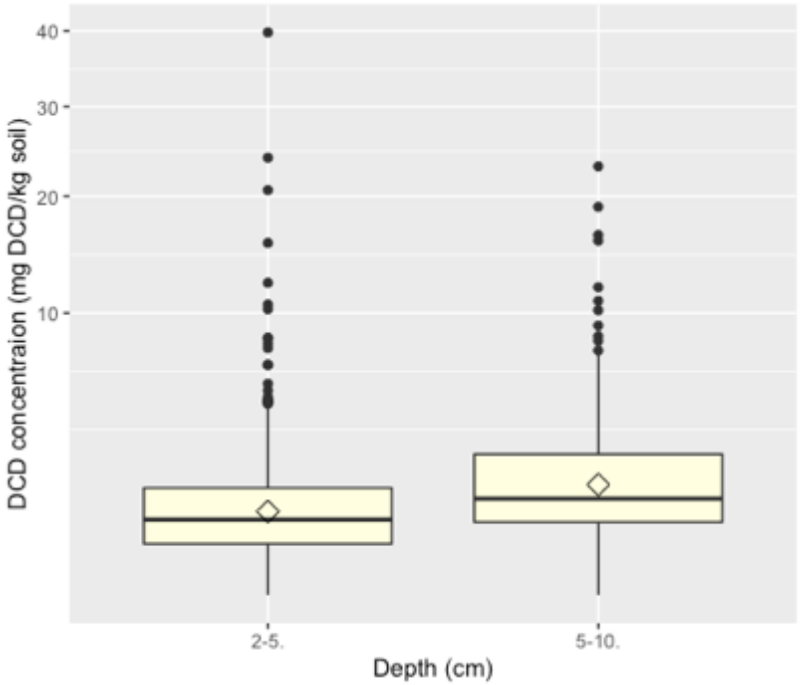

Figure 23 - DCD conc showing spatial variation across a urine patch at 2-5 and 5-10 cm. Note square root scale of y axis. Diamond shows mean

4.3.5. Post rainfall

Soil moisture did not appear to have any effect on DCD concentration or distribution in the soil ($p>0.1$). For subsequent analysis the different soil moisture treatments will be combined together.

On average 46% of the DCD applied was recovered from the soil, however, this varied significantly with application volume on Site 3.

Most of the DCD remained in the top 2 cm of soil, on average 54% of the DCD recovered from the soil was in the 0-2 cm section, 31% from the 2-5 cm section and 15% from the 5-10 cm depth. When comparing this to the pre-rainfall measurements, it appears that DCD from the top 2 cm has moved down into the 2-5 cm band. Also of interest is that there is less DCD measured in the 5-10 cm section when compared to the pre-rainfall data. It is unclear why this would be. The movement of rainfall down the soil profile reflects the recommendation to apply DCD shortly before rainfall or irrigation to "wash" the DCD deeper into the soil. Whether this recommendation is practical on-farm will depend on the rainfall pattern of the district and the presence of irrigation. In New Zealand it may be applicable in Canterbury and the west coast of both islands, but not elsewhere.

Application rate

Recovery rates on the Site 1 did not vary significantly between application rates, averaging 43%. Recovery on the Site 3 – 60 mL application was much lower than the Site 3 – 30 mL application, 65% and 34% respectively (see Table 10). Note that around 40% of the DCD was removed on the pasture in the first round of sampling (see Table 6). The low recovery of the site 3 – 60 mL treatment is a reflection of the same low recoveries from the pre-rainfall round of sampling.

Table 10 - percent recovered of DCD applied - post rainfall. Letters show Tukey HSD

	Site 1		Site 3	
Depth	**30 mL**	**60 mL**	**30 mL**	**60 mL**
0-2 cm	23% *b*	24% *bc*	37% *a*	18% *bcd*
2-5 cm	11% *bcd*	15% *bcd*	21% *bc*	12% *bcd*
5-10 cm	8% *cd*	7% *d*	8% *cd*	4% *d*
Sum	**41%**	**46%**	**65%**	**34%**

Table 11 - Percent of total DCD recovered for the two soil - post rainfall

	Site 1	Site 3	P-Value
0-2 cm	54%	53%	0.872
2-5 cm	29%	34%	0.040
5-10 cm	17%	13%	0.005

Unlike the pre-rainfall measurements, here there was a significant effect of application volume of the distribution of DCD down the soil profile on the Tokomaru soil, with DCD appearing to have moved further down the profile with the higher application volume. The proportion of recovered DCD measured in the top 2 cm was about the same across the two volumes, but a higher proportion was measured in the 2-5 cm layer (32% vs 26% for 60 and 30 mL, suggesting that the higher application rate resulted in better penetration of DCD down the soil profile after rainfall.

Increased application rate resulted in significantly higher concentrations of DCD measured in the Tokomaru soil at all depths: 7.1 vs 13.4 mg/kg soil in the top 2 cm for 30 and 60 mL respectively, and at 2-5 cm, 2.3 vs 5.5 mg DCD/kg soil for 30 mL and 60 mL respectively ($p=0.016$; Figure 24). Concentrations were also somewhat higher at 5-10 cm (1.0 vs 1.6 mg DCD/kg soil, $p=0.007$). Referring back to Table 8, if this was Manawatū silt loam, then the 60 mL application is likely to be inhibiting more than half of the potential nitrification, while the 30 mL application only a quarter. Again, specific measurements would be needed on Tokomaru

silt loam to make quantitative statements about the effectiveness of DCD at these concentrations, however, the higher concentrations will certainly be more effective, for longer (Bishop, 2010). These measurements suggest that applying higher concentrations of DCD will result in better control of nitrification at lower depths, provided rainfall is sufficient to move the DCD down the soil profile.

Table 12 - percentage of total DCD recovered – post rainfall

	Site 1		Site 3	
Depth	**30 mL**	**60 mL**	**30 mL**	**60 mL**
0-2 cm	55% *a*	52% *a*	55% *a*	51% *a*
2-5c m	26% *cd*	32% *bc*	31% *bc*	37% *b*
5-10 cm	19% *de*	16% *e*	13% *e*	12% *e*

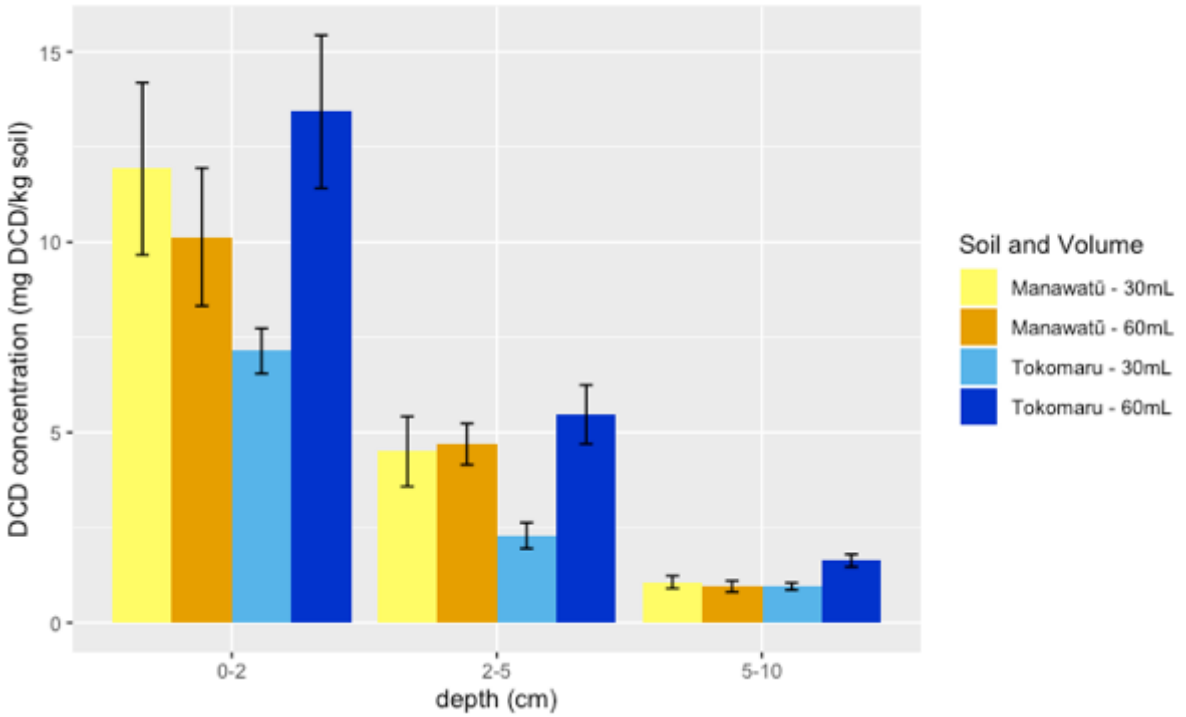

Figure 24 - DCD concentration in soil - post rainfall

Soil

There was no significant difference in the percentage of applied DCD recovered between the soils. There is a significant difference in the depth distribution of DCD between soils. There is no difference in the top 2 cm. However, a higher proportion of the recovered DCD was found in the 2-5 cm section of the Manawatū soil when compared with the Tokomaru (34% vs 29% respectively, $p=0.04$). In contrast, at the 5-10 cm depth the reverse is true, the proportion of DCD is higher in the Tokomaru soil (17% vs 13% respectively, $p<0.01$).

The contrast between the proportions at 2-5 and 5-10 cm between the two soils could be explained by the different flow paths that dominate the two soils; Manawatū silt loam, being well-drained, will be dominated by matrix flow and water (and the DCD it carries) should flow evenly and steadily through the mesopores. In contrast, Tokomaru silt loam, being poorly-drained, will be much more reliant on preferential flow, bypassing more of the soil matrix (McLaren & Cameron, 1996), meaning that DCD which is moved out the surface 2cm is being moved down deeper. However, this would require further experimentation to determine if this were true, possibly using dyes and cross sectioning soil cores.

There was no significant difference in concentrations between the soil types. Again, this is perhaps not a useful comparison, as the concentrations in each soil type will be influenced by differing pasture interception (not a function of soil type), and bulk density.

4.3.6. Learnings and gaps for future research

The spray unit did not deliver all of the DCD solution placed in the soil to the ground and performed worse with higher volumes. This needs to be addressed before any future research is carried out. Larger nozzles on the spray unit may reduce the amount of misting that occurred. Small droplets can bounce off surfaces, so larger droplet sizes would increase the chances of DCD "sticking" to the herbage and soil. This improvement has already been made to the commercial version of Spikey® (P. Bishop, personal communication, February 2020).

To provide a more realistic picture of how rainfall/irrigation impacts DCD distribution the pre- and post-rainfall, particularly how rainfall washes DCD off the herbage, measurements would have to be made on two separate sets of urine/DCD patches, so herbage could be removed in both the pre- and post-rainfall rounds of sampling. However, time prevented this; the main objective of this experiment and the needs of the GPLER programme was for data from in the hours following DCD application, and therefore, the first sampling round was given priority. The post-rainfall round was merely to give some indication of how rainfall moves DCD down the soil profile, given that DCD is recommended to be applied shortly before rainfall or irrigation. A more detailed study of post-rainfall DCD movement will require future work.

Other soil types also need to be investigated. This experiment has considered how soil drainage capacity might influence DCD movement, however, there are other soil characteristics that could be considered. In particular Allophanic soils or Organic soils may behave differently and may restrict DCD movement (Singh et al., 2008; Zhang et al., 2004). This is of particular importance as a sizable proportion of dairy production in the North Island is on these two soils.

For any future experiment, sectioning the soil core into 0-1 cm and 1-2 cm would provide more detail as to the depth distribution of DCD is the top two cm where most of the DCD appears to remain. This would result in very small amounts of soil to extract, so aggregating many samples in a single patch would be required, preventing the measurement of spatial variation in DCD concentration across the urine patch. However, this experiment has already demonstrated that this varies considerably, the remaining knowledge gap is the depth distribution of DCD within those top 2 cm.

There are also other potential nitrification inhibitors, which may behave differently to DCD, so this study should be repeated with other nitrification inhibitors. No other nitrification inhibitors are used in NZ to the extent that DCD was, so there are no obvious candidates, however, DMPP may have potential and may be a useful inhibitor to repeat these experiments with. Other novel inhibitors may also emerge.

4.4. Conclusions

Less than two thirds of the applied DCD were recovered from the soil. 40% remained on the pasture, where it would be ineffective and vulnerable to ingestion by grazing stock. Most of the recovered DCD was recovered from the pasture and top 2 cm of soil; this has implications for the effectiveness of DCD and similar nitrification inhibitors below 2 cm. Increased application rate increased DCD concentration measured in the top 2 cm on the Tokomaru soil. It did not improve depth penetration of DCD before rainfall. Soil type affected DCD distribution after rainfall. Rainfall moved DCD down the soil profile, with significantly increased concentrations between 2-5 cm.

5. General Discussion

Based on the distribution of urine in chapter 3 and the distribution of DCD is chapter 4, it is possible to estimate the proportion of urine that DCD will reach in the hours after DCD application.

Most of the DCD remained in the top 2 cm of soil up to 16 hours after application. There did not appear to be any significant difference in DCD movement between the well and poorly drained soils. These results indicate that distribution of water-soluble nitrification inhibitor DCD could be reasonably consistent across many similar soil types. However, further research on other soil types and inhibitors would be needed to confirm this. While very small amounts of DCD were measured below 2 cm, DCD concentrations were so low (<3 ppm) to have any significant impact on the rate of Urine-N nitrification. Therefore, the effectiveness of DCD will largely depend on the proportion of urine-N above and below 2 cm. Urine-N distribution data collected in the previous experiment (Chapter 3) were available for urine patch soil cores sectioned into 0-5, 5-10 and 10-20 cm. Thus, the proportion of urine-N in the top 2 cm soil was estimated. For more accurate urine-N distribution data in the top 5 cm soil further sectioning of the cores between 0-2 and 2-5 cm depth would have been appropriate. This was not possible in the Urine-N distribution experiment for two reason: first, the urine data was collected to identify 3-dimaentional movement of urine, and part of a wider GLPER programme, resulting in a very high number of samples. It was not possible to increase the number of samples with more depth measurements without increasing the time and potential for urine-N transformations. Second, the urine data was collected before the DCD data (and before the DCD experiment was conceived of), so there was no way of knowing the appropriate depths to section.

Based on the average distribution of urine between the two soils, the proportion of urine above 2 cm appears to range from 35-50% (see Figure 25). This suggests that the DCD applications applied with the Spikey® spray unit would intercept 35-50% of a urine patch. This would vary depending on the size and shape of the urine patch, which can vary considerably with urine volume, soil moisture, and other soil properties such as hydraulic conductivity (Forrestal et al., 2020; Jolly et al., 2019; Mehra et al., 2020; Selbie et al., 2015).

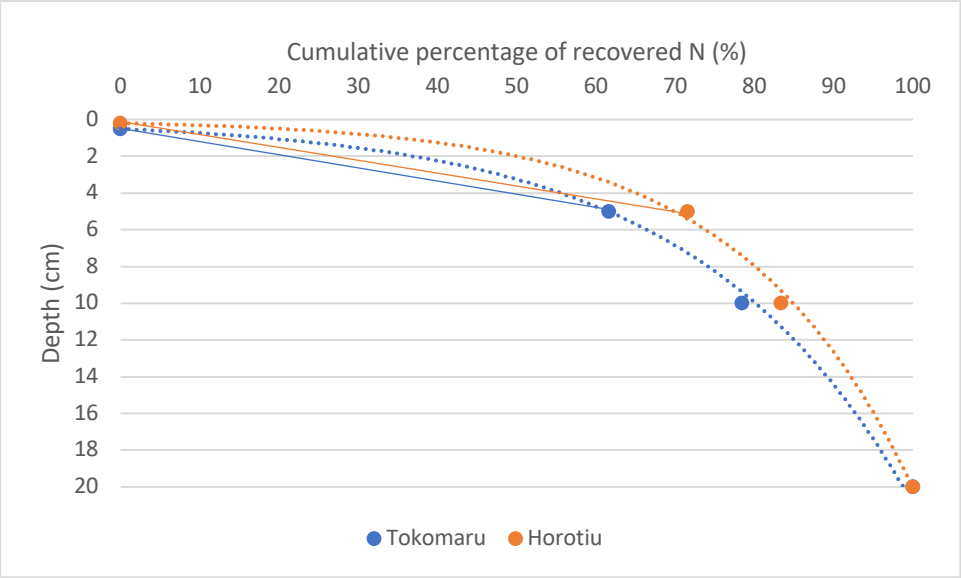

Figure 25 – Average cumulative distribution of urine down the soil profile on Tokomaru and Horotiu soils, with log relationship shown (dotted line) and linear relationship (solid line)

This can be compared with the modelling work in Giltrap, Jolly, et al. (2020); Giltrap, Portegys, et al. (2020). Here, it is estimated that only 25-35% of urine is being intercepted. This discrepancy could be explained by limitations in the modelling used. It is also possible that logarithmic relationship used might be appropriate for urine below 5 cm but not above. Drawing a linear line between 0 and 5 cm shows 20-25% of urine being above 2 cm, slightly below the estimations in Giltrap, Portegys, et al. (2020). The true proportion would require further research, sectioning the urine cores into above and below 2 cm.

The post-rainfall data can be used to indicate potential interception after rainfall, with the 60mL application resulting in moderate DCD concentrations at 2-5 cm. In this case, the DCD would be intercepting around 80% of the urine. Modelling of urine distribution in Giltrap, Portegys, et al. (2020) is again slightly lower, ranging from 60-70%. It should again be noted that the DCD measured post-rainfall does not take into account DCD that would wash of the pasture into the soil.

From this, one might assume that the DCD application would only be able to inhibit around 40% of the urinary-N and is therefore, only capable of a maximum of 40% reduction in N leaching and N2O emissions. However, this is not necessarily the case. Firstly, DCD is recommended to be applied before rainfall or irrigation. As discussed earlier, this can wash DCD deeper into the soil. In theory, because NH_4^+ is positively charged, it will remain bound in place onto the soil while the DCD moves downward, allowing the DCD to "catch up" with

the NH_4^+. How realistic this is depending on the climate of the farm in question, or the presence of irrigation.

Nitrous oxide is more complicated, N_2O emissions tend to be higher nearer to the soil surface, as nitrification rate decreases down the soil profile. Measurements on Manawatū silt loam and Dannevirke silt loam found nitrification rate below 2 cm dropped below half what was measured in the top 0.2 cm (Bishop, 2010). A similar decrease (albeit over a much larger depth profile) was observed on Oxfordshire, UK clay soils, with nitrification rates at 15-20 cm half that of 2-10 cm (Macduff & White, 1985). Further to this, N_2O can be consumed as it diffuses up the soil profile, so N_2O produced deeper down is less likely to reach the atmosphere (Clough et al., 2006; Goldberg et al., 2008), further increasing the importance of N_2O produced at the surface. Therefore, while DCD's limited depth penetration will limit its potential to reduce N_2O emissions, this limitation in unlikely to be as severe as it will be in the case of N leaching. It should be noted that DCD applied using the same method in this experiment on Tokomaru silt loam reduced N_2O emissions by 38% (S. Saggar, personal communication, January 20, 2020, unpublished data). This can be compared to 39-52% reductions on the same soil over the autumn-spring period, when DCD was mixed with urine before application (Zaman et al., 2009).

6. Conclusions

On average, around 60-70% of urine-N remains in the top 5 cm of the soil in the hours and days after urination. An estimated average of 35-50% of urinary N remains in the top 2 cm. This does not take into account pasture canopy interception and volatilisation. There is also substantial variation in urine-N distribution both within and between individual patches.

Effective DCD concentrations were only measured in the top 2 cm in the well-drained and poorly drained soils. Again, there can be substantial variation in DCD concentration within individual DCD patches.

This suggests that on most soils, the DCD sprayed onto pasture will only intercept the 35-50% of the urine patch, without rainfall. Following a significant rain event (in this case 24 mm) and with at least 13 kg DCD/ha DCD could be inhibiting 80% of the urine-N.

This will limit the effectiveness of DCD to reduce nitrate leaching as the DCD may not be able to intercept urine-N further down the soil profile. Its impact on N_2O emissions is less certain, as N near the surface is more likely to be lost as N_2O. As rainfall can move DCD down the soil profile, this limitation is likely to be more acute in low rainfall areas.

Future research could explore ways to combine emerging urine patch measuring technologies with physical soil sampling to generate more data with less time and cost. DCD movement could also be explored on different soil types such as Allophanic or Organic soils. Measuring DCD and urine-N movement at the same time in the same patches, with particular focus on the top five cm would also provide useful information that this study could not.

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Appendix 1 – Urine patches

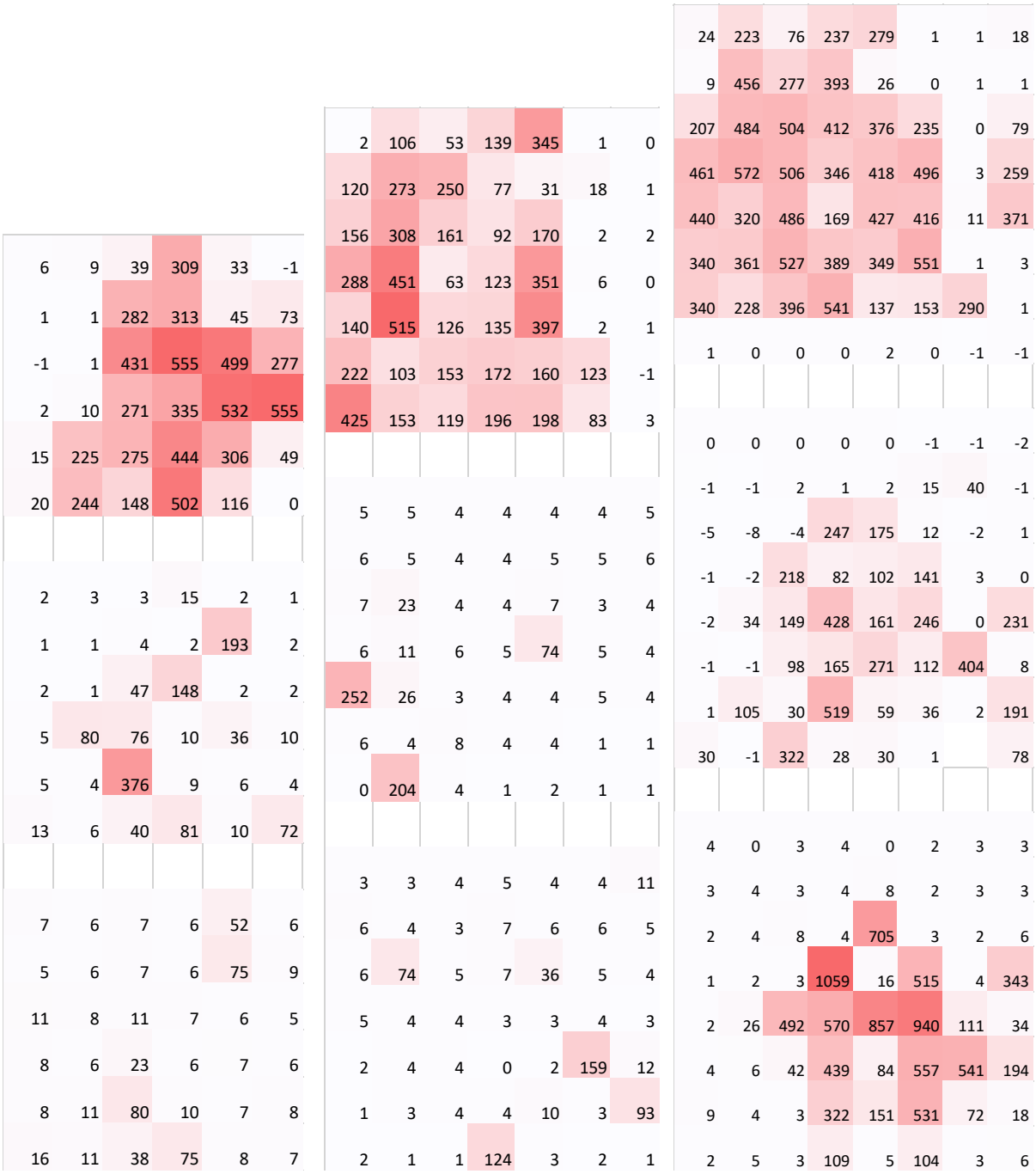

Figure 26 – NH_4^+-N concentration (mg/kg soil) in urine patches on Site 2, moist soil. From top to bottom: 0-5 cm, 5-10 cm, 10-20 cm. From left to right: 1 L, 2 L, 3 L.

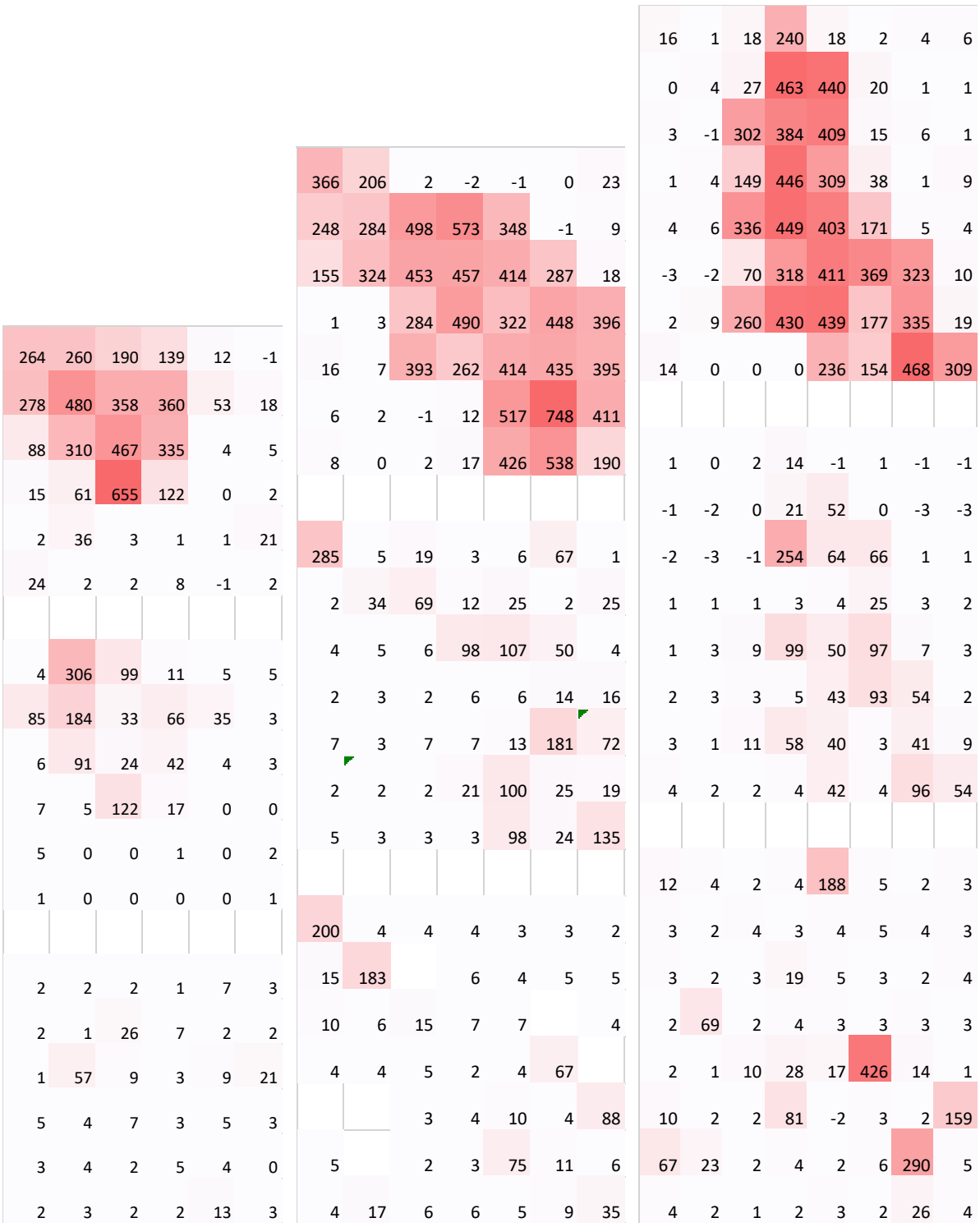

Figure 27 - NH_4^+-N concentration (mg/kg soil) in urine patches on Site 2, drier soil. From top to bottom: 0-5 cm, 5-10 cm, 10-20 cm. From left to right: 1 L, 2 L, 3 L.

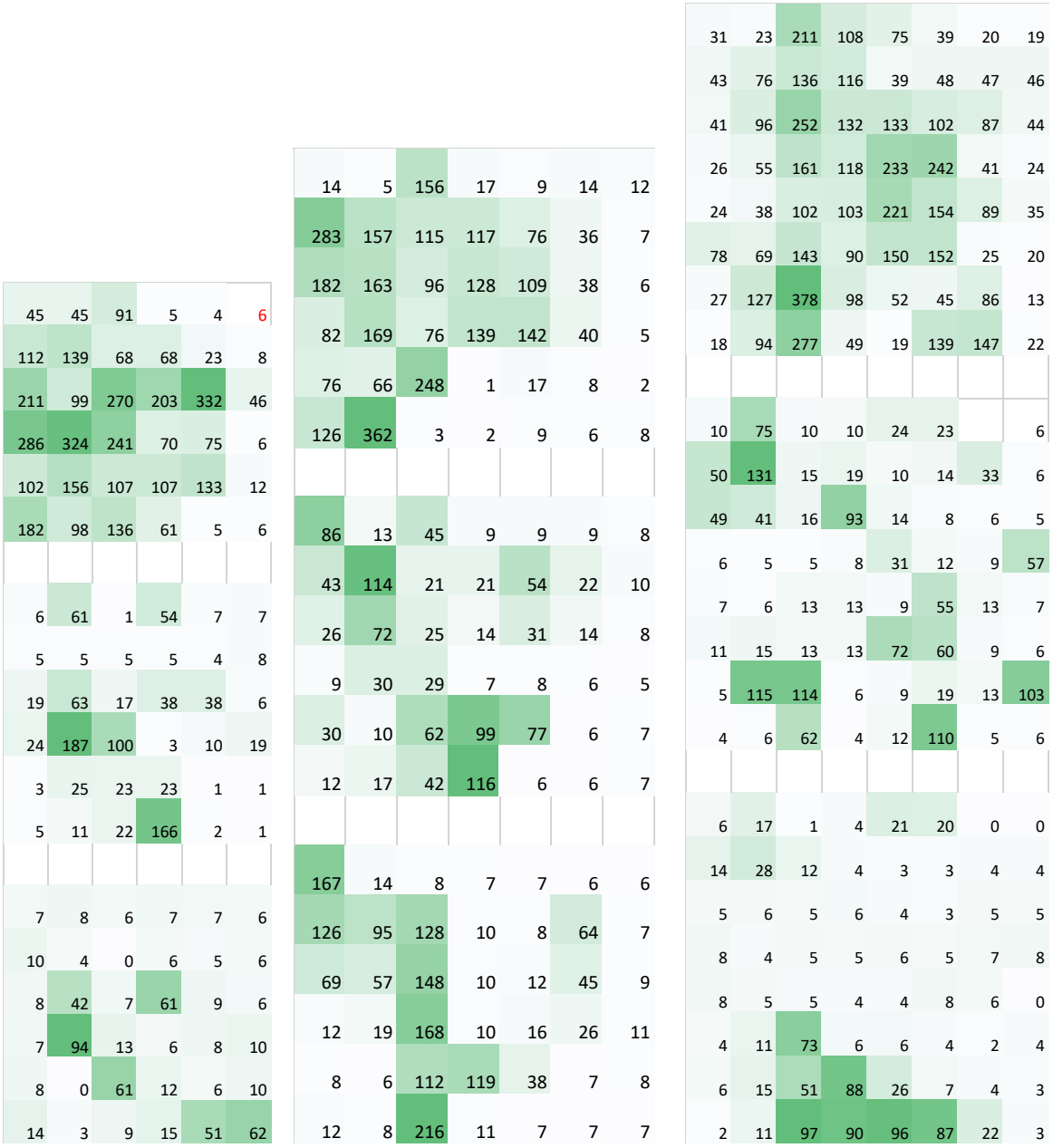

Figure 28 - NH_4^+ + NO_3^--N concentration (mg/kg soil) in urine patches on Site 1, moist soil. From top to bottom: 0-5 cm, 5-10 cm, 10-20 cm. From left to right: 1 L, 2 L, 3 L.

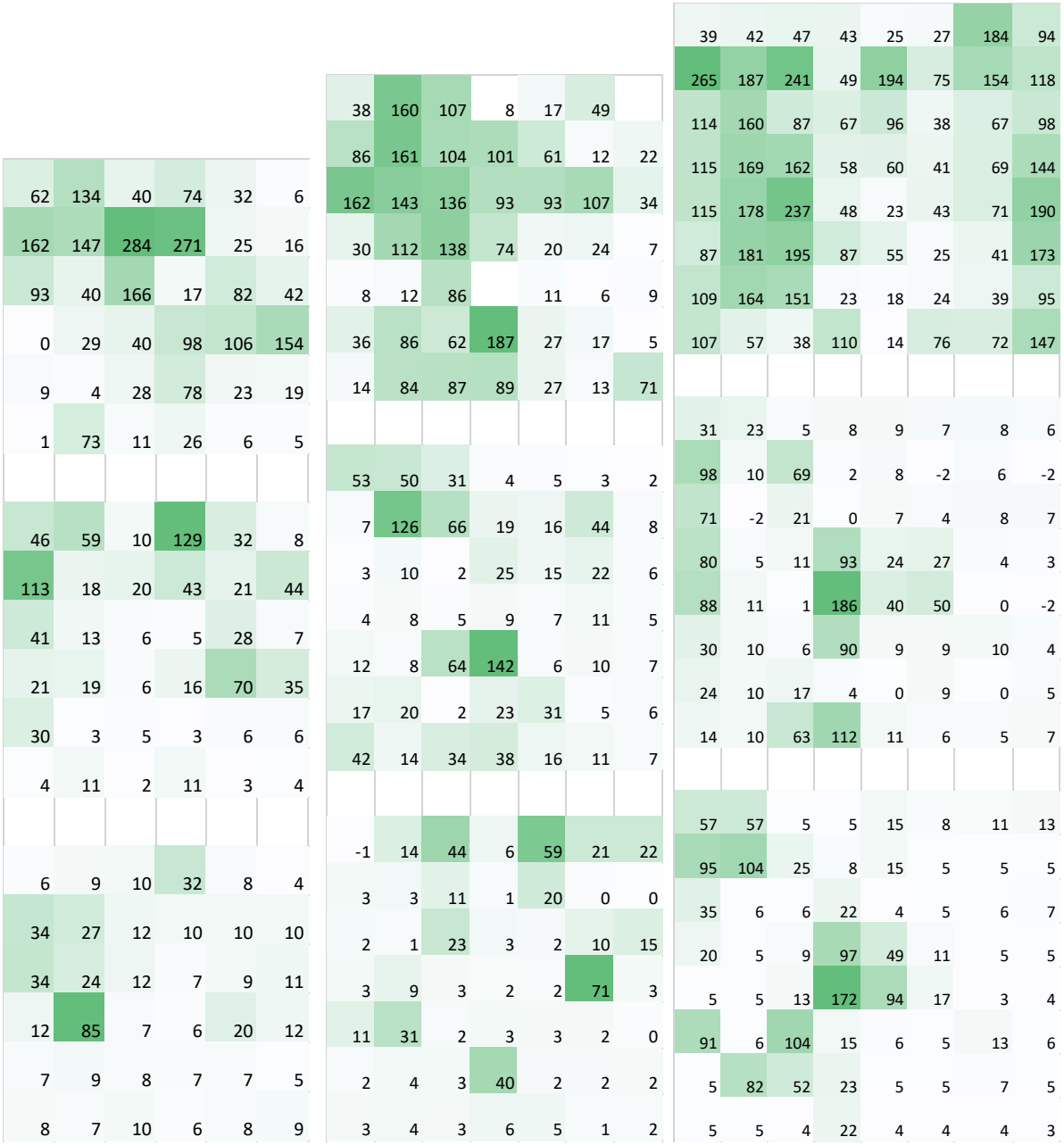

Figure 29 - NH_4^+ + NO_3^--N concentration (mg/kg soil) in urine patches on Site 1, moist soil. From top to bottom: 0-5 cm, 5-10 cm, 10-20 cm. From left to right: 1 L, 2 L, 3 L.

Appendix 2 – DCD patches

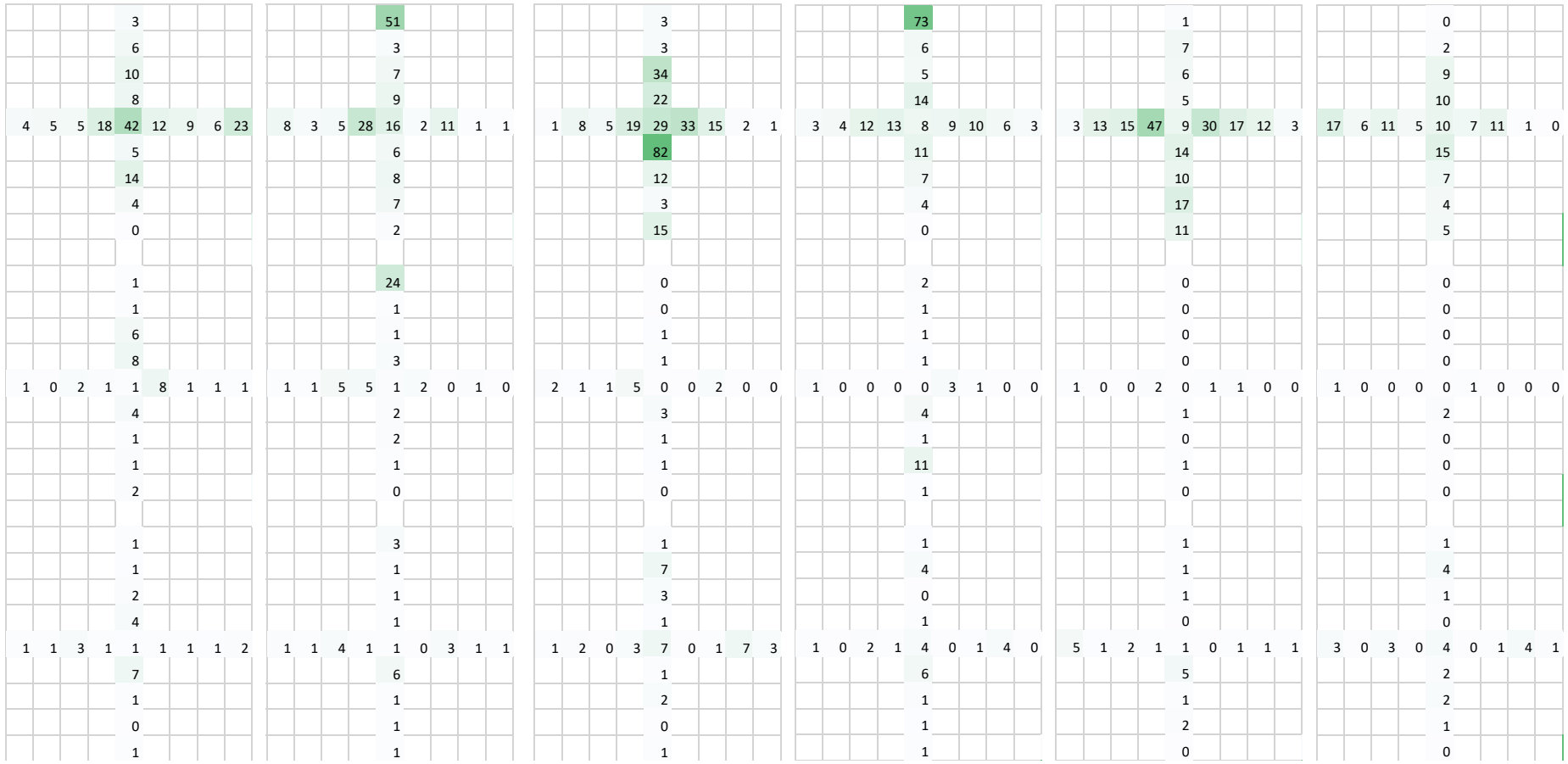

Figure 30 – measured DCD concentrations (mg/kg soil) for 30 mL DCD applications on Site 1. Top to bottom: 0-2 cm, 2-5 cm, 5-10 cm.

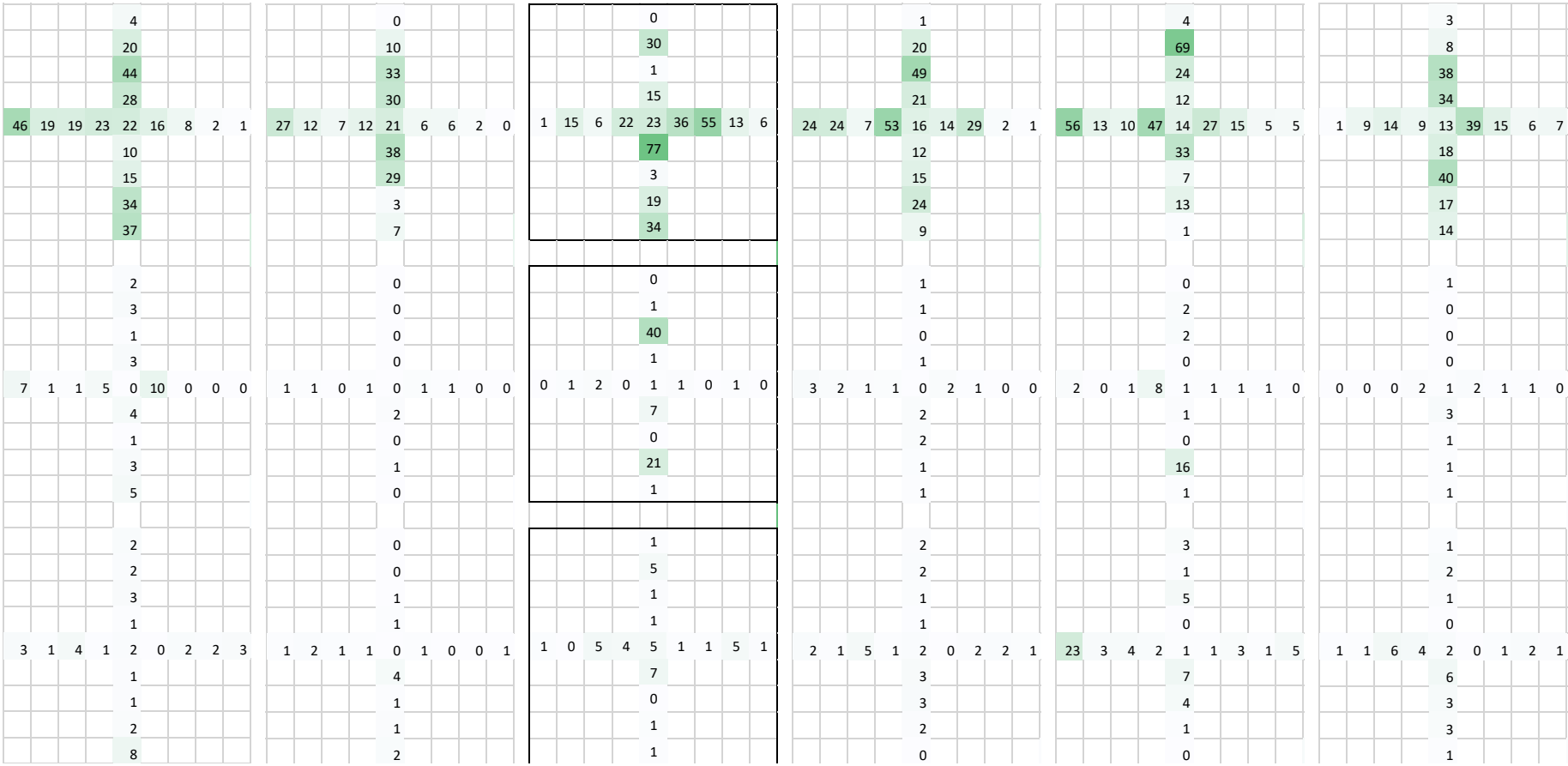

Figure 31 - measured DCD concentrations (mg/kg soil) for 60 mL DCD applications on Site 1. Top to bottom: 0-2 cm, 2-5 cm, 5-10 cm.

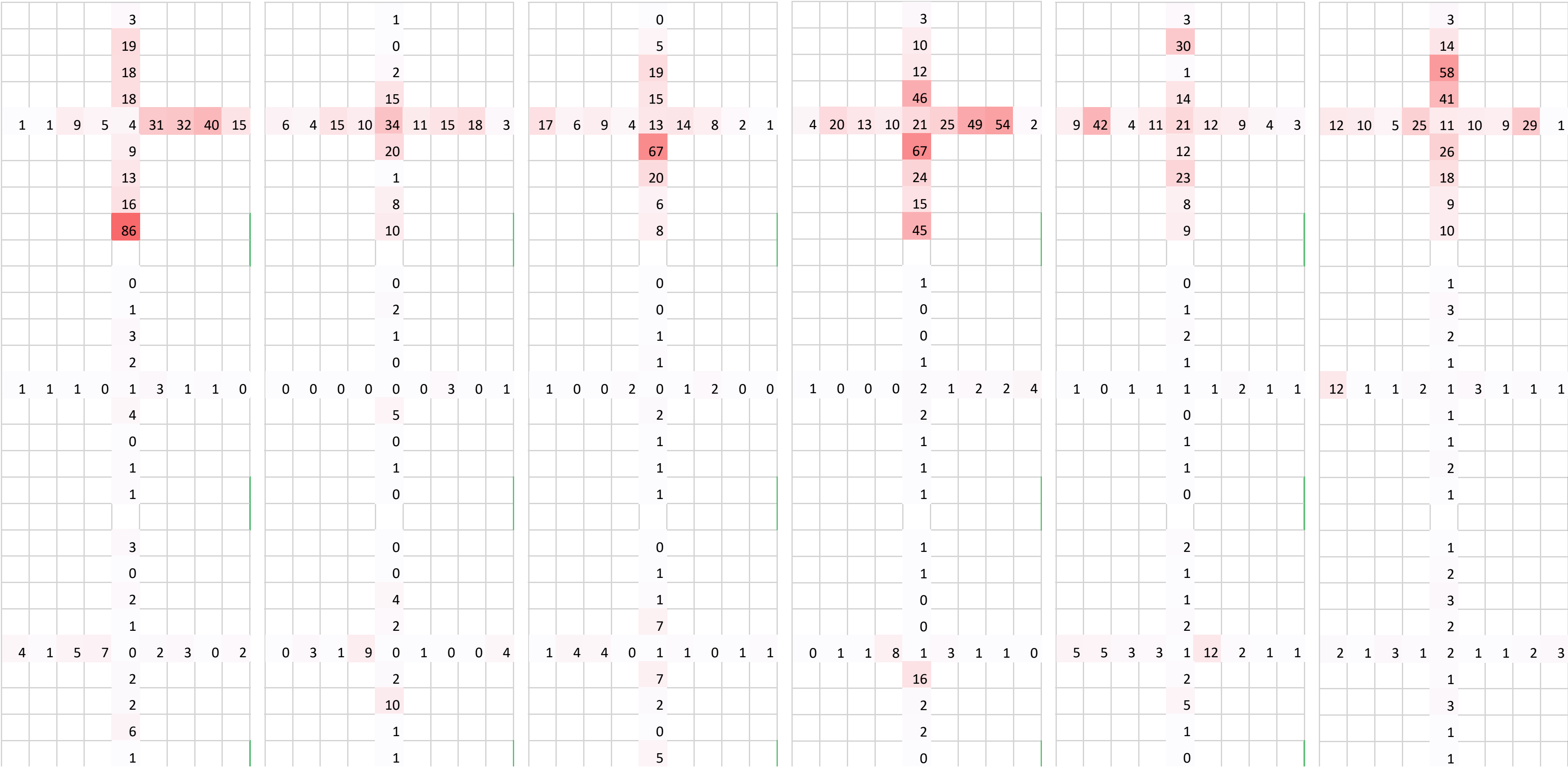

Figure 32 - measured DCD concentrations (mg/kg soil) for 30 mL DCD applications on Site 3. Top to bottom: 0-2 cm, 2-5 cm, 5-10 cm.

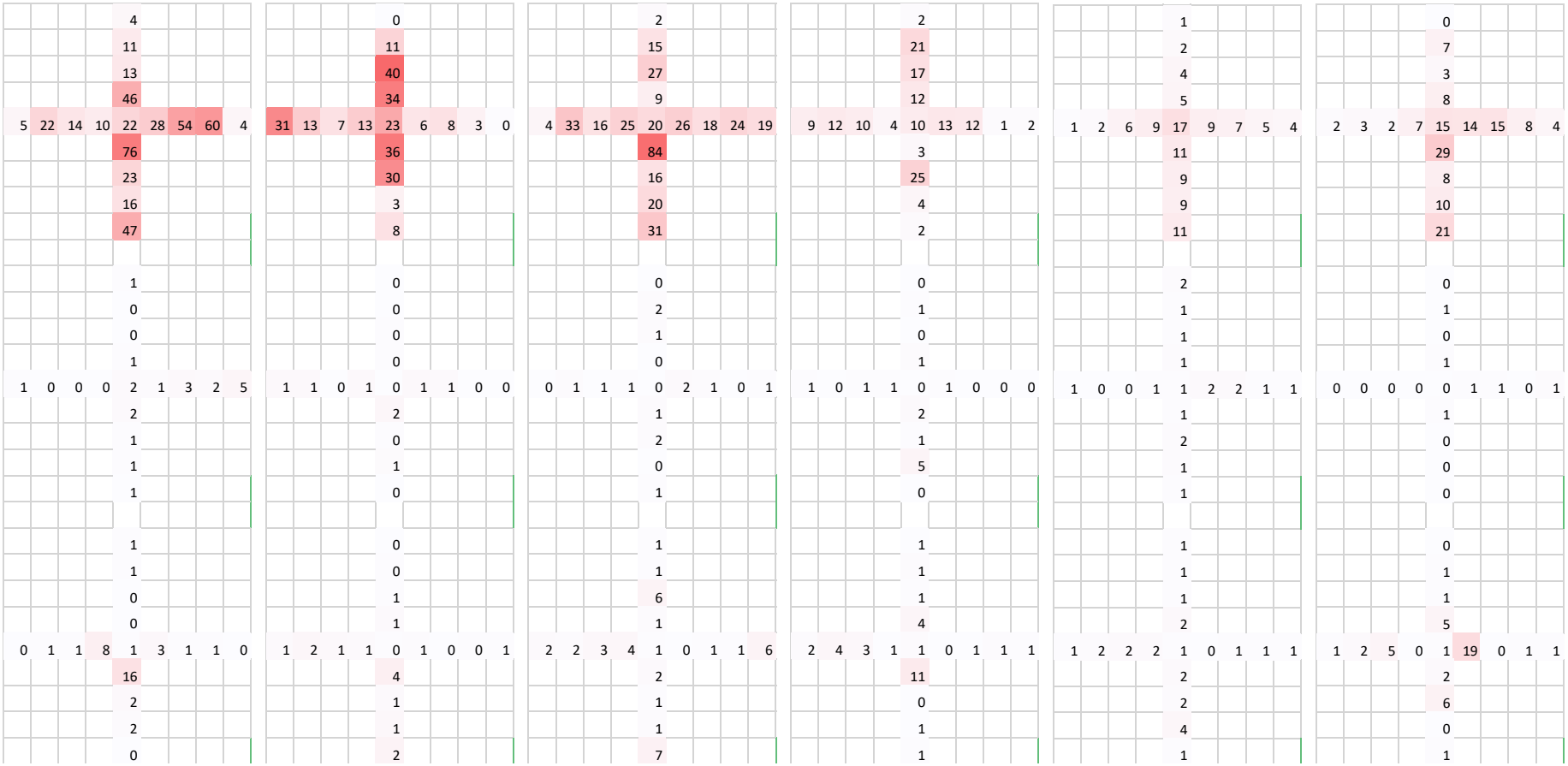

Figure 33 - measured DCD concentrations (mg/kg soil) for 60 mL DCD applications on Site 3. Top to bottom: 0-2 cm, 2-5 cm, 5-10 cm.