Effect of a Catch Crop to Reduce Nitrate Leaching Loss Following Simulated Winter Forage Grazing

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By Peter Carey

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Te Whare Wānaka o Aoraki

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Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

The Effect of a Catch Crop to Reduce Nitrate Leaching Loss Following Simulated Winter Forage Grazing

by

P.L. Carey

The growing of high yielding forages for dairy winter grazing is a common pastoral farm management practice in the temperate regions of New Zealand. The high stocking rates during winter forage grazing means that once the forage is grazed large volumes of urine are deposited. The aim of this research was to determine the effects of using a catch crop to sequester N following simulated winter forage grazing and to reduce nitrate leaching losses.

Two lysimeter trials and one growth chamber experiment were conducted at Lincoln University to measure the effect of catch crops in reducing nitrate leaching loss from a Balmoral stony silt loam (NZ classification: Acidic Orthic Brown soil). In year 1, a comparative lysimeter study was conducted between two potential catch crops, oats (*Avena Sativa* L.) and Italian (It.) ryegrass (*Lolium multiflorum* L.), that were sown at the recommended dates. Urine labelled with ^{15}N was applied at 350 and 700 kg N ha^{-1} in late June and the oats and It. ryegrass were sown 7 and 11 weeks later, respectively. The effect of the nitrification inhibitor, dicyandiamide (DCD), on reducing nitrate leaching after urine application was also investigated for each catch crop at the 350 kg N ha^{-1} rate only. In year 2, another lysimeter study was conducted to determine the effect of the most effective catch crop in Experiment #1, oats, to capture N and reduce nitrate leaching from urine application at different times over winter (early June, early July and late July). This 2nd lysimeter experiment also determined the effects of increasing the interval between urine applications and sowing date of the oats (1-63 days after application).

In year 1, only 3-4% of the urinary-N applied was captured by the catch crops (non-DCD) indicating that both were probably sown too late to make any significant impact on N uptake to reduce nitrate

leaching. However, the cool season activity of the oats increased evapotranspiration compared to the lt. ryegrass, reducing drainage and thus nitrate leaching, by 22% and 25%, respectively, over the winter-spring period (Jun-Nov). Application of DCD increased N uptake in the oats three-fold (~13% of N applied) and reduced nitrate leaching losses by over 60%.

In year 2, nitrate leaching losses from a series of winter urine applications were reduced by around a third (~34%; range 19-49%) after the sowing of an oats catch crop compared to the fallow treatments. Part of this success was likely due to a warmer and drier winter than normal but the size of the decrease suggests that there is significant potential to mitigate nitrate leaching in these low-cost winter feed systems whilst improving N-use efficiency and DM production. Later sowings of oats leached up to 25% more nitrate than the earliest sowing with earlier sowing of the oats increasing N uptake over later sowings by up to a third. Later winter urine applications had lower overall nitrate leaching losses (range 170-270 kg N ha-1). Calculations for paddock N loss indicate a potential reduction in nitrate leaching overall of ~30% from 88 to 62 kg N ha^{-1}.

In year 3, a growth chamber experiment was established to investigate in more detail the main factors controlling oats development and N uptake using ^{15}N-labelled urine. Two growth chambers set at 6°C (mean winter) and 10°C (mean spring), with two lighting levels (mid-winter, 5 MJ m^2 day^{-1} and early-spring, 10 MJ m^2 day^{-1}) each were set up to examine the soil temperature-by-light intensity interaction on oats development. Sowing oats in the 10°C and 6°C chambers reduced nitrate leaching losses, on average, by around three-quarters and one-third, respectively. Results indicated a strong interaction between temperature and light intensity at 10°C on oats development but no direct effect of light at 6°C. Nitrogen uptake by the oats treatments in the 10°C chamber was almost complete after 45 days from sowing, with soil mineral-N concentrations reduced close to zero. However, indirectly, increased evaporative loss under the spring lighting and a slower rate of nitrification at 6°C meant drainage losses of nitrate were similar for both 6 ° and 10°C oats treatments, 90 days after urine application. Where no oats were sown, nitrate leaching losses were large, particularly under the 10°C fallow treatments.

This research programme has discovered that oats have considerable potential to reduce winter forage nitrate leaching losses but they need to be established early (sown within 3-4 weeks after urine application). Cool soil temperatures are not an impediment to early establishment of oats.

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Chapter 1 General Introduction

"A concise overview of the main issues and problems around the anthropogenic effects from excess nitrate leaching within agricultural terrestrial systems and identification of gaps in the literature relating to methods to mitigate nitrate leaching loss in the New Zealand winter forage grazing system."

Although the plant-available mineral nitrogen (N) comprises only 1% of total terrestrial N it is this form of N that presents the most threat to human and livestock health and to the wider environment, largely through losses from anthropogenic activities (Haynes 1986f; Cameron et al. 2013). Generally, fluxes of mineral-N entering or leaving a natural terrestrial ecosystem are small compared to those cycling within the vegetation such that the N-cycle remains relatively closed. This contrasts, however, with the open nature of agricultural systems where there is considerable disturbance through product removal and from N losses associated with runoff, leaching and gaseous emissions. The N fluxes in agricultural systems, consequently, may be several orders of magnitude greater and the potential for losses to affect sensitive environments and/or contaminate essential ecosystem services is considerably heightened. The World Health Organisation recommends that nitrate (NO_3-N) concentrations, in drinking water, do not exceed 11.3 mg N L^{-1} to protect human health (WHO 2011) but elevated N concentrations in freshwater can also have detrimental effects on aquatic life and/or cause excessive aquatic plant growth and algal blooms, leading to eutrophication (Jenkinson 2001; Di & Cameron 2002a; Monaghan et al. 2007a; Smith & Schindler 2009; Cameron et al. 2013).

With human population exceeding 6 billion and currently consuming ~25 million tonnes of protein nitrogen annually there is considerable pressure to increase farm productivity with demand projected to exceed 45 million tonnes by 2050 (Jenkinson 2001). Intensifying farm production, however, increases reliance on imported fertilisers, particularly N, placing pressure on farm management to increase N-use efficiency and reduce N losses. Animal grazing systems may have a greater risk of N leaching loss than arable systems due to the imposition of discrete zones of high N concentration from deposited excreta (e.g. urine patches) (Di & Cameron 2002a; Selbie et al. 2015). Developing mitigation strategies and technologies to improve N cycle efficiency in soils are needed to minimise environmental damage and ultimately improve the productivity and sustainability of New Zealand agriculture, and grazing systems in particular (Monaghan et al. 2007a).

Animal grazing systems using winter forage grazing (WFG) can heighten the potential for nutrient loss as gaseous and leaching losses of N increase disproportionately because of high stocking rates and increasing fertiliser-N use (Monaghan et al. 2007a). The use of wintering forages to build

pregnant cow body score is a common farm management practice but leaching losses of nitrogen from such systems has been less well known till recently. Monaghan et al. (2007b) identified that the wintering component of dairy systems can represent as much as 60% of the farm's annual N leaching but from typically less than 15% of the grazing platform area. One means of reducing this N loss could be the use of a 'catch crop' sown following grazing. However, there is insufficient knowledge about whether plant establishment and growth can occur early enough to reduce nitrate leaching at a time of generally low plant activity and N uptake.

The use of the nitrification inhibitor dicyandiamide (DCD) to reduce NO_3^--N leaching losses from urine spots has been well documented e.g. (Di & Cameron 2002b; Di & Cameron 2005; Di & Cameron 2007; Monaghan et al. 2009; Selbie et al. 2015) but winter forage grazing systems represent a new challenge to reduce N losses from an intense event that occurs over the peak drainage period. Using catch crops plus the use of a nitrification inhibitor could be an effective combination to prevent N leaching loss and improve N use efficiency.

The main objectives of this research programme will be to determine the effectiveness of using catch crops following winter forage grazing to reduce nitrate leaching loss and to examine the interaction between urine application, drainage and timing of catch crop establishment. A second aim will be to determine the effects of using a nitrification inhibitor in addition to catch crops to reduce nitrate leaching

Chapter 2 Literature Review

"A review of established and current scientific literature that informs and explicates the principal issues and concerns around anthropogenic effects on N fluxes, cycling and losses within agricultural terrestrial systems and specifically the potential for catch crops to mitigate nitrate leaching loss in the New Zealand winter forage grazing system."

2.1 Introduction

Nitrogen (N) is one of the more ubiquitous elements in nature and its compounds are found in great quantities in all of earth's major spheres: lithosphere, atmosphere, hydrosphere and biosphere. As an elementary constituent of all amino acids, protein, enzymes, nucleic acids, chlorophyll and growth hormones, N is essential for the maintenance of life. Making up between 1-6% of a plant's dry-matter, N is often a limiting nutrient for plant growth (McLaren & Cameron 1990a) and its use in New Zealand, especially as industrially-produced fertiliser, has increased substantially over the last 20 years (IFA 2011).

Literature on the functions of nitrogen in the environment is so voluminous that it would not be possible to summarise all this content here so this literature review will primarily focus on the current knowledge of N use in the pastoral grazing system, description of the major N pathways, the factors that influence and mitigate N loss, and agricultural practice that can help to mitigate agronomic and environmental N losses.

2.2 The Nitrogen Cycle

2.2.1 Global distribution

The bulk of the earth's N (98%) is held in rocks and minerals of the crust and upper mantle (lithosphere) whilst the remainder is mostly found (~1.9%) in gaseous (mainly N_2) form within the atmosphere with a smaller amount (0.01%) present as dissolved N within the hydrosphere (earth water cycle) (Haynes 1986f). However, N held in the earth's crust contributes relatively little to the N cycle and mainly through volcanic emissions and crust out-gassing. Of the N in the atmosphere, more than 99% is the very stable N_2 molecule with significant, but very much minor, amounts of other N gases. Thus, in comparison with the N contained in the lithosphere and atmosphere, the total amount of N held in the biosphere is very small (~0.01%) and of this ~70% is held in seawater. For both oceans and land, most of this N is in organic forms (50% and 73%, respectively) with only around 1% of N in the terrestrial environment existing in mineral forms (Haynes 1986f).

2.2.2 Elements

Despite a very low overall proportion of N within the terrestrial environment there is great mobility between the elements that form the nitrogen cycle. This mobility is created by many processes and factors that control N availability and where N can accumulate in soil, exceeding microbial immobilisation, then plant growth can occur and life within the biosphere sustained. Nitrogen is frequently a key limiting factor for growth because soil-N contents are typically only 0.1-1% N with around 94% of this held in soil organic matter (SOM). Of this, only around 1% of N is available to plants and microorganisms, mainly as ammonium and nitrate ions (Rosswall 1976). In natural systems the amounts of N fixed from the atmosphere and received in precipitation are approximately equalled by that lost in denitrification and leaching so a quasi-equilibrium is formed (Foth & Ellis 1997). Furthermore, these amounts are generally very small compared to that cycled internally within the ecosystem. However, when natural systems are disturbed, as with cultivation in agriculture, then losses can be substantial through vegetation removal and soil disturbance, resulting in a decrease in soil-N. Whilst this can be reversed through re-establishment of vegetation, for an agricultural system to be sustainable and not severely N limited requires losses through crop and product removal to be balanced by external inputs to maintain a quasi-equilibrium state.

2.2.3 Cycling processes in pastoral agriculture

Movement of N within the N cycle and specifically in pastoral agriculture is controlled by a series of addition, loss and transformation processes (Figure 2.2-1). Additions of N to the biosphere are from three main categories; wet and dry deposition (and transformation) of ammonia and nitrogen oxide gases (collectively referred to as NO_x), biological N fixation and industrially-produced N fertiliser. Losses of N mainly occur through ammonia volatilisation, di-nitrogen (N_2) and nitrous oxide (N_2O) evolution via denitrification, leaching of mineral-N (mainly nitrate) and soluble organic-N in grasslands (Murphy et al. 2000), and from soil erosion (Gregg 2012).

The N fluxes passing through these processes are considerably enhanced in modern agriculture because of the removal of products containing large amounts of N and the need to replace these through biological fixation and/or fertiliser-N inputs to sustain the system. However, as the system is intensified and N inputs are increased, nitrogen-use efficiency (NUE) decreases and

opportunities for N losses rise. Indeed, fertiliser recovery under cropping is only about ~50% on average (Krupnik et al. 2004) and N losses under pastoral grazing systems similar (Ledgard et al. 1999). With increases in N turnover rates, the importance of the nitrification process in the agricultural paradigm becomes more critical.

Figure 2.2-1. The nitrogen cycle: Inputs, transformations and outputs (Cameron 1992).

The major inputs, outputs and transformations of N in the soil system influence the amount of N that is available for plants and transferred into the wider environment. The mineral-N in soil solution becomes the central pool from which the major pathways are drawn from, or contribute to, and can be summarised in the mineral N balance equation below (2.2-1) to represent the amount of mineral-N present in the soil (Cameron et al. 2013):

$$N = N_p + N_b + N_f + N_u + N_m - N_{pl} - N_g - N_i - N_l - N_e \quad \textbf{2.2-1}$$

Where: *p* is precipitation and dry deposition, *b* is biological fixation, *f* is fertiliser, *u* is urine and dung returns to the soil, *m* is mineralisation, *pl* is plant uptake, *g* is gaseous loss, *i* is immobilisation, *l* is leaching loss and *e* is erosion and surface runoff.

2.3 Soil Nitrogen Processes

2.3.1 Soil N transfers

With around 95% of N in soils held in organic forms, release of N for plant uptake depends on a range of processes critical for N cycling and long-term ecosystem survival. Internal transfers of N within the soil-plant system rely on plant uptake of N and its return as organic detritus, via plant senescence, and deposition of animal excreta. The detritus is broken down by the combined actions of the decomposer community consisting of fungi, bacteria, protozoa and invertebrate species. Established soil organic matter is a mix of cellular and humic materials that vary in stability but the most stable fractions may have half-lives of hundreds, if not thousands of years (Rosswall 1976; Haynes 1986f). At any one time, the amount of N held in mineral forms and microbial organisms is only ~1-2% of the total N pool with much of this largely derived from fresh organic matter inputs, particulates and soil biomass (Rosswall 1976). These may only amount to 10% of total soil-N, but their half-lives are relatively short (0.5-1.5 years) and consequently, supply the bulk of mineral-N. The larger stabilised SOM fraction (~35%) may supply around a third of mineral-N but old SOM (~50%) is relatively resistant to breakdown and contributes little to the mineral-N pool (Foth & Ellis 1997).

2.3.2 Soil-N accumulation and decomposition

In developing ecosystems, the initial substrate for plant establishment may be very harsh and unproductive so under those conditions the only major source of N is from biological fixation via free-living and symbiotic organisms that colonise the site. Once conditions allow their establishment, N-fixing plants can follow and soil-N accumulates more rapidly, although this may still require many hundreds, if not thousands, of years. Other non-N fixing plants eventually establish, and soil N approaches an equilibrium that is determined largely by the soil forming factors (climate, biota, relief, parent material and time) (Jenny 1961). Once this quasi-equilibrium is formed

(it does not remain static forever), plant species and their growth are generally limited by N availability because transfers of N in and out of the system, in comparison with the internal cycling of N, are small (Rosswall 1976).

To achieve this internal cycling relies on three inter-related decomposition processes: leaching, comminution and catabolism (Haynes 1986f). Leaching removes the more soluble N detritus components by physical action of water soon after deposition although this generally stops once the organic matter becomes incorporated into the soil. Comminution involves the physical reduction of the particle size of detritus, usually by the feeding activity of decomposer animals, increasing its surface area and importantly, making it more susceptible to microbial colonisation and attack. Catabolism comprises a series of enzyme-mediated reactions that release energy, transforming complex organic molecules to more simple ones. The enzymes are released by fungi and saprotrophic bacteria as well as those in the digestive systems of protozoa and feeding invertebrates. Some of these products will be inorganic, others might be intermediates that enter the metabolic pool of decomposer organisms and are resynthesized into more complex molecules whilst others may be incorporated into non-cellular organic matter (e.g. humus).

2.3.3 Soil N pools

Decomposition performs two important functions within ecosystems: 1) mineralisation of nutrient elements and 2) formation of SOM (Haynes 1986f). Soils might typically contain between 2500 and 7500 kg N ha^{-1} within the top 15 cm depth, corresponding to 0.1 to 0.6% of the biosphere's total N (Cameron 1992). The soil-N contained can be assigned to three main pools (Figure 2.3-1): (i) mineral N in soil solution (ammonium; nitrate and nitrite), (ii) ammonium ions held by clay minerals, and (iii) organic compounds in plant material, soil organisms, and soil humus (McLaren & Cameron 1990a). As discussed earlier, N held in organic compounds is mostly unavailable to plants directly so plant N uptake relies on transformations of organic material through decomposition and mineralisation to release ammonium. Although plants can take up ammonium it is usually nitrified to nitrate relatively quickly and thus, the bulk of N uptake is generally as nitrate (Haynes 1986c).

Figure 2.3-1. Soil-N breakdown and approximate fraction size (McLaren & Cameron 1990a).

2.3.4 Mineralisation and immobilisation

Mineralisation-immobilisation are two inextricably linked aspects of the final step in the catabolism decomposition process; namely the metabolising of simple organic-N compounds to ammonium (ammonification) and the release of energy for anabolic activity to build microbial cells and their components. Because the latter activity requires the products of mineralisation, namely inorganic-N (NH_4^+ or NO_3^-) and/or the simple organic-N molecule precursors, a proportion of mineralised N is always immobilised due to micro-organism demands and therefore, plant N uptake depends on mineralisation exceeding immobilisation. Both these processes occur simultaneously (Cameron 1992) and net mineralisation relies on a sufficient concentration of N present in the decomposing material (~3% of DM) and a low C:N ratio. Generally, if the latter is less than 25:1, net mineralisation occurs whilst for a ratio greater than 25:1, net immobilisation occurs (Haynes 1986d; McLaren & Cameron 1990a). Because net mineralisation rates are affected by a range of soil-N processes and effects, it can be difficult to extrapolate the likely net rate or outcome from one site or set of circumstances to another. Indeed, over the short-term, net immobilisation may be as likely to occur

as net mineralisation because the soil is heterogeneous by nature and zones of high and low nutrient status exist together, strongly affecting the intensity of N cycling.

This process of continuous transfer between mineralisation and assimilation of N by the soil microbial biomass (SMB) and immobilisation and release back into the mineral pool is referred to as “Mineralisation-Immobilisation-Turnover” (MIT) (Jansson & Persson 1982). It assumes that the bulk of immobilised-N occurs from the mineral-N pool, specifically as ammonium although nitrate can be assimilated in the presence of a readily-available C source (Recous et al. 1988). However, some direct immobilisation of small molecular weight organic-N compounds, such as amino acids, also occurs at the microsite scale (“direct hypothesis”) where the excess in SMB requirement is released as ammonium (Drury et al. 1991; Iyyemperumal et al. 2007). The mineralisation/immobilisation processes are not mutually exclusive and can run concurrently, emphasizing the heterogeneity of soils and microbial biomass (Iyyemperumal et al. 2007). More recent models that aim to calculate N mineralisation and the likely N available for a crop now allow for both processes to run in parallel (Manzoni et al. 2008).

Nitrogen mineralisation rates are affected by a range of soil factors notably soil temperature, soil moisture and soil texture (Haynes 1986d; Andersen & Jensen 2001; Dessureault-Rompré et al. 2011), the forms, amounts and quality of C and N (Barrett & Burke 2000; Bengtsson et al. 2003; Schütt et al. 2014), cultivation (Silgram & Shepherd 1999), land-use intensity (Stempfhuber et al. 2014), microbial factors (Hamer et al. 2008), and contaminants such as heavy metals (HM) (Dai et al. 2004). Generally rates increase with optimal soil moisture (~field capacity) and in warmer soils whilst HM content is negatively correlated with N mineralisation (Haynes 1986b; Sauvé et al. 1999). Soil pH seems less influential on organic-N decomposition and N mineralisation rates (Aciego Pietri & Brookes 2008) but more critical to nitrification rates (Haynes 1986b).

2.3.5 Nitrification

Nitrification is the biological oxidation of ammonium (NH_4^+) to nitrate (NO_3^-) and is driven in the soil by the activity of autotrophic bacteria that derive their energy solely from these oxidation reactions. The oxidation occurs in two steps: ammonium (NH_4^+) is converted to nitrite (NO_2^-) (2.2-1), and NO_2^-

is further oxidised to NO_3^- (2.3-2). The second step of the reaction is generally rapid and nitrite seldom accumulates in soil.

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2 + 2H_2O + 4H^+ + energy$$

2.3-1

$$2NO_2^- + O_2 \rightarrow 2NO_3 + energy$$

2.3-2

Up to five genera of autotrophs are known to be able to oxidise NH_4^+ to NO_2^-: *Nitrosomonas, Nitrosospira, Nitrosococcus, Nitrosolobus and Nitrosovirio* but only one genus, *Nitrobacter*, is known to oxidise NO_2^- to NO_3^-. Generally, *Nitrosomonas* and *Nitrosospira* are the most commonly represented in agricultural soils but *Nitrosomonas* is the more dominant in manured agricultural land (Haynes 1986b). As only one genus of autotrophs is involved in the second oxidative process, the conversion of nitrite-to-nitrate is generally more strongly influenced by external factors such as moisture, soil pH and temperature. The more diverse heterotrophic biomass responsible for the oxidation of ammonium is less influenced and able to cope with a wider range of soil conditions (Haynes 1986b). Recent soil and molecular biology research and phylogenic analysis has shown that in many New Zealand dairy pasture soils the dominant ammonia oxidising bacteria are *Nitrosospira* species, rather than the *Nitrosomonas* species (Di et al. 2009b).

As can be seen in equations 2.3-1 and 2.3-2, two moles of hydrogen ions are produced for every mole of ammonium ions converted to nitrate. However, if the ammonium ion is sourced from the mineralisation of SOM then a proton is consumed in the ammonification process and the net effect is one proton produced per molecule of nitrate produced. Uptake of nitrate by plants tends to counteract this effect, as for every NO_3^- ion taken up by the roots a hydroxide (OH^-) or bicarbonate (HCO_3^-) ion is released. Where nitrate accumulates and downwards movement through drainage occurs, then there is usually an associated loss of cations (Ca^{2+}, Mg^{2+}, K^+ and Na^+) as accompanying counter-ions. Thus, nitrification and nitrate leaching can have a net acidifying effect on soil and can be a major cause of declining soil pH and base cation saturation (Haynes 1986b).

2.3.6 Factors affecting nitrification

Rates of nitrification are sensitive to several factors, notably soil moisture content, pH, temperature and soil nutrient status; consequently, there are optimal ranges for most of these. Maximum rates of ammonium oxidation occur when soil moisture content is around 'field capacity' (i.e. ~-10 kPa soil matric potential) (Haynes 1986b) and generally decline when approaching saturation or beyond permanent wilting point (-1500 kPa; Figure 2.3-2) (Malhi & McGill 1982; Monaghan & Barraclough 1992). This is primarily because increasing soil wetness is associated with decreasing oxygen availability from reduced aeration and slowing gas diffusion. Conversely, increased aeration increases soil nitrification rates, especially in arable soils where cultivation exposes SOM to oxidative attack (Haynes 1986b).

Many studies have shown optimal soil pH for nitrifying bacteria lies generally between 4.5 and 7.5 (Table 2.3-1) where the inhibitory effects of Al toxicity are least (Haynes 1986b; Aciego Pietri & Brookes 2008). Many soil processes produce surpluses of H^+ ions and have net acidification effects on soils (Thomas & Hargrove 1984), including nitrification; consequently, there is a feedback effect on nitrification rates as soil pH and fertility decreases. Similarly, the optimal soil temperature range for nitrifying bacteria lies between 20°C and 30°C (Figure 2.3-2) (Malhi & McGill 1982; Haynes 1986b). Nitrification may still occur at soil temperatures below 5° C but the rates may be considerably slower, especially for more recalcitrant SOM (Sims 1986; Andersen & Jensen 2001).

In situations where the supply of surplus ammonium is low, then most products of the mineralisation cycle may be assimilated as amino acids, rather than ammonium ions. This can lead to a nitrification inhibition effect because of low nitrifier population numbers present (Haynes 1986b). Conversely, large concentrations of ammonium can inhibit *Nitrobacter* activity through a toxicity effect of ammonia at high pH and/or an increase in soil salinity with increasing rates of ammonium addition (Monaghan & Barraclough 1992). Similarly, increasing soil heavy metal concentrations, whether from industrial contamination or from long accumulations of sewage sludge etc., can also affect *Nitrobacter* activity and inhibit nitrification (Haynes 1986b; Sauvé et al. 1999). The sensitivity of nitrifiers to HMs varies considerably (Figure 2.3-3) but generally *Nitrobacter* are affected more that ammonia oxidisers (i.e. *Nitrospira*) and potentially an accumulation of NO_2^- could occur. However, many variables such as clay sorption, SOM complexation and soil pH can affect soil

solution HM concentrations whilst bacterial adaptation to HM concentrations will also affect nitrification rates (Haynes 1986b).

Table 2.3-1. Net NH_4^+-N, NO_3^--N and net total inorganic N (NH_4^+-N + NO_3^-N) concentrations in 2M KCl soil extracts from an arable Hoosfield soil acidity gradient strip incubated for 50 days with L (+)-arginine (Aciego Pietri & Brookes 2008).

Figure 2.3-2. Effect of soil temperature and matric potential (soil moisture content) on soil nitrification rates of added ammonium (Haynes 1986b).

Differences in soil type have also been shown to affect nitrification rates (Monaghan & Barraclough 1997; Decau et al. 2003) although some of these differences may relate to soil conditions created by differences in moisture regime, texture or management (Monaghan & Barraclough 1995). For example, banding of ammonium or urea fertilisers can create high local concentrations of ammonia that inhibit *Nitrobacter* bacteria whilst soil environments with very low inputs of ammonium may have few nitrifying organisms present and/or have other nutrient deficiencies that inhibit nitrification (Haynes 1986b). The latter situation, however, is unlikely to occur in most agricultural soils.

Autotrophic bacteria involved in nitrification processes are some of the most sensitive soil micro-organisms to soil-applied agrochemicals (e.g. fumigants, insecticides, herbicides, fungicides), especially fumigants due to their potent nitrification inhibiting effects. However, at normal application rates, insecticides, herbicides and fungicides are unlikely to majorly effect nitrification (Goring & Laskowski 1982).

Figure 2.3-3. Relationship between increasing DTPA-extractable heavy metals and percentage inhibition of nitrate production in a silt loam (Haynes 1986b).

2.4 Nitrogen Losses from Soils

2.4.1 Ammonia volatilisation

Agricultural sector emissions of ammonia represent a major nitrogen loss in the New Zealand pastoral system with a number of associated downside effects via soil acidification and eutrophication of aquatic systems (Saggar et al. 2004a). Ammonia volatilisation is commonly used to refer to the process by which gaseous NH_3 is emitted from the soil surface to the atmosphere and is a complex process involving physical, chemical and biological factors. A supply of free ammonia (i.e. $NH_{3\ (g)}$ + $NH_{3\ (aq)}$) near the soil surface is a prerequisite to gaseous loss and is favoured by high pH via the reaction between ammonium and hydroxide ions (2.4-1).

$$NH_4^+ + OH^- \rightleftarrows NH_3 \uparrow + H_2O$$

2.4-1

Major ammonium sources include animal urine and faeces, farm effluent, organic residues and native SOM but also ammonium (NH_4^+)-incorporated fertilisers (e.g. NH_4NO_3, NH_4Cl, $(NH_4)_2SO_4$, and $(NH_4)_2HPO_4$,) and urea ($(NH_2)_2CO$). Urea (fertiliser or urine) undergoes hydrolysis readily in soils (2.4-2), catalysed by the enzyme *urease* to form ammonium carbonate $(NH_4)_2CO_3$:

$$(NH_2)_2CO + 2H_2O \rightarrow (NH_4)_2CO_3$$

2.4-2

The hydrolysis reaction in soil is often rapid and zero order (Vlek & Carter 1983), with half-lives of urine-urea measured commonly in hours. Sherlock and Goh (1984) calculated half-lives for urea in urine of 3.0 and 4.7 hours for summer and autumn in New Zealand, respectively, the higher value attributable to lower soil temperatures. The release of ammonium ions after hydrolysis allows interaction with those on the cation exchange complex, resulting in electrostatic binding to soil colloids and equilibrium reactions with NH_4^+ ions in soil solution. The cation exchange capacity (CEC) of the soil buffers to some extent, this increase, and any associated rise in soil pH. Consequently, high cation exchange capacity (CEC) soils (e.g. clay loam) tend to have lower NH_4^+ solution concentrations than soils with a low CEC soil (e.g. sandy loam).

Soil pH has a significant influence on ammonia volatilisation and soils with naturally high pH (>7; e.g. calcareous soils) are capable of losing significant amounts of ammonia gas (Bishop & Manning 2011). However, neutral or acid soils can also lose significant amounts via the reaction shown in equation 2.4-3 due to the sharp increase in pH from the reaction of the carbonate ion with water. Localised zones of high pH (>8) form close to the site of hydrolysis and these favour production of ammonia gas (Figure 2.4-1) and thus, increased volatilisation (Sherlock & Goh 1984; Black et al. 1987; Sherlock et al. 1995; Sommer et al. 2004). Indeed, Black et al. (1985b) found there was a strong positive relationship between volatilisation and maximum surface pH achieved by the fertiliser used. With the onset of nitrification processes, soil pH eventually decreases, reducing the volatilisation rate.

$$(NH_4)_2CO_3 + H_2O \rightleftarrows 2NH_4^+ + HCO_3^- + OH^- \rightleftarrows NH_4^+ + NH_3 \uparrow + H_2O + CO_2$$

2.4-3

Figure 2.4-1. Ammonia volatilisation loss and soil pH around a broadcast urea granule (McLaren & Cameron 1990a).

The conversion of NH_4^+ to NH_3 regulates the potential loss of NH_3 to the atmosphere through the various equilibria involved in the transfer of ammonium held on the exchange complex through soil

solution, release into the soil atmosphere, and diffusion away from the soil surface. Generally, the higher the concentration of ammonium in soil solution, the greater the potential ammonia emission rate. Rising air and soil temperature, windspeed, soil moisture (rapid hydrolysis), roughness (increased turbulence), and porosity (gas diffusion rate) are all environmental and soil factors shown to increase ammonia volatilisation rates (Vlek & Carter 1983; Black et al. 1987; Sommer & Olesen 1991; Sherlock et al. 1995; Sommer et al. 2004). Conversely, mitigation methods to reduce ammonia losses include transporting urea to depth via rainfall or irrigation soon after fertiliser application (Sherlock & Goh 1984), incorporation below the surface (Sommer et al. 2004), and the use of urease inhibitors such as N-(n-butyl) thiophosphoric triamide (NBPT) to coat urea granules (Watson et al. 1994). The latter has been shown to reduce NH_3 volatilisation by up to 95%. Although dry soils slow the initial hydrolysis of solid urea fertilisers over the short-term, losses over the long-term may be similar if the NH_4^+ ions remain largely on or near the soil surface (Sherlock et al. 1995).

Volatilisation losses from N fertilisers or animal excreta applied to soils can vary widely depending on the amount, form, concentration and degree of incorporation. Bishop and Manning (2011) recently summarised published results on urea fertiliser volatilisation losses for NZ, the most commonly used N fertiliser, for both pastoral and arable cropping. Mean N volatilisation losses for acid soils were 20±10% for grasslands (15-500 kg N ha^{-1} applied) and 14±7% (46-200 kg N ha^{-1} applied) for arable cropping. These are comparable with losses reported in New Zealand of typically 5-15% of the N applied where urea application rates are <50 kg N ha^{-1} (Black et al. 1985b; Haynes & Williams 1993; Di & Cameron 2004b). Volatilisation losses from dung deposited whilst grazing are regarded as less significant than those from urine due to the fewer applications and smaller area covered by dung, their slower decomposition and the majority of N occurring in less mineralisable forms (Haynes & Williams 1993). Where excreta storage is required (common in European systems), volatilisation losses of the ammoniacal-N present can be significant (19-100%) (Sommer & Olesen 1991) but these same wastes applied to pastures can also incur large losses, although this is very dependent on the waste type and ammoniacal-N present (Bolan et al. 2004).

In the pastoral system the grazing animal is estimated to return up to 85-90% of the ingested N in urine and dung (Cameron et al. 2013). Of this amount, it has been observed that sheep and dairy

cattle excrete 70–75% and 60–65% of N in urine, respectively, when grazing N-rich grass/legume pastures (Oenema et al. 1998). Whilst the majority of the N present in both dung and urine is in organic forms, typically 70% of the N in urine is present as easily hydrolysable urea and the rate of N application may be as high as 1000 kg N ha^{-1} in a urine spot (Oenema et al. 1998; Di & Cameron 2002a). Ammonia volatilization losses from urine, consequently, can range from 2-46% and commonly 15-25% of the N present, with losses typically greatest in the first few weeks after application (Haynes & Williams 1993). Losses are favoured under hot, dry summer conditions but minimised under cooler, moist winter conditions (Haynes & Williams 1993; Di et al. 2002). Sherlock and Goh (1984) reported losses of 22-25% over summer/autumn in New Zealand but only 12% in winter, whilst Di et al. (2002) found volatilisation losses from an autumn urine application of only 2% of total-N. Total volatilisation losses for pastoral grazing systems in New Zealand have been shown to range from 15-68 kg N ha^{-1} for intensive dairying systems (Ledgard et al. 1999) and around ~13 kg N ha^{-1} for hill country sheep pastures (Haynes & Williams 1993).

2.4.2 Gaseous N_2 and NO_x emissions

A characteristic of intensively managed pastoral grazing systems is a surplus of N once plants, micro-organisms and soils can no longer assimilate or retain the excess N. This surplus is lost via two main ways, by nitrate leaching and through gaseous N emissions. Nitrogen gas emissions as ammonia (section 2.4.1), dinitrogen (N_2), nitric oxide (NO) and nitrous oxide (N_2O) gases can all represent a significant loss of N from the soil/plant system but emissions of N_2O particularly represent a major contributor to climate change due to N_2O's large radiative-forcing potential. This potential means that N_2O has about 300-times the warming potential of carbon dioxide (CO_2). About half of New Zealand's agricultural greenhouse gas (GHG) inventory is from agriculture (Figure 2.4-2) and is dominated (~90%) by emissions of N_2O and methane (Ministry for the Environment 2013a). Nitrous oxide makes up about 16% (on a CO_2-equivalent basis) of New Zealand's greenhouse gas emissions (Saggar et al. 2002) consequently, efforts to reduce this contribution have dominated New Zealand's efforts in GHG-soils research (Bolan et al. 2004; Saggar et al. 2004a; Cameron et al. 2013; Saggar et al. 2013).

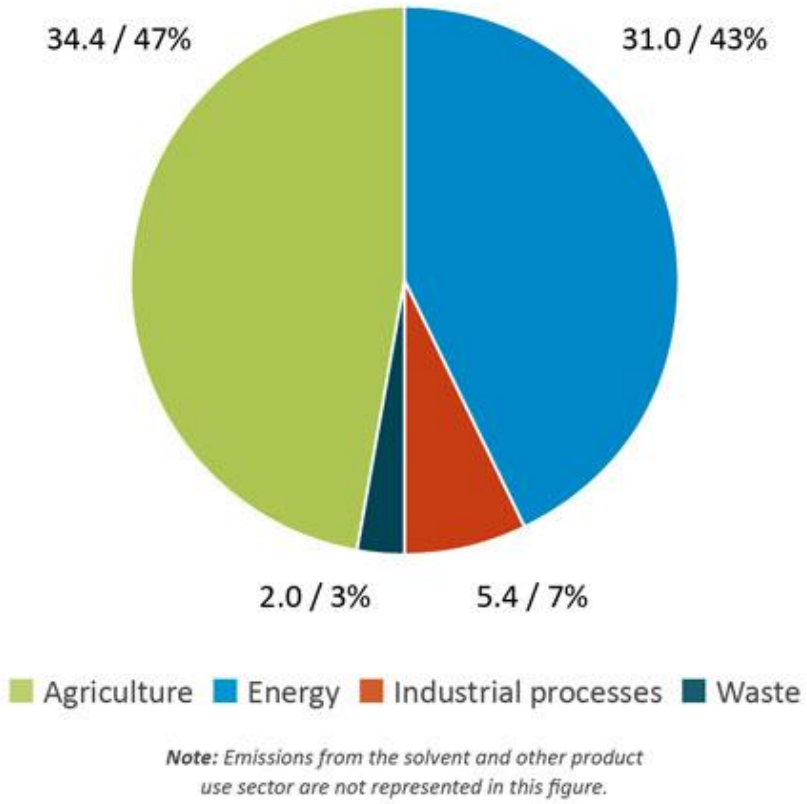


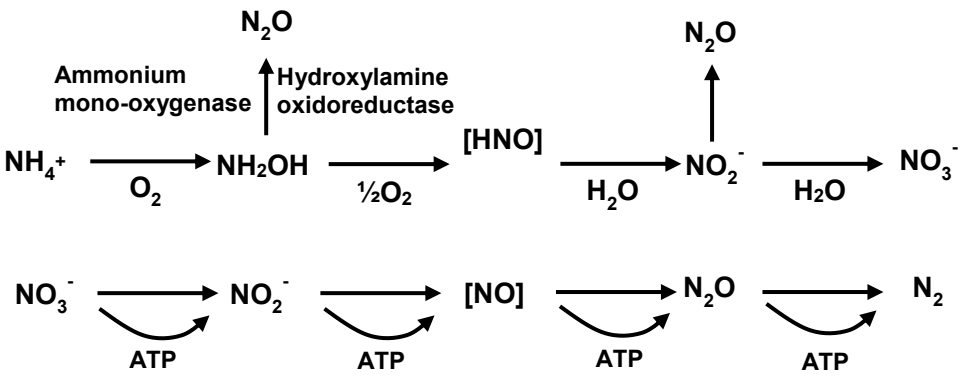
Figure 2.4-2. New Zealand's greenhouse gas emissions (by sector, in million tonnes of CO2 equivalent) in 2011 (Ministry for the Environment 2013b).

Emission processes

Gaseous nitrogenous emissions are produced by three groups of organisms: dissimilatory denitrifying bacteria (i.e. the products are not assimilated), non-denitrifying fermentative bacteria and fungi, and autotrophic nitrifying bacteria (Haynes & Sherlock 1986). In anaerobic conditions, denitrifying bacteria are thought to be the most important organisms contributing to gaseous N losses (Payne 1981; Firestone 1982) but in oxidative environments nitrifying bacteria may be the biggest source of nitrogenous gas loss and an equal contributor to total N_2O loss (Carter 2007). Whilst the process of nitrification has already been discussed, the mechanism described in equation 2.4-3 can be expanded (equation 2.4-4) to show that the NH_4^+-oxidising bacteria *Nitrosomonas, Nitrosospira* and *Nitrosolobus* have the capacity to produce N_2O from NH_4^+ or hydroxylamine (an intermediate in the oxidation of NH_4^+ to NO_2^-) under most conditions (Haynes & Sherlock 1986). It is also thought that N_2O is evolved from a second intermediate compound,

nitroxyl (HNO), dismutating under low O_2 concentrations, or from the nitrate reductase enzyme using NO_2^- as the terminal electron acceptor instead of O_2 when the latter is in low concentrations during metabolic processes (Schmidt 1982; Haynes & Sherlock 1986). Nitric oxide (NO) may be evolved in this step and preferred, as the O_2 concentration in the medium decreases (Lipschultz et al. 1981).

Denitrification is a major biologically-mediated process in agricultural soils, occurring mostly in poorly drained soils where both low O_2 availability and low redox conditions (<320 mV) are present. Essentially the last step in the N cycle, where fixed N is returned to the atmospheric pool of N_2, biological denitrification (2.4-4) is defined as the dissimilatory reduction of NO_3^- or NO_2^- by essentially anaerobic bacteria producing molecular N_2 or oxides of N when O_2 is limiting (Payne 1981). These respiratory denitrifiers gain energy by coupling N-oxide reduction to electron transport phosphorylation (i.e. ATP generation) and are present in nearly all soils (Payne 1981). The main genera capable of denitrification comprise mainly facultative anaerobes (i.e. normally aerobic but can switch to anaerobic respiration or fermentation if O_2 is absent) and include *Pseudomonas, Bacillus, Alculigenes* and *Flavobacterium* genera (Payne 1981; Firestone 1982).



2.4-4

Denitrification as shown in 2.4-4 (adapted from Saggar et al. (2004a)) is mediated by a series of enzymes that requires a supply of free electrons at each stage for complete reduction to N_2 (+5→0). The supply of these electrons in soil environments is usually soil organic matter and can be described in the following stoichiometric equation (2.4-5):

$$5(CH_2O) + 4NO_3^- + 4H^+ \rightarrow 2N_2 + 5CO_2 + 7H_2O$$

2.4-5

Many soil bacteria seem capable of denitrification or at least partial denitrification (i.e. they can mediate at least one step in the process) but exhibit a variety of reduction pathways and differing end-products, some producing N_2 only while others produce a mixture of N_2O and N_2. Consequently, varying ratios of N_2O/N_2 are produced depending on soil conditions, the organisms involved and the substrate used (Bolan et al. 2004) although the relative proportion of N_2O/N_2 evolved during denitrification generally increases as the soil becomes more aerobic (Firestone 1982). Nitric oxide (NO) can also be emitted in the denitrification process (2.4-4) but the amounts are generally low compared to the quantities of N_2O that can be emitted under field conditions and before it can be converted to N_2 (Cameron et al. 2013).

Dissimilatory reduction of nitrate to ammonium (DNRA) is also recognised as another part of the denitrification process that occurs after the dissimilatory reduction step of nitrate to nitrite (i.e. $NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+$) but the diversity of enzymes and organisms that can carry out this process means that the process is less well characterised than for respiratory denitrification (Tiejde 1988). Generally, however, it is a less favourable process due to less ATP production and thus, less important in discussing losses from pastoral grazing systems.

A range of other contributing processes can also be implicated in gaseous N emissions. Chemodenitrification is a term commonly used to describe various chemical reactions of NO_2^- ions within soils that result in emission of a variety of nitrogenous gases (e.g. N_2, NO, NO_2 and N_2O) but aren't biological in origin. These reactions only occur where there is a build-up of nitrite and thus, don't normally occur in well-aerated, unfertilised soil environments where the rate of $NO_2^- \rightarrow NO_3^-$ conversion is faster than the $NH_4^+ \rightarrow NO_2^-$ step. However, if conditions produce high localised concentrations of NH_4^+ ions and high pH from, for example, the banding of ammonium-producing fertilisers or within urine patches, then activity of the oxidiser *Nitrobacter* can be inhibited leading to high concentrations of nitrite. In the periphery between the acid soil environment and the localised nitrite concentration, the nitrite ion may be unstable and gaseous N emissions may occur as a result (Haynes & Sherlock 1986). Fungal denitrification may also be implicated in gaseous N loss. Most fungal-denitrifying activity reduces only $NO_2^- \rightarrow N_2O$ but some fungi can reduce both NO_2^- and NO_3^- (Shoun & Tanimoto 1991). In wet soils, the anaerobic oxidation of NH_4^+ directly to N_2O or N_2, called 'anammox', has been identified as another possible process for gaseous N loss

although the fungi responsible and the anammox reaction have not been identified together (Hayatsu et al. 2008). Its importance in temperate pastoral systems, therefore, may be slight.

In recent years, research identifying the genes in microorganisms that encode specific denitrification enzymes has enabled greater differentiation between denitrifier communities and their relative contribution to N_2O production (Saggar et al. 2013). The relative importance of both nitrification and denitrification to gaseous-N emissions, and N_2O in particular, will depend on the system and the soil condition but experimental evidence shows that in NZ pastoral systems where the main inputs of N are from urine, and water filled pore space is less than 60% of maximum, then nitrification contributes little to gaseous-N emissions and the primary source is denitrification (Saggar et al. 2002). In summary, denitrification requires four main requirements to proceed:

1. A readily-available carbon supply
2. The presence of soil microorganisms possessing the necessary metabolic capacity
3. Anaerobic conditions and/or a reduced O_2 supply, and
4. A supply of N oxides (usually nitrate) to act as terminal electron acceptors.

Factors affecting denitrification

Environmental factors that affect denitrification can be divided into two types: proximal regulators - those that affect denitrification rates almost immediately, leading to instantaneous changes in denitrification rates; and distal regulators – those that affect denitrification rates over wider scales of distance and time as influenced by land management and soil factors (Saggar et al. 2013). Proximal regulators include nitrate supply, carbon availability, O_2 concentration and temperature whilst distal regulators include plant growth, soil texture, soil pH, water availability and land management practice (i.e. grazing and/or cultivation practice). The myriad of compounding factors means it is difficult to predict the effect of a combination on N_2O/N_2 ratios and denitrification rates but, generally, complete denitrification will be promoted where there is high soil water content, neutral-to-slightly basic soil pH, high soil temperature, low rates of O_2 diffusion and a presence of labile C (Saggar et al. 2013).

Range of gaseous N losses

Reported gaseous N losses for both N_2O and N_2 in temperate grassland vary widely due to the range of inputs, land management, climatic zones, soil moisture and soil physical conditions that impact upon emissions (Table 2.4-1). This also means gaseous N loss can vary greatly within a single field (Velthof et al. 1996), potentially requiring many measurements to achieve the accuracy and precision to confidently up-scale values to farm and regional level. Generally, higher rates of denitrification are associated with grazed and fertilised pastures with temporal changes often related to soil moisture and temperature variables (Bolan et al. 2004). Total N losses through denitrification in unfertilised dairy-grazed pastures in New Zealand were found to be ~5 kg N ha^{-1} on an annual basis (Ledgard et al. 1999; Luo et al. 2000) but the addition of up to 400 kg urea-N ha^{-1} increased denitrification to 19 kg N ha^{-1} (Ruz-Jerez et al. 1994) and 25 kg N ha^{-1} (Ledgard et al. 1999) (about 5 and 6%, respectively, of the N applied). Saggar et al. (2002; 2004b) reported N_2O losses of 1.8 and 1.6 kg N_2O-N ha^{-1} for well and poorly drained ungrazed pastures, respectively, and 9.7 and 11.7 kg N_2O-N ha^{-1}, respectively, for the same pastures when grazed. These emissions represented on average 2.0 and 2.5% of the applied excretal and fertiliser N in these two soils, respectively, and were attributed mainly to denitrification. Gaseous losses of N_2O from the nitrification process can also occur under urine patches, and where soil moisture content is intermediate (~45% WFPS), N_2O losses from both nitrification and denitrification may be similar (Carter 2007). Losses, however, usually represent a considerably lower overall proportion of total nitrification (<0.5%) (Koops et al. 1997; Carter 2007) and under temperature pastures in New Zealand, the greatest losses seem to originate mainly from denitrification (Saggar et al. 2002).

Table 2.4-1. A range of studies measuring N_2O and total denitrification losses in temperate grasslands. Adapted from Saggar et al. (2009) and (2013).

Location	Soil texture	N treatments (kg N ha^{-1} applied)	Method	Denitrification (kg N $ha^{-1}d^{-1}$) mean (min-max)	Denitrification - % of total-N input mean (min-max)	Reference
N_2O only						
Germany	Sandy silt loam	CAN (120: 4x 30)		-	0.3	(Anger et al. 2003)
New Zealand	Stony silt loam	Cow urine (1000)		-	2.2	(Di & Cameron 2003)
New Zealand	Stony silt loam	Cow urine (1000)		-	1.9	(Di & Cameron 2006)
New Zealand	Silt loam	Cow urine (1000)		-	3.1	(Di & Cameron 2006)
New Zealand	Silt loam	Cow urine (1000)		-	2.0	(Di et al. 2007)
New Zealand	Sandy loam	Cow urine (1000)		-	0.8	(Di et al. 2007)
New Zealand	Sandy loam	Cow urine (1000)		-	0.6	(Di et al. 2007)
New Zealand	Clay	Synthetic urine (1000)		-	1.9	(Clough et al. 1998)
UK	heavy clay loam	Cow urine (700 spring)		-	0.0	(Allen et al. 1996)
UK	heavy clay loam	Cow urine (700 autumn)		-	1.5	(Allen et al. 1996)
Total						
Denmark	Loamy sand	U, ammonium soln. (529)	Soil core	0.014	-	(Carter 2007)
Germany	Loam	AN, PS, CS (450)	Chamber	0.003 (0.001-0.008)	0.32 (0.1-0.7)	(Schwarz et al. 1994)
New Zealand	Silt loam	U (500)	N balance	1.414 (0.863-1.974)	27.0 (16.4-37.3)	(Clough et al. 1995)
New Zealand	Silt loam	Natural rainfall	Soil core	0.02 (0.003-0.247)	-	(Luo et al. 2000)
New Zealand	Fine sandy loam	U (500)	Soil core	0.014 (0.005-0.031)	3.61 (2.6-4.8)	(Ruz-Jerez et al. 1994)
New Zealand	Silt loam	KN (200)	Sieved soil	1.48 (0.80-2.33)	24.7 (20.0-32.0)	(Zaman et al. 2008)
New Zealand	Silt loam	U, KN (400)	Soil core	0.0145 (0.0009-0.03)	0.59 (0.39-0.84)	(Zaman & Nguyen 2010)
New Zealand	Fine sandy loam	U, DE (1000 & 400)	N balance	-	9.6 (5.3-13.9)	(Di et al. 2002)
Netherlands	Sand, loam, peat	KN (40, 80)	Soil core	1.41 (0.03-3.6)	-	(de Klein & Van Logtestijn 1996)
Netherlands	Heavy clay	CS, KN (33-264)	Soil core	0.276	20	(van der Salm et al. 2007)
UK	Clay, clay loam	CAN, U (100, 200, 300)	Soil core	0.07 (0.004-0.216)	11.4 (3.3-26.3)	(Jordan 1989)
USA	Unknown	U (510)	Soil core	0.0008 (0.00025-0.0015)	0.002 (0.006-0.002)	(Frank & Groffman 1998)
UK	Loam over clay	AN (250, 500)	Chamber	0.038 (0.004-0.08)	5.1 (4.4-5.8)	(Ryden et al. 1984)

Fertiliser type: AN- ammonium nitrate, AS- ammonium sulphate, CAN- calcium ammonium nitrate, CS- cattle slurry, DE- dairy effluent, KN- potassium nitrate, PS- pig slurry, U- urea/urine.

2.4.3 Nitrate Leaching

Although surpluses of N in natural ecosystems are seldom large and external N transfers relatively slight, this is often not the case in highly productive soils. As the intensity of land-use increases it is inevitable that a rise in fertiliser application and N particularly, will lead to a heightened potential for nitrate losses. Whilst this is an agronomic and economic loss, it is the wider threat to the environment and human health that is of most concern (Wild & Cameron 1980; OECD 1982; Di & Cameron 2002a). Excess nitrate in water supplies can present a risk to human new-borns through the condition methaemoglobinaemia and is also linked to cancer and heart disease (Cameron et al. 2013). An increasing number of tested groundwater sites in New Zealand now show elevated nitrate concentrations (Figure 2.4-3) with a number of these worsening in recent years (Figure 2.4-4) (Ministry for the Environment 2010). Increasing nitrate in the environment is a leading cause of anthropogenic eutrophication (Smith & Schindler 2009) with nitrate from pastoral agriculture blamed as a leading contributor to decreasing water quality over the last 10-15 years in New Zealand rivers, lakes and groundwater (Hamill 2006; Daughney & Randall 2009; Ballantine et al. 2010).

Often fertiliser use is based on what is agronomically beneficial. However, N use efficiency (NUE) decreases with increasing application rates and the excess N in the system can present a harmful output to the environment (Havlin 2004). These surpluses of N under grazed grassland, from a combination of fertiliser-N, animal excreta, legume activity and cultivation, can be large and nitrate can build up well in excess of plant demand (Ledgard et al. 1999; Di & Cameron 2002a).

When N and moisture conditions in the soil exceed pasture requirements, leaching losses of N may occur via soil drainage, mainly as nitrate since the negatively charged ion (NO_3^-) is not retained in most soils. Nitrate leaching losses can be exacerbated by combinations of circumstances that produce both large nitrate concentrations and excessive drainage. For example, large N fertiliser applications on free-draining light-textured soils can produce a high potential for nitrate leaching losses whilst small N applications on high water retention soils will tend to have a lower loss potential. Using appropriate fertiliser rates and timing application with plant development will

minimise leaching losses. Similarly, optimal irrigation rates, timed to occur with plant requirements as opposed to excessive and/or ill-timed irrigation, can also reduce N leaching losses.

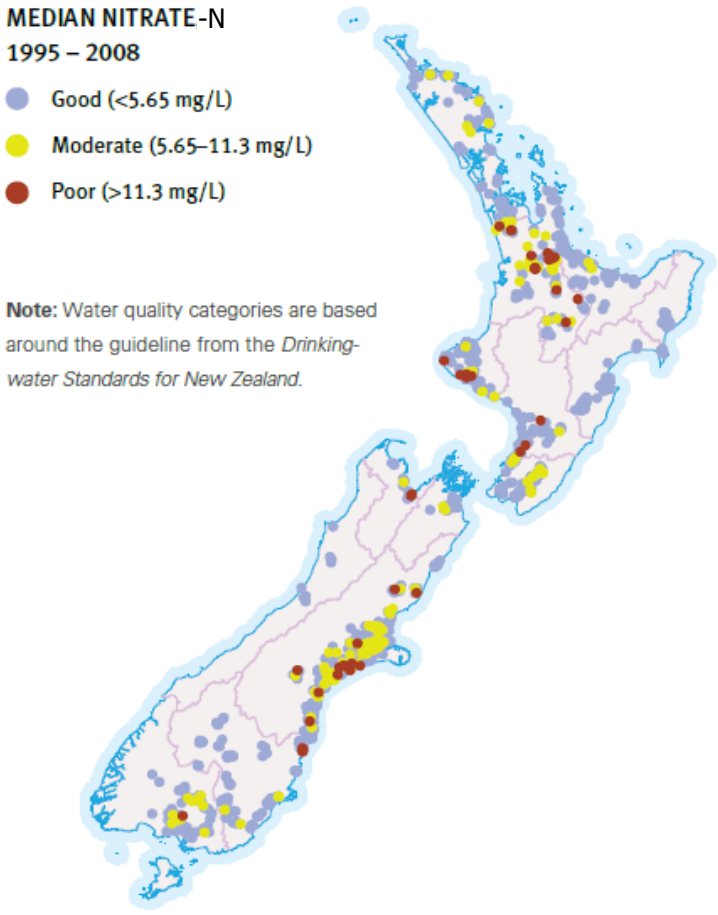


Figure 2.4-3. Median nitrate-N levels for the period 1995 to 2008 for 914 groundwater sites in New Zealand (Ministry for the Environment 2010).

Appropriate use of N fertilisers and skilfully managed farms means high fertiliser-N use efficiency can be obtained (Vibart et al. 2012) but N loss from animal excreta, and urine patches in particular, will always prove problematic due to their deposition in excess of plant demand. As discussed in section 2.4.1, approximately 85-90% of N ingested by the grazing animal is returned to the soil-pasture system of which ~70% is passed out in the urine; with over 70% present as urea (Saggar et al. 2013). Under a urine spot an effective dairy cattle urine-N application rate may be as high as 1000 kg N ha^{-1} and, consequently, the potential for leaching losses below the root zone is large (Haynes & Williams 1993; Di & Cameron 2002a). There is considerable data on nitrate leaching losses under grazed pastures both in New Zealand and overseas and generally losses appear to

increase with the intensity of the management system and the N loading from urine deposition (Di & Cameron 2002a).

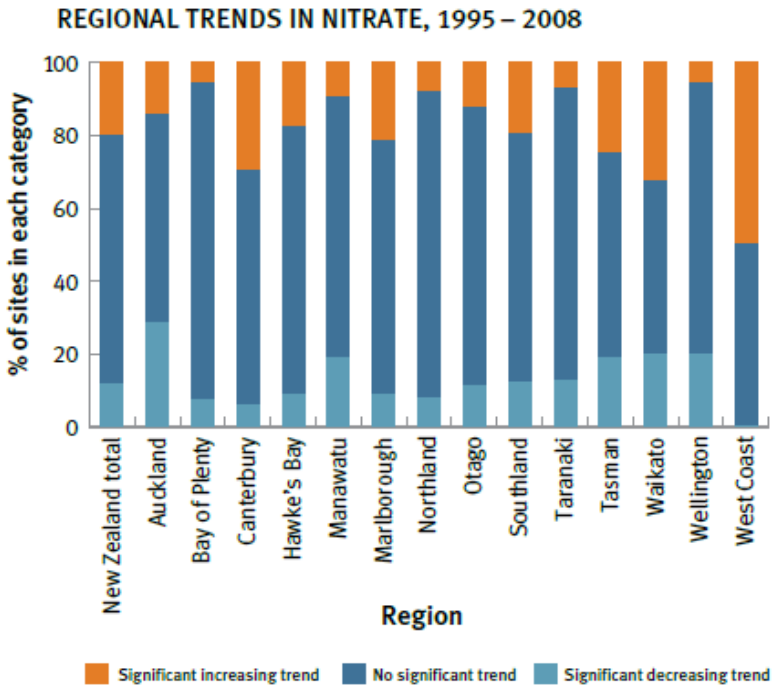


Figure 2.4-4. Regional changes in nitrate-N levels in groundwater 1995-2008 (Ministry for the Environment 2010).

Table 2.4-2. Measured nitrate leaching losses under grazed pastures (Di & Cameron 2002a).

N applied (kg N ha^{-1}	Soil texture	Grazing system	Drainage (mm)	Leaching loss (kg N $ha^{-1}y^{-1}$)	Reference
0	Clay loam	Cattle	300	30	(Monaghan et al. 2005)
Urea 100	Clay loam	Cattle	300	34	(Monaghan et al. 2005)
Urea 200	Clay loam	Cattle	300	46	(Monaghan et al. 2005)
Urea 400	Clay loam	Cattle	300	56	(Monaghan et al. 2005)
Dairy shed effluent	Sandy loam	Dairy cows	350	47	(Silva et al. 1999)
Urea 200	Sandy loam	Dairy cows	350	54	(Silva et al. 1999)
Urea 225	Silt loam	Dairy cows	200	57	(Ledgard et al. 1996)
Urea 360	Silt loam	Dairy cows	200	110	(Ledgard et al. 1996)
NH_4NO_3 200	Clay	Beef cattle	300-500	39	(Scholefield et al. 1993)
NH_4NO_3 400	Clay	Beef cattle	300-500	134	(Scholefield et al. 1993)
NH_4NO_3 420	Loam	Beef cattle	330	162	(Ryden et al. 1984)
NH_4NO_3 450	Loam-clay	Beef cattle	170-240	11-48	(Ryden et al. 1984)
None	Sandy loam	Sheep	220-270	6-7	(Ruz-Jerez et al. 1995)
Urea 400	Sandy loam	Sheep	220-270	11-41	(Ruz-Jerez et al. 1995)

The nature of New Zealand's agriculture and its almost totally outdoor-based pastoral livestock system means it is difficult to significantly change the scale of these N losses under current farm management practices. Therefore, the challenge is to find mitigating technologies or management systems that can reduce leaching losses and/or recycle the N deposited in these areas back into the grazing system more efficiently, whilst improving productivity.

2.4.4 Solute transport mechanisms

Given steady-state water conditions in the soil and no interaction between solute and soil, movement of the nitrate ion can be described by a combination of three main mechanisms: convection, diffusion and hydrodynamic dispersion (McLaren & Cameron 1990b; Hillel 1998a).

Convection transport

Any initial discussion of the mechanisms of solute movement in soils first needs to consider convection alone. Convection or mass flow of water (sometimes called *Darcian flow*) carries with it a convective flux of solutes J_c, proportional to their concentration *c;* (2.4-6); (Hillel 1998a):

$$J_c = qc = -c\left(K\,\frac{dH}{dx}\right)$$

2.4-6

where $q = -K\,(dH/dx)$ is Darcy's law that describes the steady state flux q of water for saturated flow in porous media over hydraulic gradient dH/dx (for a fixed hydraulic head H over distance x). The proportionality factor K, otherwise known as the soil's *hydraulic conductivity*, describes the ability of the soil to transport the liquid and thus is related to the soil's pore space distribution and continuity. The distance travelled by solute per unit time depends on the average pore water velocity, U, described below (2.4-7):

$$U = \frac{q}{\theta}$$

2.4-7

where q is as described above, and θ is the volumetric water content. Convection transport implies uniform displacement of the band of solute (Figure 2.4-5a).

Diffusive transport

In actuality, the band of nitrate transported by the convection process is subject to separate diffusion and dispersion processes that cause it to spread out (Figure 2.4-5b). Diffusion processes commonly occur in gas and liquid phases because of random thermal motion from repeated collisions and deflections between molecules. In solute transport, there is a net tendency to even out the spatial distribution of a solute along a concentration gradient (i.e. from a zone of high solute concentration to one of low) so that the solute is evenly distributed within the liquid medium. This process can be described by Fick's law below (2.4-8).

$$J_c = -D_o \frac{dc}{dx}$$

2.4-8

where J_c is the rate of diffusion, D_o is the diffusion coefficient of solute in the soil and depends on the soil moisture content, and *dc/dx* is the solute concentration gradient. As the soil dries, the ability of the solute to diffuse throughout the liquid phase decreases and pathway *tortuosity* increases. Consequently, the diffusion coefficient in soil D_s, is related to D_o, Fick's law coefficient, the volumetric water content θ, and the empirical tortuosity factor ξ, through $D_s = D_o \cdot \theta \cdot \xi$.

Dispersive transport

Hydrodynamic dispersion describes the mechanical mixing of a solute in the soil liquid phase where the same conditions exist as in diffusive transport but the movement of the liquid itself through the soil pores creates the solute mixing effect analogous to diffusion. This occurs because of the range of velocities of moving water, within and between soil pores of difference sizes. Within a pore, the liquid at the centre will tend to move faster than that at the edges where friction and other forces create a drag on the liquid, thus, solute held at the centre will tend to travel further than that at the edges. Similarly, the range of velocities between pores is related to the 4th power of the radius such that the flux q, increases by a factor of 16 for every doubling of the pore radius. Mathematically, hydrodynamic dispersion can be described in an analogous manner to diffusion except that a *dispersion* coefficient D_h, is used instead of a diffusion coefficient. Because of the

similarity of effect, if not mechanism, the two are combined together into a single term called the *diffusion-dispersion* coefficient D_{sh}, represented by equation 2.4-9 (Hillel 1998a):

$$D_{sh}(\theta, U) = D_s(\theta) + D_h(U)$$

2.4-9

where D_{sh} is a function of both the fractional water volume, θ, and the average pore water velocity, U.

Combined convective-diffusive-dispersive transport

Considering all three processes discussed thus far in the transport of solutes, the mass flow (convection) is combined with molecular diffusion and hydrodynamic dispersion equations to create a combined solute flux equation (2.4-11). This equation takes account of the solute flux for each of the three components and since in practice the diffusion and dispersive phenomena cannot be separated, the equation (2.4-10) is usually written in the form (Hillel 1998a):

$$J = \theta v c - D_{sh}(\theta, U)\frac{dc}{dx}$$

2.4-10

where J is the total solute mass transported across unit cross-sectional area of soil per unit time, D_{sh} is the lumped diffusion-dispersion coefficient (a function of volumetric wetness θ and average pore-water velocity, U), v is pore water velocity, c is the solute concentration, and dc/dx is the solute gradient (Hillel 1998a). Most parameters in the above equation however, can only be defined in macroscopic terms as gross spatial averages and is only an approximation of the process it's intended to represent. With transient-state processes where fluxes and concentrations can change in space and time, the conservation or continuity principle is invoked, which for combined convection-diffusion-dispersive transport (2.4-11) can be written as (Hillel 1998a):

$$\frac{\partial(c\theta)}{\partial t} = -\frac{\partial J}{\partial x}$$

2.4-11

For steady-state water flow (but not necessarily steady-state solute movement), θ, U and D_{sh} are taken as constant and equations 2.4-10 & 2.4-11 are combined and simplified to a Convective-Dispersive-Equation (CDE) equation (2.4-12) (Hillel 1998a):

$$\frac{\partial c}{\partial t} = D_{sh}\frac{\partial^2 c}{\partial x^2} - U\frac{\partial c}{\partial x}$$

2.4-12

Classically, solute transport in porous media described using the above equation can be exemplified in Figure 2.4-5b but as alluded to in the previous paragraph this assumes there are no other mechanisms operating that affect gains, losses or transport of solute. This is obviously untrue for many solutes, including nitrate, where a number of soil-driven processes occur that can affect solute concentration and transport, but due to their complexity and transient nature, are difficult to describe mathematically. Often a sink-source (S) term is added to the CDE to sum the possible solute sinks, sources and storage changes but the total effect on nitrate leaching processes is invariably difficult to calculate accurately as the processes are often spasmodic and/or temporal (Hillel 1998a).

Soil charge characteristics

There are two main charge effects in soils that affect nitrate transport, exclusion and sorption. In exclusion, the process is driven by the net negatively-charged soil surfaces that dominate most temperate soils, repelling the negatively-charged nitrate ion towards the faster moving centre of the soil pore. This reduces the effective pore volume of the soil and thus the nitrate ions travel faster than that predicted by convection theory, as shown in Figure 2.4-5c. Sorption on the other hand occurs in soils with significant anion exchange capacities, such as in some tropical and/or allophanic soils, where non-specific adsorption of nitrate ions retards their leaching, increasing the effective pore volume beyond that predicted by convection theory (Figure 2.4-5d) (Haynes 1986e).

Macropore leaching

Soil structure is invariably affected by natural and man-made management processes. Thus, it is not one homogenous range of soil pores but has cracks, earthworm burrows, old root channels and fissures that may form a contiguous network that can conduct water under saturated conditions

(Kanchanasut et al. 1978; Scotter & Kanchanasut 1981; Kanchanasut & Scotter 1982; Francis & Fraser 1998; Nuutinen et al. 1998). Under unsaturated flow, these pores may be largely air-filled but under heavy rainfall will conduct water to depth more quickly if open to the surface. In these conditions, there are two main effects on nitrate leaching, if the infiltrating water has a high concentration of nitrate, then macropore flow will lead to extensive leaching at a faster rate than predicted by the CDE (Figure 2.4-5e). Conversely, nitrate sitting within soil micropores will be largely bypassed by the bulk of flowing water and a slower than predicted rate of leaching will occur (Figure 2.4-5e).

Figure 2.4-5. Schematic representation of solute (nitrate) transport processes in soils: a) convective transport, b) convection-diffusion-dispersion, c) anion exclusion, d) anion adsorption, and e) macropore bypass and macropore leaching. Adapted from Cameron and Haynes (1986) and McLaren and Cameron (1990b)

2.4.5 Factors affecting nitrate leaching

Season and climate

The greatest potential for nitrate leaching losses occurs with a build-up of nitrate within the soil profile and an excess of soil water. Consequently, the greatest nitrate leaching losses usually occur during the late autumn, winter and early spring months when plant growth is slow and uptake of nitrate is low, and soil drainage is occurring due to a surplus of rainfall over plant evapotranspirative demand. Timing of rainfall and plant N demand, therefore, is critical to the size of losses from N fertiliser and/or urine application. For instance, Di et al. (1999) compared N leaching losses from

autumn-applied N fertiliser (NH_4Cl) to that applied in spring and found that these ranged between 15-19% in autumn but 8-11% in spring. Similarly, Decau et al. (2003) found that timing of urine application to pastures between autumn and spring had a significant effect on urinary-^{15}N recovery in drainage, with an order of magnitude greater loss in leachate collected after the autumn application (0.7% vs 16.7%, respectively).

Higher residual soil nitrate left after a crop has been harvested or from the ploughing-in of pasture prior to sowing of a crop in autumn can lead to significant nitrate leaching losses (Cameron & Wild 1984; Adams & Pattinson 1985; McLenaghen et al. 1996). Urine deposited over a summer-dry period may also lead to increased nitrate leaching losses due to low pasture growth and a lack of N uptake. This N can leach over the succeeding winter (Scholefield et al. 1993; Shepherd et al. 2011; Snow et al. 2011) and indeed losses can be considerably higher than from an irrigated system on the same soil-type (Burgess 2003). Similarly, optimally-applied N fertiliser and irrigation can reduce N leaching losses by more efficient plant uptake of N (Hahne et al. 1977). Excess irrigation, however, has also been implicated in increased nitrate loss (Haynes 1986e).

Seasonal effects on soil temperatures can also influence nitrification as when ammonium is not limiting, and nitrite is not accumulating, rates can be shown to be largely zero-order and increase linearly with temperature (Figure 2.4-6) (Flowers & O'Callaghan 1983; Macduff & White 1985). This is more likely in grassland topsoils where the *Nitrosomonas* and *Nitrobacter* populations are near maximal and consequently the nitrification rate for urea-N in urine, for example, can be largely predicted based on prevailing soil temperatures under moist conditions (Macduff & White 1985). If soil temperatures remain low (~5°C) the nitrification rate in a urine patch is likely to be slowed, and the nitrate leaching potential less, than in warmer conditions where nitrate will tend to accumulate until the pool of ammonium is largely exhausted.

Generally, there is a relationship between the timing and size of rainfall events and nitrate leached where the latter increases in direct response to increased drainage, especially under fallow conditions after cropping or pasture renewal (Scholefield et al. 1993; Francis 1995). The source of N in many cases is from residual nitrate after harvesting or from grazing and/or mineralisation of organic-N and low N uptake over the winter period (Francis 1995; Drury et al. 2014). Nitrogen fertiliser can also incur large nitrate losses if rainfall occurs soon after application (Haynes 1986e).

Many factors therefore can influence nitrate leaching, depending on soil condition, the climate pattern and prevailing land management.

Figure 2.4-6. Nitrification rates vs. soil temperature for a Dunkeswicke soil after addition of either 50 or 250 mg N kg^{-1} soil as ammonium sulphate or pig slurry. Adapted from McDuff and White (1985).

Soil properties

Soil texture and structure have an important influence on nitrate leaching, with sandy, coarse textured soils generally having higher hydraulic conductivities than finer-textured silt or clay soils (McLaren & Cameron 1990b). Slower water movement in clay soils can create conditions that promote higher rates of denitrification (Saggar et al. 2013) and hence, fewer nitrate ions are available for leaching. Di et al. (2009a) showed that nitrate leaching from urine application to three soils of different textures under the similar leaching regimes was about 25% less for the finer, silt-dominated soil compared with the coarser-textured soils, with the difference attributed to denitrification in the silt-dominated soil.

Differences in leaching rates can be substantial due to hydraulic conductivities varying by several orders of magnitude between sandy and clay-textured soils (Lin et al. 2001). However, macropores, cracks and fissures can affect actual nitrate loss through the by-pass and preferential leaching mechanisms described in section 2.4.4. For example, Silva et al. (2000) showed

increased N leaching from macropore flow of urine under field-saturated flow conditions in a NZ pastoral soil. Similarly, agricultural drainage systems can increase nitrate leaching in heavy soils through a combination of shortened flow paths and improved aeration that decreases denitrification and increases N mineralisation (Scholefield et al. 1993).

A considerable quantity of nitrogen emanating from pastoral and cropping soils can be attributed to mineralisation and nitrification of SOM rather than from fertiliser application. Cropping soils in particular can leach large quantities of nitrate if cultivated and/or left fallow over winter (Dowdell & Webster 1984; Adams & Pattinson 1985; Francis et al. 1998) with N fertiliser potentially only a small part of total N losses (Dowdell & Webster 1984; Adams & Pattinson 1985). However, research has

also shown that N fertiliser can also act as a “primer” for increased SOM mineralisation and not just as part of normal immobilization-mineralisation turnover (Haynes 1986d).

2.5 Nitrogen leaching within the pastoral grazing system

2.5.1 Landuse effects

Whilst essentially the same N processes occur in agricultural systems as those in the natural world, the magnitude of the N fluxes involved tend to increase as the system becomes more reliant on increased N inputs to improve production, such as in intensive pastoral grazing systems (Ledgard et al. 1999; Jarvis & Ledgard 2002; Bolan et al. 2004). Such systems amplify the N transfer fluxes of herbage consumption, excreta return and animal product removal, and in turn increase opportunities for N losses. Extensive grasslands usually have quite low nitrate leaching losses due to rapid plant uptake and immobilisation of N that keep soil solution nitrate concentrations low (Woodmansee et al. 1981). The lower stocking density of extensive systems means urine patches are more spatially distant and leaching losses considerably lower over a unit area (Soussana & Lemaire 2013). Similarly, pastures that are cut for silage or hay also tend to experience low leaching losses because grass and pasture plants are usually very efficient at capturing N from applied fertiliser, or N fixed by pasture legumes such as clover and cycled back into the soil pool (Cameron et al. 2013). Di et al. (1998b), in a fertiliser and dairy-shed effluent (DSE) lysimeter study, found that pasture removed approx. 300-400 kg N ha^{-1} after 400 kg N ha^{-1} of either amendment was applied in two split applications. Matching N application with plant demand is important if nitrate leaching losses are to be avoided or kept to a minimum. For instance, in a similar study Di et al. (1998a) found that splitting an application of 200-400 kg N ha^{-1} as either urea or DSE into four, rather than two, applications made a significant reduction in nitrate leaching losses, from 13-49 kg N ha^{-1} to 6-17 kg N ha^{-1}.

The potential scale and factors affecting nitrate leaching losses were discussed in section 2.4.5 but essentially where N is applied in excess of plant demand and/or at times of low pasture growth, and significant drainage can occur, then nitrate leaching losses can be large. Such conditions exist on farms where a grazing cow, on average, may deposit the equivalent of 700-1200 kg N ha^{-1} (Haynes & Williams 1993; Di & Cameron 2002a; Selbie et al. 2015) within a patch of around 0.4

m^2 (Moir et al. 2011). With a ruminant animal producing, on average, 8-12 urinations per day (Haynes & Williams 1993; Selbie et al. 2015), 20-30% of the paddock surface could be covered by these concentrated urine patch areas annually, depending on stocking rate (Moir et al. 2011). The largest proportion of the applied urine-N will be nitrified to nitrate although some N loss will occur via volatilisation (Sherlock & Goh 1984; Ledgard et al. 1999) and from gaseous N loss during the nitrification process itself (Colbourn 1992; Carter 2007). Given that plant uptake of N from a urine patch is usually <600 kg N $ha^{-1}y^{-1}$ (Moir et al. 2006; Moir et al. 2011), a large pool of nitrate remains for potential leaching unless soil conditions promote denitrification (Fraser et al. 1994; Clough et al. 1998; Di et al. 2002; Carter 2007) or immobilisation. These losses might be typically 80-120 kg N $ha^{-1}y^{-1}$ under the urine patch itself (Silva 1999; Di & Cameron 2004b) but of course the whole paddock surface is not covered by a urine patch and is effectively 'diluted' by the inter-urine areas. Thus, the whole paddock leaching loss can be calculated by a simple proportional coverage equation (2.5-1):

$$\boldsymbol{N_L = (N_{L1} \times P_1) + (N_{L2} \times P_2)}$$

2.5-1

where N_L = the annual average nitrate (NO_3^--N) leaching loss for a grazed field, N_{L1} and N_{L2} are the leaching losses from urine and inter-urine areas, respectively, and P_1 and P_2 are proportional factors representing the relative coverage of urine and inter-urine areas, respectively.

Using this equation Silva et al. (1999) calculated the overall nitrate leaching loss was 33 kg N ha^{-1} for an irrigated Canterbury Templeton soil although the under-patch leaching loss was 124 kg N ha^{-1}. However, the addition of N fertiliser increased this loss to between 36-60 kg N ha^{-1} depending on fertiliser-N rate. Similarly, Ledgard at al. (1999) found that mean leaching losses for a Waikato dairy pasture over three years were 40 kg N $ha^{-1}y^{-1}$ (range 20-74) but fertiliser at 200 and 400 kg N $ha^{-1}y^{-1}$ increased these to 80 (range 59-101) and 150 kg N $ha^{-1}y^{-1}$ (range 100-204), respectively. Monaghan et al. (2005) found nitrate leaching losses under cattle grazing in Southland increased with an increase in N fertiliser use, averaging 30, 34, 46 and 56 kg N $ha^{-1}y^{-1}$ when 0, 100, 200 and 400 kg N ha^{-1} of N fertiliser was applied annually, respectively. The relationship between nitrate leaching losses and increasing N fertiliser use, stocking rate, and urine-N losses, although subject to a range of climatic and soil conditions, clearly increases with total-N input (Figure 2.5-1).

Figure 2.5-1. Relationship between annual N inputs and nitrate-N leaching losses from grassland (C=clover; G= grass) (Cameron et al. 2013).

Management of grasslands also includes periodic pasture renewal and/or cropping rotations where mineralisation of the N contained in SOM can result in high soil inorganic-N concentrations. This introduces a window of vulnerability where, if this N is not taken up in a subsequent crop or the land is left fallow, large nitrate leaching losses can occur (Cameron et al. 2013).

Francis et al. (1992) reported average nitrate leaching losses of 60 kg N ha^{-1} when a 3-year leguminous pasture was cultivated and left fallow for increasing periods (5, 40 and-78 kg N ha^{-1} for March, May and July, respectively). Where the land is left to fallow after a crop is harvested or grazed, the sowing of a cover crop as early as possible is desirable to prevent residual soil-N from mineralising and being leached in the subsequent winter period (McLenaghen et al. 1996; Francis et al. 1998; Thorup-Kristensen et al. 2003).

2.5.2 Irrigation and fertiliser efficiency

Irrigation, as discussed previously, can improve the efficiency of N use and reduce nitrate leaching losses by increasing plant-N uptake (Hahne et al. 1977; Burgess 2003). However, the timing and intensity of irrigation can be important factors in preventing leaching, especially in stony soils. For instance, Silva et al. (2000) found that recently applied cow urine was at significant risk of leaching (as urea and ammonium) when saturated flow was occurring through the larger soil pores. Close et al. (1986) found nitrate leaching losses under flood irrigation of 7-70 kg N ha^{-1} on a stony soil, in part caused by significant saturated flow. The use of drainage systems can also exacerbate N leaching losses due to the creation of preferential flow paths that carry solutes more quickly from slow-draining soils (Monaghan et al. 2007b). The application of dairy shed effluents that are often rich in N and usually irrigated onto the paddock can also find their way through these drainage systems, especially if applied in excess to wet soils (McDowell et al. 2011). Thus, excess irrigation can be a significant factor that increases N leaching losses where the rate of application and/or the total quantity of water is more than can be held in the soil pores.

Nitrogen fertiliser efficiency can range widely due to the many plant, soil and climatic factors that affect utilisation, some of which have already been discussed. Overall, N recoveries in crops of 30-70% are typical (Goh & Haynes 1986). Indeed, Chien et al. (2009) found in over 800 cereal experiments that N utilisation averaged only just over half (51%). Recoveries under grazing, however, are often even lower and considerable research has been undertaken to increase efficiency, especially regarding animal products leaving the farm. In cropping, if N fertiliser is applied at rates that match crop demand then little mineral-N is left in the soil at harvest to leach (Jenkinson 2001). However, on intensively grazed pastures where high N rates are combined with

high stocking rates, then leaching losses can be considerable (50-200 kg N $ha^{-1}y^{-1}$) (Ledgard et al. 1996).

2.5.3 Farm dairy effluents and manures

Under intensive dairying significant amounts of nutrients, N especially, are contained in Farm Dairy Effluents (FDE) but with the advent of herd shelters and winter stand-off pads, increasing amounts of nutrients are also retained in organic materials from these systems (Longhurst et al. 2006; Luo et al. 2006). Such management systems can generate large quantities of organic materials that need to be applied to the land later. Much of the N retained in these materials can be mineralised in the soil and released for pasture uptake but the rate can be difficult to predict due to their variable composition (Vanderholm 1985). Rapid mineralisation can potentially result in N leaching if there is an excess over pasture uptake (Cameron et al. 1996) but the judicious use of such materials can benefit soil condition and fertility and are a valuable adjunct to soil-N supply and the general recycling of nutrients (Wang et al. 2004). Determining N leaching losses from the land application of such materials is difficult and will depend on several factors:

1. The amount of N present and proportion in mineral and readily-mineralisable forms
2. The timing of application and effect of soil temperature
3. The soil moisture content and amount of rainfall received soon after application, and
4. The N application rate relative to plant-N uptake (Chambers et al. 2000).

Modelling nutrient availability from FDE and other composts can help avoid over-application by providing calculators that aid in ensuring adequate N nutrition without excessive nitrate leaching (Wheeler et al. 2012).

2.5.4 Methods to reduce N losses

The need to reduce all sources of nitrate leaching losses has led to the development of technologies and farm management strategies to increase N use efficiency. These methods can be briefly summarised as follows:

1. Applying the correct amount of N fertiliser to match plant demand to reduce excessive N inputs;

2. Accurate application of N fertiliser and the use of split applications (i.e. little and often);
3. **Maintaining an active plant cover over the drainage period (e.g. a cover crop in any arable phase);**
4. **Early sowing of crops to ensure sufficient growth and less bare ground;**
5. **Use of a nitrification inhibitor to slow down the rate of nitrification under urine patches, fertilisers and manures, reducing the pool of nitrate available for leaching;**
6. Renewing pasture swards frequently to maximise plant N uptake and cultivating soil in spring rather than autumn to reduce N losses from mineralisation of SOM;
7. Maximising plant N uptake, for example, by applying optimal rates of irrigation to increase plant growth or controlling pasture pests and diseases; and
8. Application of animal wastes and slurries at times when the risk of leaching is low, and at rates and times that match plant N demand (Cameron et al. 2013).
9. Reduced urinary-N returns in autumn

Many of these methods have already been discussed and it is not intended to review all in depth but it is apparent that there are a significant number of research opportunities and gaps to be investigated. The use of cover crops after harvesting or grazing a crop is an area where there is little current research, especially in winter forage grazing, and there are few established strategies to reduce nitrate leaching losses. The subsequent focus of this review is now to concentrate on the use of cover crops and nitrification inhibitors (3-5) as management tools to reduce nitrate leaching.

2.6 Nitrogen capture using cover crops

2.6.1 Introduction

The use of cover or catch crops is a technique whose origins can be found in green-manuring practices at the beginning of settled agriculture and records in China show it has been in use for over 3000 years (Allison 1973). Where green leguminous material was incorporated, there was a benefit to the production of a subsequent crop although the basis of that benefit was largely unknown. Pound et al. (1999) in their review of the use and relevance of catch crops in agricultural systems concluded that catch crops became highly important for maintaining production in many

communities as their farming systems moved from extensive to more intensive practices but used few external inputs. Organic farming systems still largely operate under the same principles but pressures on externally-based input agricultural systems to reduce nutrient loss, particularly mobile nutrients such as nitrate, has renewed interest in catch or cover crops to help deal with imposed environmental or production constraints (Thorup-Kristensen et al. 2003). This has exposed large knowledge gaps in whether catch crops have a part to play in strategies to reduce nutrient losses under some intensive farm management systems, especially those involving winter forage cropping.

In temperate climates, once the summer's crop has been harvested, the winters are generally too cold for most crops to grow and under these conditions the soil can be left with no vegetative cover over the winter period. With winter the time of greatest soil drainage, considerable quantities of nutrients can potentially be leached out of the root zone. During the autumn period, sufficient light and heat may remain to allow some plant growth but usually not enough to provide a commercial crop. The reason for sowing a green manure or catch crop is somewhat different in each case. In green manuring the goal is to incorporate a young green crop, usually a legume that decomposes sufficiently quickly over the spring and mineralise the N contained for uptake by the subsequently sown crop. Whilst a part of this N may be sourced from residual mineral soil-N from the previous crop and mineralisation of organic residues, a sizable proportion may be fixed-N. Sowing a catch crop is aimed more at retaining nutrients and/or improving or preserving soil condition (e.g. improve SOM or prevent erosion), usually in situations where a considerable proportion of the N requirement for the previous crop was imported. Thus, by definition, a catch or cover crop is a non-commercial crop intended mainly to capture available-N, thereby limiting the potential loss of nitrate (and accompanying cations) in drainage (Thorup-Kristensen et al. 2003).

2.6.2 Catch crops in winter forage grazing

The growing of winter forage is a common practice in the South Island of NZ where, for example, of the 250,000 ha of winter forage brassicas grown nationally, 75% (185,000 ha) is grown in the South Island (Statistics New Zealand 2012a). With South Island dairy cattle numbers having increased by more than 50% in the period 2007-12 (Statistics New Zealand 2007, 2012b), the

demand for winter forage has increased. It is difficult to accurately determine how much of that grown is used directly by the South Island dairy industry, given that a significant proportion is grown off-farm on dairy support land, but it is likely to be more than 50%. The main forage crops grown are kale (*Brassica oleracea* L.) and fodder beet (*Beta vulgaris* L.), which are grazed over the winter to achieve body condition score targets for pregnant, non-lactating dairy cows prior to the start of calving in early spring (Judson et al. 2010). These are normally sown in the previous spring (~November), and grazed for 8-12 weeks from late May/early June (Edwards et al. 2014b), after which the cropped area remains fallow until the next crop is sown in late spring (2-3 months later). There is considerable risk of large nitrate leaching losses from the large volumes of urine deposited during grazing over the winter (Beare et al. 2006; Monaghan et al. 2007b).

Although almost any crop, grown satisfactorily, can be used as a green manure or catch crop, in practice the selection is dictated by the intention and situation and hence, more limited. Plant species selection affects almost every aspect of catch cropping so in terms of N capture there are several factors that need to be considered:

1. **Speed of establishment, growth rate and rooting depth**
2. **Cold tolerance**
3. **Is the crop to be harvested, incorporated or grazed?**
4. Nitrogen fixing capacity - legume or non-legume?
5. Quality of the catch crop material – as forage or a harvested crop, mineralisation potential, C/N ratio etc. (Thorup-Kristensen et al. 2003).

Points 1-3 are of more interest as they constitute a considerable knowledge gap in whether catch crops can help control nitrate leaching in temperate forage grazing systems, post-winter grazing. In Canterbury, soil temperatures (0-10 cm) over winter months average 5-8°C (NIWA 2016); to fulfil the first two criteria, the sown catch crop must therefore be capable of germinating and growing sufficiently to take up N in this temperature range. This effectively restricts selection to winter cereals such as wheat and oats whose roots remain active over this period (Thorup-Kristensen et al. 2003; Thorup-Kristensen et al. 2009) and where sufficient dry-matter might be grown by late spring to graze or harvest for silage. Points 4 & 5 are generally less important in the winter forage grazing situation as the main intention is to sequester N rather than provide than a source of

mineralisable N for a following crop and the early harvesting means the quality of the material is generally high.

2.6.3 Cool season cereal development in New Zealand

A number of yield models for cool season forage oats developments have been reported previously in New Zealand (Taylor & Hughes 1979; Hughes et al. 1984) where prediction of yield is correlated with a combination of heat units and solar radiation (Taylor & Hughes 1979). These invariably produce a range of yield curves that differ by sowing date and region due to differences in soil temperature and radiation data. Consequently, Hughes et al. (1984) used the familiar degree-days as a means to relate yield in the early development stages across a range of regions and sowing dates and to predict overall yields.

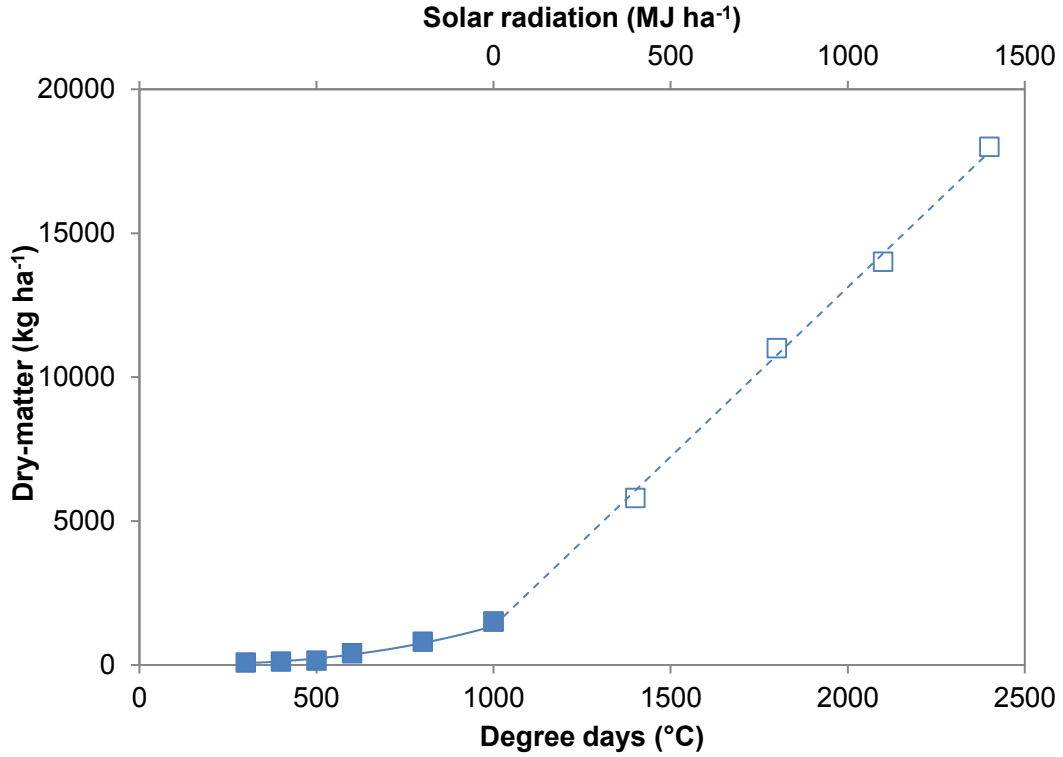


Figure 2.6-1. Representative cool season forage oats yield development yield curve after Hughes et al. (1984).

Essentially there are three parts to the forage oats model developed by Hughes (1984) and represented in Figure 2.6-1; the first, measured in degree days, covers the period when the crop emergences after sowing but essentially the yield is zero. In the second part, yield builds

proportionally with degree days (i.e. aggregated daily average temperature) and is largely controlled by soil temperature until coverage is sufficient to intercept a large proportion of the available solar radiation. In the final phase, the model switches from one where yield is proportional to temperature to one controlled by the received photosynthetically-active radiation component.

2.6.4 Catch crop N uptake and mineral-N depletion

The primary requisite for any catch crop is to take up N from the soil thereby reducing nitrate in drainage waters. Although catch crops can increase the recycling of N within the cropping system and therefore, increase N-use efficiency, results for N uptake can vary considerably, and by more than an order of magnitude (~10-200 kg N ha^{-1}) (Thorup-Kristensen 1994; Francis et al. 1995; Richards et al. 1996). Underlying such variation is the fact that catch crops are usually sown in conditions that aren't optimal for growing and consequently, N uptake is often limited by a lack of growth. This variation in N uptake can be attributed to three main factors:

1. Variable catch crop growth and potential to take up N under the prevailing climatic conditions.
2. Variable catch crop root growth and contact with available soil-N.
3. Spatially variable amounts of available-N in the soil (Thorup-Kristensen et al. 2003).

Figure 2.6-2. Mean two-year catch crop capture of N at high or low N availability for two experiments at adjacent sites (Thorup-Kristensen et al. 2003).

A catch crop well supplied with N can produce 1000 kg DM ha^{-1} in two weeks of active growth, taking up 3-4 kg N/day (3-4% N content) (Vos & van der Putten 1997). Thus, a few weeks' active growth could theoretically take up all the available-N within a soil profile. In reality catch crop growth and N content is often much lower than this due to a lack of available-N (McLenaghen et al. 1996; Vos & van der Putten 1997). Indeed, the lack of available-N can be more limiting than insufficient N uptake capacity and in trials where fertiliser N is applied, or where a legume catch crop has been used in preference to a non-legume (Figure 2.6-2), there is generally a greater DM response (Vyn et al. 2000; Thorup-Kristensen et al. 2003). Obviously where catch crops receive too short a growing season their ability to deplete soil inorganic-N is reduced. For example, Vos and Van der Putten (1997) found a strong relationship between day of autumn planting and DM and N uptake accumulation in winter rye (Figure 2.6-3).

Figure 2.6-3. Dry matter and nitrogen accumulation for three winter rye field trials sown at approximately 235 (S1 —), 256 (S2 – –) and 272 (S3 •••) days (autumn). Expt. 1- □, Expt. 2- ○ and Expt. 3- △ (Vos & van der Putten 1997).

2.6.5 Root growth and development

To deplete the soil of available-N, a catch crop must develop a root system to enable N uptake. Since nitrate is a highly mobile ion it is not necessary to have a high root density to enable effective soil-N depletion and, accordingly, studies that have attempted to show a correlation with root length density have often proved inconclusive (Robinson et al. 1994; Vos et al. 1998; Malcolm et al. 2014). A combination of plant activity with deep rooting architecture has proved a better predictor of N uptake and consequently, a better correlated (negative) relationship with nitrate leaching. Thorup-Kristenson (2001) found that soil-N depletion by catch crop species was highly correlated to their rooting depth but only weakly with root intensity. Popay and Crush (2010) and Crush et al. (2005), however, found for a given rooting depth, those grass species with more finely divided root systems and larger surface area-to-weight ratios generally had significantly higher nitrate interception rates and greater N uptake. However, applying these latter, largely lab-based studies to field soils with their more complicated water and solute transport effects, and predicting the interaction of rooting

depth, architecture, activity and all the genetic species factors that might influence N uptake, can be more problematic. For instance, Malcolm et al. (2014) has recently found that a winter-active Italian. (It.) ryegrass/white clover combination (It. ryegrass WC) leached 24-54% less nitrate-N over two consecutive winters than perennial ryegrass/white clover (P. ryegrass WC), tall fescue/white clover (T. fescue WC), or perennial ryegrass/It. ryegrass/white clover/red clover/chicory/plantain mixed pasture (Diverse) (Figure 2.6-4). Despite the considerably greater root mass in the 0-40 cm depth of the P. ryegrass WC and T. fescue WC pasture species compositions (Figure 2.6-5), the important factor for greater N uptake over the winter drainage period was the higher plant winter activity (plant growth/root metabolic activity) of the It. ryegrass, rather than specific root architecture (e.g. deep roots of the T. fescue).

Figure 2.6-4. Total nitrate-N leaching losses leached in 2010-11 (a) and 2011-12 (b) for a range of pasture species after autumn urine application of 1000 kg N ha^{-1} (Malcolm et al. 2014).

Figure 2.6-5. Average root density for a range of pasture species in 2010-11 (a) and 2011-12 (b) after autumn urine application of 1000 kg N ha^{-1} (Malcolm et al. 2014).

Nevertheless, for significant N uptake to occur it will require plant roots near the nitrate ions and thus, the rate and depth of root establishment is important if the crop or pasture is still in a development phase. Although rooting depth is controlled by several factors, the actual species and duration of growth are probably the two more important and the most controllable in terms of farm practice. Thorup-Kristensen (2001) calculated that 1000 degree-days after sowing, a crucifer catch crop would typically root to a depth of 1.5 m, winter rye and oats to 0.9-1.0 m, but only 0.6 m for ryegrass (Figure 2.6-6) although the crucifer crop allocated a smaller proportion of its biomass to its root system than grasses or rye (Lainé et al. 1993). Similarly, Herrera et al. (2010) found that nitrate leaching from different catch crops planted after spring wheat was not related to the static characteristics of the root system but inversely related to early root growth, where the brassica crop generally outperformed phacelia and sunflower crops (Table 2.6 1).

Figure 2.6-6. Root development of catch crops showing different rates of rooting depth development with cumulative temperature (Thorup-Kristensen et al. 2003).

The ability of crops to take up N also differs between species and particularly between non-leguminous dicots, such as the crucifer species (e.g. kale, forage rape), and cereal monocots (e.g. rye, oats). Crucifer crops potentially have higher nitrate uptake potentials than cereals and grasses

(Lainé et al. 1993) and appear to be less sensitive to low temperatures than monocots (Laine et al. 1994). However, this is dependent to some extent on duration of crop establishment. Thorup-Kristensen (2001) found that although crucifers had larger potential rooting depths than oats or rye at 1000 degree-days, it took 600 degree-days to reach the same root depths as those of the cereals (Figure 2.6-6). This was attributed to the greater seed size and sowing rates of cereals (100-200 kg ha^{-1}) that give them a head start compared with smaller-seeded crucifers or fine grasses (5-10 kg ha^{-1}). The latter will require a 20-fold increase in weight before they would surpass the biomass a cereal crop has at the outset of sowing.

Table 2.6-1. Mean rooting depth at harvest and their parameters of change over time for three catch crops as influenced by year and level of N supply (Herrera et al. 2010).

In Canterbury, NZ, Francis (1995) found that the effectiveness of a cover crop in preventing nitrate leaching depended on early planting and/or a delay in major rainfall events to enable sufficient time for crop development and significant uptake of soil-N. The sowing of oats did not significantly reduce leaching losses in 1991 as the major leaching events occurred in early winter (June), before the oats developed sufficiently to take up the bulk of the soil NO_3^--N. Conversely, in the following year (1992) when major drainage events occurred mainly in September, leaching losses were reduced by ~60% by the presence of an oat cover crop. McLenaghen et al. (1996) found

Canterbury autumn-sown cover crops were effective in preventing nitrate leaching after ploughing-in of a 2-year pasture, particularly those crops that had high surface coverage. Losses ranged in the order: fallow > bean > lupin > mustard > ryecorn > ryegrass, from 33 to 2.5 kg N ha^{-1} and were better correlated when root yields were included with plant shoot yields (R^2 improved from 0.465 to 0.627).

2.6.6 Modelling N leaching losses

Modelling the effects of catch crops on nitrate leaching losses is problematic because of the many variables involved e.g. climatic zones, time of sowing, crop type, soil temperature etc. Nevertheless, agricultural systems models can be used to simulate cover crop performance and impact on nitrate losses over a variety of locations, soils, management practices, and climate scenarios. They can also be used to identify research needs and to estimate the potential impacts of soil, air and water quality across a wider geographic area. For instance, Meisinger et al. (1991) using the Erosion Productivity Impact Calculator (EPIC) model (Williams et al. 1984), assessed the effect of winter cover crops by running simulations for 10 representative US sites, including two in the Corn Belt (Ames, IA; Jackson, IL). For these two sites, a barley winter cover crop following corn was predicted to reduce nitrate-N leaching by 66% for a sandy soil and by 70% for a clay-loam soil. Similarly, Malone et al. (2007), using a modified Agricultural Production Systems Simulator Model (APSIM), was able to predict that using a wheat cover crop would reduce nitrate leaching by 38% (341 vs. 537 kg N ha^{-1}) under 41-years of corn-soybean rotations and 150 kg N ha^{-1} applied to corn. However, in high nitrate loss situations the same authors found there remained a reasonably high degree of variability (~30%) (Malone et al. 2007).

Teixeira et al. (2016) recently calibrated and applied a biophysical model to isolate the impact of potential drivers of variability in the effectiveness of catch crops to reduce nitrate leaching in the Canterbury Plains of NZ. A sensitivity analysis of simulated results showed that sowing dates were the main contributor to total variability, followed by weather, factor interactions and soil WHC. The analysis showed that, compared to fallow treatments, winter cover crops reduced N leaching by an average (±95% CI) of 17 ± 8.2 kg N ha^{-1}. This represented a median N leaching reduction of ~50% with a wide interquartile range (6–75%).

Figure 2.6-7. Simulated distribution of nitrate leaching (90 cm depth) for fallow and winter cover crops sown at different dates on two Canterbury soils with contrasting water-holding capacities (WHC 80 and 160 mm m^{-1}) in response to 30 years of historical data. (Teixeira et al. 2016)

2.6.7 Winter forage cropping in dairy farm systems and nitrate leaching

A common goal for many New Zealand dairy systems is to have a fodder crop component in the winter diet of dairy cows, particularly in the South Island, so body condition can be regained after lactation ceases. Typically, cows are dried off at a body condition score (BCS) of 4.0-4.5 and then, ideally, built up again over the winter months to 5.0-5.5 to maximise milk production and reproduction potential in the following lactation (Judson & Edwards 2008; Houlbrooke et al. 2009a). Kale (*Brassica olercaea*) is a popular winter forage that lends itself to such usage because it is able to produce a large quantity of high quality DM per unit area and retain this over the winter (Brown et al. 2007). Sown in October/November, yields of 15-20 tonnes of DM ha^{-1}, 150-220 days after sowing, are not unusual and the forage is generally grazed only once (Brown et al. 2007; Judson & Edwards 2008). Because of the large quantities available, high stocking densities (10 m^2/cow on kale vs. 35-120 m^2/cow on pasture) are required to ensure high utilisation at a time when soil moisture levels are at, or close to, field capacity. The high water intakes contained in the crops

means that there are frequent cow urinations, around nine to twelve daily (Jenkinson et al. 2014). Unsurprisingly, the potential for drainage and N leaching losses over this period are high.

The initial potential for nitrate leaching from a urine patch is determined by both the volume and N concentration of the urination event. Li et al. (2012) modelled pastoral N leaching loss on two major New Zealand soil types, varying both volume and N concentration, showing that N leaching loss increased logarithmically with an increase in urine volume but exponentially with an increase in N concentration. Recent research by Jenkinson et al. (2014) suggests that average urinary-N concentrations from cows feeding on winter forage might be less than 3 g N/L and thus, is only half of average pastoral urinary-N concentrations. This effectively reduces N loading under the urine patch to ~300 kg N ha^{-1} but high stocking rates during grazing means overall urine volume and

coverage might be large (with the complication of urine patch overlap). This might occur quite frequently under winter forage grazing but there is little by way of predictive modelling for describing these effects on nitrate leaching (Li et al. 2012).

Many soil and management factors can interact with, and thus affect, nitrate leaching from winter forage grazing. For instance, many stony soils in Canterbury are expressly used for winter forage production because they provide some resistance to pugging but also because kale is drought tolerant and can be grown without irrigation, albeit for lower yields (<10 T ha^{-1}) (Stewart et al. 2014). However, these same soils have characteristically low water holding capacity and high drainage volumes over the autumn-winter-spring period and, consequently, nitrate losses post-winter grazing, are potentially large (Malcolm et al. 2015). Conversely, on soils of finer texture, an outcome of high stocking rates is the significant soil physical damage that may occur from surface treading (pugging) (Houlbrooke et al. 2009a) (Figure 2.6-8). This may reduce nitrate leaching losses through impeded drainage and increased denitrification losses (Van der Weerden & Styles 2012) but at the expense of increased runoff losses of P and sediment and reduced soil productivity (McDowell et al. 2003; Houlbrooke et al. 2009b).

Figure 2.6-8. Differences in (a) macroporosity (b) and bulk density values at 0–50 mm depth between pre- and post-grazing of a winter forage crop. Error bars represent LSD values (P =0.05) (Houlbrooke et al. 2009a).

Monaghan et al. (2007b) have identified that the dairy wintering forage component of dairy systems could represent as much as 60% of the farm's annual N leaching but from typically, less than 15% of the grazing platform (Figure 2.6-9). Water quality was shown to be degraded by comparison with less intensively farmed catchments, especially regarding N, P and faecal coliforms. Monaghan et al. (2013) attributed the greater N losses from the wintering part of these systems to (i) the relatively large amounts of mineral N remaining in the soil in late autumn following pasture cultivation and forage crop establishment the preceding spring and (ii) the deposition of much excretal N onto the grazed forage crop during winter when plant uptake is correspondingly low. There is, therefore, a need for mitigation strategies and policies to reduce N emissions from farms using winter forage grazing systems. Mitigation measures might take the form of low-emission wintering systems, such as confined feedpad operations, but there are added costs with harvesting winter forages and soil physical damage from mechanical harvesting may be as severe as animal trampling. Another option identified and considered more cost-effective was the use of a nitrification inhibitor to delay nitrification sufficiently long enough for uptake by a catch crop.

Figure 2.6-9. Relative area occupied and predicted contribution to stream N load of the different modelled land uses within the Bog Burn catchment, Southland (Monaghan et al. 2007a).

Shepherd et al. (2012) and Monaghan et al. (2013) have both recently investigated the use of nitrification inhibitors after winter grazing of brassica and beet crops and reported large N leaching losses from field trials after urinary-N deposition. Losses from experimental plots averaged ~150 (Taupo) and ~85 kg N ha^{-1} (Southland), respectively, with the application of the nitrification inhibitor DCD differing in its effectiveness in each study. In the Taupo trial, when DCD was applied as directed, within two days after urine application and then 6 weeks later, it was found to reduce soil NO_3^--N concentrations from a mean of 26 down to 17 mg N/L and N leaching losses by 20-27% (Table 2.6-2). In the Southland trial where DCD was only applied once, and up to 12 days after

urine deposition, it was considerably less effective and did not significantly reduce nitrate leaching losses.

The lack of any published data on the use of catch crops in winter forage grazing means a considerable knowledge gap exists about what mitigation strategies might best combat high nitrate leaching losses after grazing. Given that the benefits of a catch crop are optimised with early sowing and rapid establishment over the winter-early spring period, a number of research questions need to be answered. These questions are:

1. What crops are likely to be the most responsive over the drainage season to offer the best chance for significant N uptake?
2. How dependent is this response to soil temperatures and timing of urine deposition?
3. Could this be practically achieved in the field?

Table 2.6-2. Summary of N leaching losses (kg N ha^{-1}) post-grazing of the forage crops and pasture treatments in the study reported by Shepherd et al. (2012).

2.7 Nitrification inhibitors

2.7.1 Action modes

Nitrification rates can be affected by a number of factors (section 2.3.6) but there are a range of chemicals and conditions where nitrification can be directly supressed in soils. There are two basic modes of action associated with nitrification inhibition: (1) non-specific biological inhibitors and (2) chemically-based specific inhibitors. Non-specific factors inhibit nitrification by creating soil environments unfavourable to nitrifying organisms, through growth of competing micro-organisms, disruption of membranes and cell ultra-structure, interference with the reductive assimilation of carbon dioxide, respiration, or other metabolic activities common to other autotrophic micro-organisms. A range of compounds are known to inhibit nitrification including phenol, acetone, sulphides, sulfones, sulfoxides, dithiol, mercapto derivatives, azides, urethanes, guanidine, and some amino acids such as cysteine, methionine, and histidine (Hauck 1980). Specific inhibitors are generally chemicals that inhibit the activity of the autotrophic NH_4^+ oxidisers, *Nitrosomonas, Nitrosospira and Nitrosolobus* but ideally not nitrite oxidation (to prevent a toxic build-up of nitrite). They do so by a single action, or combination of actions, to bind enzymes, hemeproteins and/or chelate metals (such as Cu), that are involved in the oxidative process, or affect uncouplers of oxidative phosphorylation or electron transfer, or act in trapping free radicals (Hauck 1980). A range of chemicals can act as both specific and non-specific inhibitors but non-specific inhibitors

may affect other unrelated soil biological processes or interfere in nitrite oxidation; most research has therefore concentrated on the action and effect of specific inhibitors.

2.7.2 Effect of nitrification inhibitors on plants and soil-N processes

The major use of nitrification inhibitors, until relatively recently, has generally been in cropping where research on inhibitors such as nitrapyrin (2-chloro-6-trichloromethyl pyridine) have shown increases in crop N responses, particularly in high rainfall areas or years, or for autumn or spring sown crops (Haynes 1986a; Rodgers 1986). For instance, Swezey and Turner (1962) found nitrapyrin, at 1% of fertiliser-N content, significantly increased crop response for urea and aqueous ammonia application (Figure 2.7-1). The use of nitrapyrin has also been shown to decrease nitrate leaching. Owens (1987) found that the 6-yr annual average NO_3^- losses from lysimeters growing a corn crop and fertilised with nitrapyrin-coated urea and untreated urea were 117 and 160 kg N ha^{-1}, respectively. Similarly, Ronaghi et al. (1993) conducted a corn study investigating the effect of nitrapyrin on NO_3^- leaching from pots containing a sandy clay loam soil. . Nitrogen fertiliser in $(NH_4)_2SO_4$ form was added at 5 different rates (0, 50, 100, 200, and 400 kg ha^{-1} of N) and three rates of nitrapyrin (0, 2.36 and 4.72 L ha^{-1}) were used, with two leaching rates (0 and 25 mm per week over field capacity in two 500 ml increments at 3 and 6 weeks after planting). Average results across all five N application rates showed that the proportion of NO_3^- leached was reduced by approximately 60% when nitrapyrin was applied at 4.72 L ha^{-1} (Figure 2.7-2).

The effectiveness of such nitrification inhibitors, however, has been found to be subject to modification by many factors. For instance, the activity of nitrapyrin is reduced by adsorption onto SOM, chemical hydrolysis and volatilisation whilst the various genera and strains of nitrifiers differ in their sensitivity to such chemicals (Haynes 1986a). Sahrawat (1980) reviewed a number of cropping trials using nitrapyrin and found growth responses and effects on nitrate leaching varied considerably from none to significant depending on fertiliser type, soil conditions and temperature.

Figure 2.7-1. Cotton yield for urea and aqueous ammonia fertiliser treated with 0, 0.5 and 1% nitrapyrin. An uncommon letter within a column series denotes a significant difference at 5% level. Adapted from Swezey and Turner (1962).

Figure 2.7-2. Recovery of applied N fertiliser (averaged over all rates) in leachates, roots/soil, denitrification gas and plant uptake for untreated and nitrapyrin-treated soil after leaching with 1 pore volume of water. An uncommon letter between recovery fractions denotes a significant difference at 5% level. Adapted from Ronaghi and Soltanpour (1993).

Research in NZ using the nitrification inhibitor dicyandiamide (DCD) has demonstrated that treating effluent with DCD could reduce N leaching, albeit at large concentrations due to a high degradation potential at temperatures over 16°C (Williamson et al. 1996). In a subsequent lysimeter study, Williamson et al. (1998) measured nitrate leaching from a high total effluent loading of 1100 kg N ha^{-1} over seven months (fortnightly application) was reduced by 18% after applying DCD in May, 130 days after starting the effluent application. However, direct leaching losses of nitrate from applied N fertiliser and/or farm dairy effluent are usually comparatively small relative to that leached from animal urine patches (Cameron et al. 1999; Ledgard et al. 1999; Silva et al. 1999; Di & Cameron 2002a; Cameron & Di 2004). It is the surplus of N in urine patches, when oxidised to nitrate, that is most likely to leach once the onset of soil drainage occurs over the NZ autumn-winter-spring period. Soil profiles at this time are generally at, or near, water-holding capacity (WHC) so the leaching potential is particularly high as excessive soil water drains from the rooting zone. Similarly, the potential for nitrous oxide emissions is also enhanced due to the simultaneous presence of high NO_3^- concentrations and reduced air-filled pore space leading to conditions where denitrification can occur (section 2.4.2). In intensively grazed pasture systems the adoption of nitrate leaching and nitrous oxide mitigation technologies requires a focus on reducing losses from the highly N concentrated urine patch areas. Consequently, this is where most recent research in NZ on the use of nitrification inhibitors in intensive pastoral grazing has been targeted e.g. (Di & Cameron 2002b; Di & Cameron 2003, 2004b, 2005; Monaghan et al. 2009; de Klein et al. 2011; de Klein & Monaghan 2011; Monaghan et al. 2013).

2.7.3 Dicyandiamide

In recent years, one of the most examined specific inhibitors has been dicyandiamide (DCD, Figure 2.7-3), a dimeric form of cyanamide, created from calcium cyanamide ($CaCN_2$) that is manufactured from atmospheric nitrogen and calcium carbide (CaC_2) (Slangen & Kerkhoff 1984). The compound is completely biodegradable in soil (Williamson et al. 1996) and is transformed, mainly by micro-organisms, through several intermediaries by the addition of water and decarboxylation to guanylurea and guanidine and, finally, urea. The latter is quickly degraded by the enzyme urease so the end products of DCD degradation are CO_2, NH_3, and H_2O (Amberger 1989).

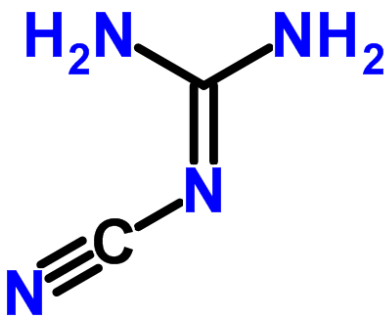


Figure 2.7-3. Diagrammatic representation of the dicyandiamide (DCD) molecule.

Dicyandiamide acts by occupying the site at which NH_4^+ ions are converted into NO_2^- ions, on an enzyme called ammonia monooxygenase contained within the *Nitrospira* and *Nitrosomonas europaea* bacteria (Figure 2.7-4). Specifically, the inhibition of *Nitrosomonas* is mediated by the reaction of the C≡N group of DCD with the sulfhydryl or heavy metal groups of the bacteria's respiratory enzymes (Amberger 1989).

Figure 2.7-4. Depiction of DCD's action on the enzyme ammonia monooxygenase (Christie & Roberts 2004).

Research into DCD use in intensive grazed pastures has enabled the application of DCD on its own (i.e. without fertiliser) to be used to inhibit nitrification in animal urine patches. Dicyandiamide diffuses into the urine patch area, interrupting the ammonium oxidative process, the first stage of nitrification (Figure 2.7-5). One of the main benefits of DCD used in this way is the increase in the efficiency of the N cycle and reduced environmental impacts of dairy farming in particular, and consequently, increased pasture production (Cameron & Di 2004; Moir et al. 2007; Carey et al. 2012; Monaghan et al. 2013). Conserving the N supply already present in soil generally means lower N losses from the soil by leaching and gaseous emission (Di & Cameron 2002b; Di & Cameron 2004a, 2005, 2006).

Figure 2.7-5. Depiction of DCD inhibitor activity slowing ammonium oxidation in the nitrification process (Christie & Roberts 2004).

2.7.4 Effect of DCD on soil processes and pasture production

Two main effects occur with the application of DCD to urine-affected soil, NH_4^+-N concentrations remain higher for longer, whilst NO_3^--N concentrations increase more slowly than in a urine-affected soil without DCD (Di & Cameron 2004c). The effects of DCD on reducing nitrate leaching and nitrous oxide emissions have been shown to be substantial in perennial ryegrass/white clover pastures, the main plant species used in NZ intensive pastoral systems. However, the magnitude of this effect has been shown to vary depending on soil, climatic and application conditions (Di et

al. 2007; de Klein et al. 2011; Di & Cameron 2012; Carlson et al. 2013). Application of DCD to dairy pastures has been shown to reduce average annual NO_3^- leaching losses on a range of soils, 58-72% on a Canterbury Templeton sandy loam (Figure 2.7-6), 45-83% (av. 63%) on a Canterbury Lismore stony silt loam, 56-71% on a West Coast Hari Hari recent silt loam, and 44-67% on a Southland Mataura recent sandy loam (Di & Cameron 2002b, 2007; Di et al. 2009a). In a national series of nitrous oxide mitigation research (NOMR) trials in South Otago, Canterbury, Manawatu and Waikato regions, mean reductions in nitrate leaching from DCD treatments on autumn-applied urine were reported from 9-68% for a range of soil types and climatic conditions (Table 2.7-1).

Figure 2.7-6. Effect of the nitrification inhibitor DCD on the total amounts of NO_3^--N leached from urea/urine-treated lysimeters containing a Canterbury Templeton sandy loam. Vertical bars indicate SEM (Di & Cameron 2004b).

Nitrous oxide emissions from urine patches in intensive dairying have also been shown to be significantly reduced after DCD application and in many cases by more than 50% (Di & Cameron 2003, 2006; Ball et al. 2012; Gillingham et al. 2012; Baral et al. 2014; de Klein et al. 2014) (Figure 2.7-7). In the series of national NOMR trials, mean reductions in N_2O emission factors (EF) from DCD treatments ranged from 51-68% for autumn-applied urine (Table 2.7-1). The build-up in soil nitrate concentrations under a urine patch, and the wet conditions that can prevail over the autumn-winter-spring period, encourages denitrification and high N_2O loss but DCD application has been found to reduce these, especially in the first few months after urine application (Ball et al. 2012; de

Klein et al. 2014). Although many factors affect N_2O loss, including soil temperature and DCD degradation rates (Kelliher et al. 2008), the ability of DCD to substantially reduce these under the field-saturated conditions that prevail over this period is potentially an important tool for control of GHG emissions (Ball 2013; Saggar et al. 2013).

Table 2.7-1. Mean and range of nitrate leaching and nitrous oxide emission factor (EF) reductions for DCD treatments applied to urine patches over autumn (April/May) compared to control (no-DCD) treatments for the NOMR national series of trials (2009-12). Data from de Klein et al. (2014), Cameron et al. (2014), Kim et al. (2014) and Ledgard et al. (2014).

	NOMR trial region			
	Otago	**Canterbury**	**Manawatu**	**Waikato**
Reduction due to DCD	%			
Nitrate leaching	9 [a] (8-11)	49 (48-69)	22 [b] (-)	58 (48-68)
Nitrous oxide EF	52 (33-70)	66 (41-82)	68 (54-78)	51 (19-77)

[a] low overall nitrate leaching (<15 kg N ha^{-1}); [b] 2011 data only;

Pasture production has been shown to increase under DCD use, especially on urine-treated areas (Di & Cameron 2002b; Di & Cameron 2004b; Moir et al. 2007; Sprosen et al. 2009), with increases in annual pasture yield reported from 15-36% (Figure 2.7-8). Further work by Moir et al. (2007) suggested that DCD use not only increased production on the urine-treated areas but also the inter-urine areas (Figure 2.7-9) between patches suggesting DCD application improves the efficiency of nutrient cycling in the non-urine patch areas too. However, other researchers have found variable responses to DCD use in a recent national series of small plot trials ranging from nil to significant (Cameron et al. 2014; de Klein et al. 2014; Kim et al. 2014; Ledgard et al. 2014). Recent reported field measurements on DCD-treated dairy pastures from farms around the country have suggested that the DCD effect on pasture yield is significant (Figure 2.7-10) and is probably because of more efficient N cycling within the pasture (Carey et al. 2012). Moir et al. (2007) has also suggested that DCD use can reduce high NO_3^- levels in pastures (Figure 2.7-11), a known problem for grazing animals, especially in spring or after dry periods (Vermunt & Visser 1987).

Figure 2.7-7. Effect of DCD on nitrous oxide emissions from two Canterbury soils treated with urine and/or urea in May (a -Lismore), August (b -Lismore) and May (c -Templeton) (Di & Cameron 2006).

Figure 2.7-8. Total annual N offtake (a) and pasture yield (b) for lysimeters treated with urea/urine only or urea/urine plus a single (May) or a double (May and August) DCD (eco-n) application containing a Templeton sandy loam soil (Di & Cameron 2004b).

Figure 2.7-9. Average annual DM yields (2002/3-2004/5) for urine and inter-urine areas (a) and estimated cumulative pasture growth curves for non-treated and DCD-treated pastures using regression of field data (b) (Moir et al. 2007).

Figure 2.7-10. Mean percentage increase in DM yield responses for DCD-treated pastures for spring-only, full year and both (mean of spring and full year trials) for North Island, South Island and New Zealand (overall means) (Carey et al. 2012).

Figure 2.7-11. Pasture NO_3^--N levels on all treatments on 4 August 2005, three months after last grazing and treatment application (Moir et al. 2007).

2.7.5 Factors affecting DCD effectiveness

The stability of DCD is an important factor in maintaining the nitrification inhibitory effect, with temperature an important arbiter of degradation rates (Kelliher et al. 2008). Temperature-dependent degradation of DCD has been shown for bacterial isolates under culture conditions, with optimal rates around 25°C (Hauser & Haselwandter 1990). Indeed, Di and Cameron (2004c) found that the half-life ($t^{½}$) of DCD was reduced from 111-116 days at 8°C to 18-25 days at 20°C (Figure 2.7-12). Although maximum leaching potential usually coincides with the coolest time of the year when soil temperatures are less than 10°C, urinary-N applied over the autumn period is still at considerable chance of leaching due to the soil being warm enough to allow rapid nitrification unless DCD has been applied recently. Kelliher et al. (2014) recently reported first-order relationships for DCD degradation for a range of soil temperatures at one field grazing site. Half-lives decreased with increasing temperature where, for example, $t^{½}$ for values at 8°C and 16°C were 39 ±6 and 25 ±3 days (±95% confidence limit), respectively. A comparison of two sites separated by 1000 km found $t^{½}$ differed significantly for the same temperature and was attributed to differences in microbial activity between the sites. Part of the reason might lay in the explanation that DCD degradation is not solely mediated by one type of microbial organism and that syntrophy or cross-feeding between different bacteria consortia influence degradation rates (Schwarzer et al. 1998). Because soils differ in these respects it is important to apply sufficient DCD to maintain an inhibition effect long enough to cover the main part of the leaching season. Di and Cameron (2005) found for a Canterbury grazed pasture that 10 kg a.i. ha^{-1} provided a satisfactory nitrification inhibitory effect but 5 kg AI ha^{-1} was far less effective (Figure 2.7-13). In practice, the rate of nitrification and magnitude of the inhibitory effect is generally much lower under high soil temperatures than low (Figure 2.7-14), and requires application rates of 10 kg AI ha^{-1} applied twice over the autumn-winter-spring period to achieve a sufficient effect (Di & Cameron 2005). Other factors also influence degradation rates. Rodgers et al. (1985), for example, found that DCD persistence was greater in acid than in near-neutral soils presumably influenced by differences in microbial activity. Soil organic matter has also been implicated in DCD effectiveness, with degradation rates increasing with increasing SOM and explained by way of increased sorption and subsequent microbial degradation (Slangen & Kerkhoff 1984; Kelliher et al. 2008).

Figure 2.7-12. Effect of soil temperature on DCD degradation rates at 8°C (a) and 20°C (b) over time for two different DCD application rates (7.5 and 10 kg a.i. ha^{-1}) (Di & Cameron 2004c).

Figure 2.7-13. Effect of DCD rate (0, 5 and 10 kg Al ha^{-1}) on total nitrate leached from a urine-treated pasture (Di & Cameron 2005).

Figure 2.7-14. Effect of soil temperature at 8°C (a) and 20°C (b) on soil ammonium and nitrate concentrations over time for two different DCD application rates (7.5 and 10 kg a.i. ha^{-1}) (Di & Cameron 2004c).

2.7.6 Future of nitrification inhibitors in NZ agriculture

Whilst the benefits of nitrification inhibitors, and DCD in particular, as a means to reduce nitrate leaching have been demonstrated, their use in NZ agriculture has been complicated because DCD sales have been voluntarily suspended after trace amounts of DCD were detected in NZ milk powder (Cronshaw 2013; Ministry of Primary Industries 2013). Although only trace amounts were found and no food safety issues are associated with DCD ingestion, a decision was made to suspend its use because no internationally-agreed standard exists for DCD presence in milk and thus there is no recognised acceptable maximum residue limit. Until DCD is registered on the CODEX Alimentarius standard for food, its use remains suspended. However, if/when DCD is registered on the CODEX, there will be a need for its effectiveness in reducing nitrate leaching losses from winter forage grazing systems to be accurately quantified.

At present, there is conflicting data from the small number of published studies on the effectiveness of DCD to reduce nitrate leaching losses under winter forage grazing and this represents a considerable knowledge gap that remains to be quantified over the smaller, and larger, scale.

2.8 Summary

A review of the literature shows that nitrate leaching losses in dairy wintering systems using winter forage grazing are potentially large and could potentially represent almost half of a farm's total N leaching loss from a small fraction of the farm's total grazing area (Monaghan et al. 2007a). Strategies to combat these losses could include planting a catch crop to capture a significant fraction of the N applied in urine and excreta before it can be leached and/or using a nitrification inhibitor to reduce nitrate leaching losses. However, efforts to reduce these losses using DCD-based nitrification inhibitors have been stopped because of the voluntary withdrawal of DCD from the market due to traces being found in milk products. Planting catch crops over the winter may prove problematic, with too little growth to prevent significant nitrate leaching; currently there is a paucity of data on their potential effectiveness under the various scenarios, however.

The main conclusions drawn from this literature review are:

- Although it is hypothesised that catch crops could reduce N losses from winter forage grazing systems, there is no published data available for the effectiveness of catch crops sown post-winter forage grazing to reduce nitrate leaching losses.
- There are very few published papers reporting the use of DCD to reduce nitrate leaching from winter forage grazing systems in NZ; the few published data shows conflicting results on the effectiveness of DCD.
- There is no published data quantifying the effect of DCD in conjunction with a winter-sown catch crop to reduce nitrate leaching losses from winter forage grazing systems.
- Although drainage soon after urine application will contribute to increased N leaching losses, there is relatively limited understanding and little published data on the sensitivity of drainage pattern, soil temperature and urine application (both volume and loading) to catch crop sowing date (i.e. speed of catch crop establishment), especially as it relates to South Island dairy wintering systems.

The main objectives of this PhD research programme will therefore be:

1. To quantify nitrate leaching losses from a simulated winter forage grazing system and to determine the effect of sowing a catch crop and/or applying a nitrification inhibitor (DCD)
    - *Hypothesis 1: Nitrogen leaching losses from single and double urine applications to a stony, free-draining Balmoral soil after winter forage grazing are additive.*
    - *Hypothesis 2: Sowing of a winter/spring catch crop and/or applying DCD after winter forage grazing will have separate and additive effects on reducing nitrate leaching losses.*
2. To quantify the main effect and interaction effect of date-of-urine-application and date of catch crop sowing on N leaching losses.

- *Hypothesis 3: Early establishment of a catch crop following winter forage grazing reduces nitrate leaching losses.*

3. To achieve a better understanding of the effects of soil temperature, drainage, light intensity and catch crop establishment on nitrate leaching losses from winter forage grazing systems and the chief processes involved.
    - *Hypothesis 4: Timing of urine application, catch crop establishment and interactions with soil temperature and light intensity following winter forage grazing will be critical factors influencing nitrate leaching losses.*

This PhD study will primarily focus on discovering new knowledge and understanding of the above whilst providing insight into the mechanisms and importance of factors in minimising nitrate leaching from winter forage crops.

Chapter 3 General Materials and Methods

"A summary of soil descriptive data, field techniques, trial implementation and analysis methods used to conduct the field lysimeter experiments."

3.1 Introduction

Three experiments were conducted in total; two field lysimeter experiments (2013-14 -year 1; 2014-15 -year 2) and one repacked soil column experiment in a temperature, humidity and light-controlled growth cabinet facility (2015-16) at Lincoln University (Field Research Centre). Each experiment received a single initial application of urine, except in year 1 when two treatments received a second application a week after the first. The soil for all lysimeter experiments was collected *in situ* from the Ashley Dene research station, near Springston, Canterbury (43° 39' 2" S, 172° 19' 45" E) (Appendices A & B) . Soil descriptions, properties and classification are discussed in section 3.3. Details of materials and methods specific to each experiment are provided in the relevant chapter.

3.2 Lysimeter collection

The collection and installation of the lysimeters used in experiments 1 and 2 occurred during the summers of 2012-13 (early January) and 2013-14 (early February), respectively. Collection was carried out essentially following the method of Cameron et al. (1992) where a single lysimeter unit comprised a cylindrical steel casing (0.5 m diameter and 0.7 m deep) with an internal cutting ring, a base plate and four steel bars to secure the base plate.

A field site was identified and fenced off from stock grazing approximately three months before lysimeter collection. The site was an established pasture, consisting of a mixture of perennial ryegrass (*Lolium perenne* L., cultivar Prospect) and white clover (*Trifolium repens* L., cultivar Kopu 11). Using a digger, a trench was dug on either side of the sampling area and the pasture where the lysimeters were to be taken from, was cut to ground level using hand clippers. The lysimeter collection procedure involved placing the hollow lysimeter casing on the pasture/soil surface with the internal cutting ring on the bottom edge. A small trench was dug around the casing to expose a 100-mm depth of soil (Plate 3.3-1) and then the casing was gradually pushed down around the exposed soil column to minimise soil structural disturbance within the casing. This step was repeated, with any stones protruding from the side of the monolith removed as required, until the surface of the soil was within 25 mm of the top of the casing (Plate 3.3-1). The internal cutting ring within the casing created a 5-mm gap between the side of the casing and the soil monolith.

Liquefied (50°C) petroleum jelly was injected into the gap until it reached the top of the casing. On cooling, the petroleum jelly prevented edge flow between the soil monolith and the side of the casing. At a depth of 700 mm, a hydraulically-assisted cutting plate was used to separate the soil column from the subsoil. Each lysimeter was uplifted and inverted and c. 50 mm of soil and gravel removed from the base and replaced with a layer of coarse gravel before attaching a drainage base plate to collect leachate (Plate 3.3-2). The installation of a gravel layer at the base of each lysimeter reproduced a situation common to the Canterbury Plains where soils often overlie coarse gravels. The gravel layer also allowed a free drainage system to be used in the lysimeter study, as matric potential in this layer was assumed to be zero (Clothier et al. 1977).

After collection, the lysimeters were lifted onto a specially designed trailer with air-bag suspension to minimise soil disturbance and transported to a field trench lysimeter facility located at the Field Research Centre, Lincoln University, 20 km south of Christchurch (43° 38’ 52” S; 172° 28’ 7” E). A 10-mm drainage tube was connected to the base of each lysimeter prior to installation that then drained into a sealed 10 L collection can. The top of each lysimeter was positioned flush with the surface of the soil surrounding it to ensure that the lysimeters were exposed to the same environmental conditions as the rest of the field (Plate 3.3-2).

3.3 Soil

3.3.1 Classification

The soil used in the lysimeter experiments was a Balmoral stony silt loam, classified as an Acidic Orthic Brown soil in the NZ classification system (Hewitt 1993). These soils sit in the high terraces of the Canterbury Plains and are derived from greywacke alluvium built up from the remnants of huge alluvial gravel fans deposited around 20,000 years ago, after the last Otiran Glaciation. The key properties of this stony silt loam are its friable nature with stones present throughout the profile, free-draining nature and unlimited aeration in the root zone (Plate 3.3-3). Historically dedicated to dryland farming for sheep meat and wool, they are droughty without irrigation but the implementation of irrigation schemes has enabled increased diversification into dairying and dairy support (Molloy 1988).

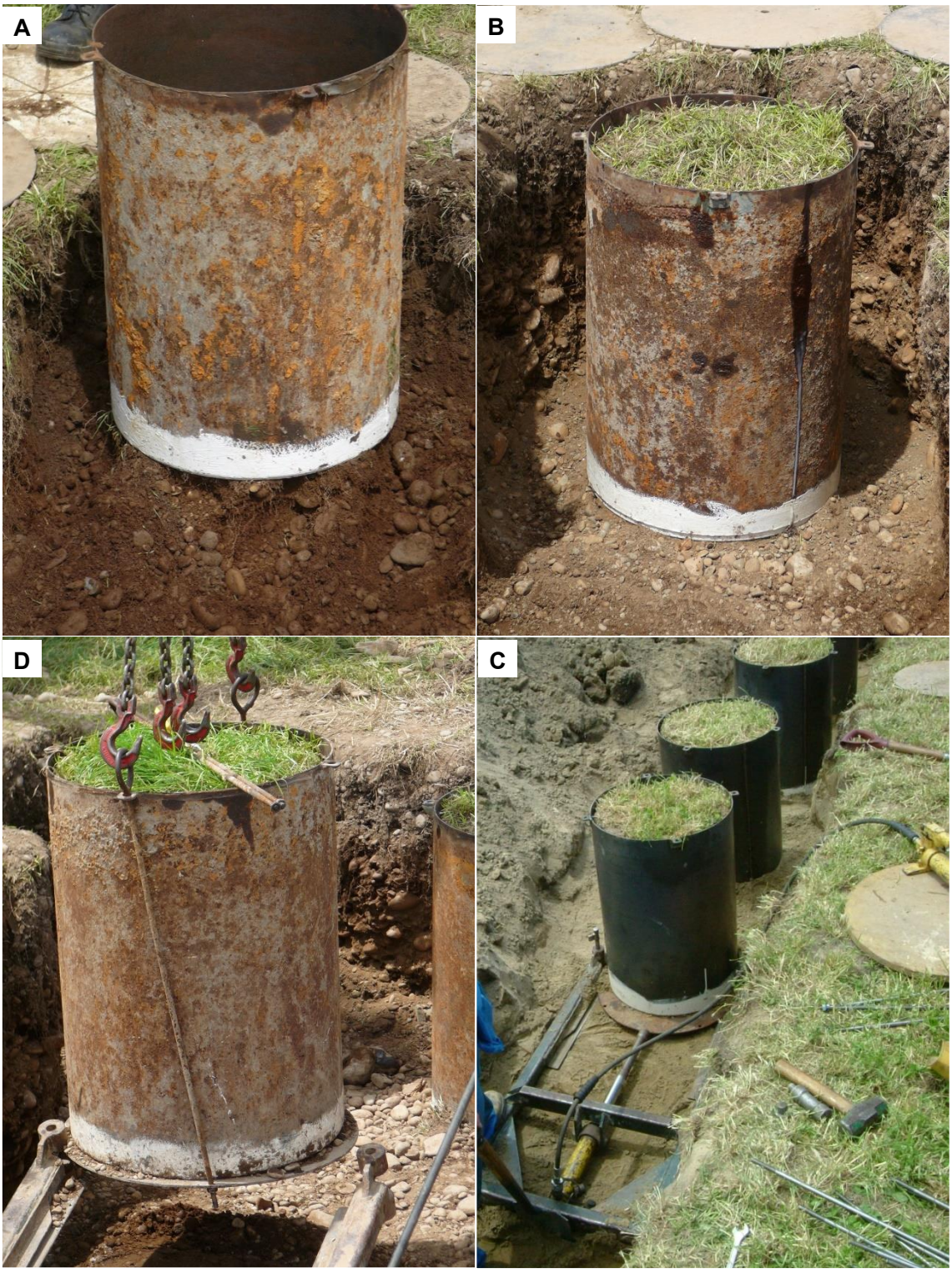


Plate 3.3-1. Positioning, collection and removal of lysimeters from the field site. From top left, clockwise: A) positioning and digging down of lysimeters into Balmoral soil pasture site; lysimeter case fully down, B) annular gap filled with vaseline and prior to insertion of cutting plate, C) insertion of cutting plate below casing using hydraulic ram; D) tractor removal of soil monolith.

Plate 3.3-2. From top-left clockwise; A) inversion of lysimeter in preparation of installation of gravel drainage base, B) lysimeters installed in field trench with transplanted kale, C) pugging of lysimeter surface with steel hoof post-kale removal, D) application of urine after treading.

Plate 3.3-3. Profile of Balmoral soil

Location: Ashley Dene research station, Springston, Canterbury.

(43° 38’ 41” S, 172° 20’ 33” E).

Soil Group: Acidic Orthic Brown Soil (Cutler 1968; Hewitt 1993)

Classification: Dystrudepts Inceptisol (Soil Survey Staff 2014)

Table 3.3-1. Soil profile description of Balmoral soil (Webb & Bennett 1986).

Horizon	Depth (cm)	Description
A_p	0-18	Very dark greyish brown (10YR 3/2); stony silt loam; friable; weakly developed fine nut and granular structure; many fine roots; 10% stones.
B_w	18-34	Dark yellowish brown (10YR 4/4); very stony silty loam; friable; weakly developed very fine nut structure; many fine roots; many casts; 40% stones.
B_{w2}	34-56	Yellow-brown, nutty/crumb, stony, fine sandy loam, friable.
C_u	56-70	Dark greyish brown to olive brown (2.5 Y4/3); very stony sand; loose few fine roots; 50% stones.
C	70-90	Gravels with pale yellow-brown silt/sand matrix.

3.3.2 Physical and chemical properties

Soil bulk density was calculated from samples collected during the deconstruction procedure of the lysimeters used in experiment 1 (section 4.2.6). From 0-40 cm, 10 cm increments were sequentially exposed and removed into bins where the stones were separated from the finer soil fraction (< 5 mm). Sub-samples of the soil fraction were air-dried for soil moisture content (30°C) and remaining stone above 2 mm sieved out. The 40-60 cm soil fraction was analysed as a single layer. Soil bulk density (SBD) was calculated using equation 3.3-1:

$$Soil\ bulk\ density\ (P_b) = \frac{(M_s + M_{st})}{V_i}$$

3.3-1

where P_b is the dry bulk density (g cm^{-3}), M_s is the weight of dry soil (105°C), M_{st} is the weight of stone and V_i is the volume of the depth increment.

Total soil porosity for each depth, and the mean for each lysimeter (0-60 cm), was calculated as follows (3.3-2):

$$Total\ soil\ porosity\ (F) = \sum_{n=1}^{N} n\left(1 - \frac{P_b}{P_d}\right)$$

3.3-2

where F is total soil porosity (cm cm^{-3}), n is the number of depth increments (40-60 cm = 2n), P_b is dry bulk density (g cm^{-3}) and P_d is particle density nominally assumed as 2.65 g cm^{-3}, mid-point in the range (2.6-2.7 g cm^{-3}) commonly found in mineral soils (Hillel 1998b).

Table 3.3-2. Mean Balmoral soil bulk density, porosity, stone volume and soil texture.

Soil depth (mm)	Soil bulk density (g cm^{-3})	Porosity (% v/v)	Stone vol. (% v/v)	[1] Soil texture
0-10	1.10	58	11	stony ZL
10-20	1.54	42	30	stony ZL
20-30	1.88	29	51	stony SL
30-40	2.02	24	54	stony S
40-60	1.93	27	51	gravelly S
Mean	**1.73**	**35**	**39**	

[1] ZL -silt loam; SL -sandy loam; S –sand

Table 3.3-3. Key soil fertility and physical properties (0-7.5 cm) of Balmoral stony silt loam (fine earth fraction <2 mm ie. excluding stones).

Analysis	Mean value (Units)
pH	5.9
Olsen-P	24 (mg P L^{-1})
ASC[1]	42 (%)
Exch-K^+	0.9 ($cmol_c$ kg^{-1} soil)
Exch-Ca^{2+}	7.8 ($cmol_c$ kg^{-1} soil)
Exch-Mg^{2+}	0.7 ($cmol_c$ kg^{-1} soil)
Exch-Na^+	0.1 ($cmol_c$ kg^{-1} soil)
CEC[2]	17 ($cmol_c$ kg^{-1} soil)
Total BS%	57 (%)
Volume weight	0.9 (g ml^{-1})
Sulphate-S	10 (mg kg^{-1})
Extractable Org-S	2.0 (mg kg^{-1})
TBK[3]	3.8 (cmol kg^{-1} soil)
Organic matter	72 (g kg^{-1})
Total-C%	4.5 (%)
Total-N%	0.41 (%)
C/N ratio	11.0

[1] Anion storage capacity; [2] Cation exchange capacity; [3] Reserve-K analysis.

3.4 Experimental treatments

3.4.1 Field experiment structure

Two field lysimeter experiments were conducted over the winters of 2013 (year 1) and 2014 (year 2) comprising of 32 and 48 lysimeters, respectively, with established kale plants in place. In year 1, there were a total of eight treatments consisting of two plant types (i.e. catch crops): Italian (It.) ryegrass *(Lolium multiflorum* L.) and oats (*Avena sativa* L.); three rates of urine application (control, 350 and 700 kg N ha^{-1}) and two dicyandiamide (DCD) treatments (0 and 20 kg N ha^{-1}). The second rate (700 kg N ha^{-1}) of urine application was spit over two application dates, the first on June 26, the second 7 days later, on July 3. This was intended to simulate a double application of urine that might occur during "break feeding" of the cows. The DCD treatment was applied only to the 350 kg N ha^{-1} rate of urine application for both catch crops. (Table 3.4-1).

Table 3.4-1 Field lysimeter treatments, catch crops and dates for urine and DCD application in year 1 (2013; table A), and urine application and sowing day in year 2 (2014; table B).

A: Year 1

Treatment ID	Crop	Urine rate (kg N ha^{-1})	DCD rate (kg a.i.ha^{-1})
IR: Control	Italian ryegrass	0	-
IR: U350		350	0
IR: U700		700	-
IR: U350+DCD		350	20
OT: Control	Oats	0	-
OT: U350		350	0
OT: U700		700	-
OT: U350+DCD		350	20
Urine/DCD rates		**Application dates**	
U350	(2x 350)	Jun 26	-
U700		Jun 26	Jul 3
DCD		Jun 27	-

B: Year 2

Treatment ID (oats)	Urine date	Sowing day
E: fallow	Early (Jun 6)	∞
E: day 1		1
E: day 21		21
E: day 42		42
E: day 63		63
M: fallow	Mid (Jul 6)	∞
M: day 1		1
M: day 21		21
M: day 42		42
L: fallow	Late (Jul 25)	∞
L: day 1		1
L: day 21		21

In both years, each treatment was replicated four-times, and in blocks of four-to-eight, within the field lysimeter trench facility (Appendix C). Although lysimeters were randomly assigned within the trench, treatments were not in a randomised block design due to difficulties this would present in management although separation distances between lysimeters were generally small.

3.4.2 Urine collection

Fresh cow urine was collected, by hand, from a Friesen/Jersey cross herd, grazing winter kale on the Lincoln University Dairy farm. Collection was at afternoon milking with the urine bulked and mixed, prior to total-N analysis (Elementar Vario-Max CN elemental total carbon and nitrogen analyser, Elementar GmbH, Hanau, Germany), and then stored at 4°C for 1-2 days before application. Urine-N concentration was diluted with deionised water from 4.0 g N/L and 5.6 g N/L in years 1 and 2, respectively, to 3.5 g N/L, to obtain values that were more representative of those reported for urinary-N concentrations for winter forage grazing cows (Edwards et al. 2014a). Urine was applied at a rate of 2 L per lysimeter for an approximate depth of 10 mm.

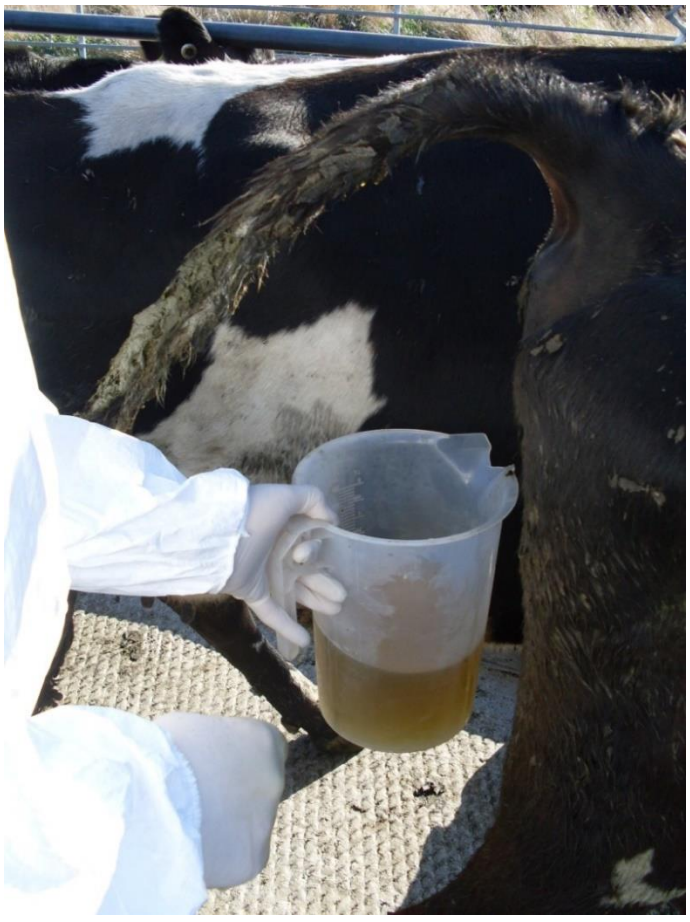


Plate 3.4-1. Urine collection in milking yard for lysimeter application.

Urine application involved pouring 2 L of the urine from a height of about 500 mm directly to the surface of the lysimeter (equivalent depth of 10 mm at 350 kg N ha^{-1}). Control treatments received 2 L of deionised water but containing the same quantity of ^{15}N as applied in the urine treatments (equivalent to 32 kg N ha^{-1}). For the 700 kg N ha^{-1} treatments in year 1, a second 2 L of similarly ^{15}N-labelled urine was applied one week later to provide an additional 350 kg N ha^{-1}.

3.4.3 Fertiliser application

Prior to lysimeter collection in 2012, the site received 3 T ha^{-1} of lime in October 2011 and 200 kg ha^{-1} of 15% potassic superphosphate in August, 2012. After transplanting of the kale in January, 2013, 50 kg N ha^{-1} of urea was applied in two applications (2x 25 kg N ha^{-1}), in February and April, 2013, to help plant establishment (Table 3.4-2). Basal fertiliser application was similar in year 2 although due to the shorter duration of the trial, there were no further applications once it began.

Table 3.4-2. Basal lime and fertiliser applications to year 1 and 2 trials.

Fertiliser	Rate	Nutrients		Date
Year 1				
Lime	3 T ha^{-1}	kg ha^{-1}		18/10/2011
15% potash super	200 kg ha^{-1}	P	15	30/8/2012
		K	18	
		S	15	
Urea	110 kg ha^{-1}	N	25	20/2/2013
		N	25	2/4/2013
30% potash super	100 kg ha^{-1}	P	6	23/9/2013
		K	15	
		S	8	
Di-ammonium phosphate	200 kg ha^{-1}	N	36	6/12/2013
		P	40	
Year 2				
15% potash super	200 kg ha^{-1}	P	15	12/2/2014
		K	18	
		S	15	
Urea	110 kg ha^{-1}	N	50	27/3/2014

3.5 Climate data

Local rainfall, air and soil temperature data were collected using the climate station at the Lincoln University research dairy farm (Plate 3.5-1). Rainfall was measured using a Hydrological Services (Sydney, Australia) model TB3 tipping bucket collector while solar radiation was measured with a CMP3-L Pyranometer by Campbell Scientific. Ground (at 10 cm depth) and air temperature were determined by 107 Campbell Scientific probes that incorporate a Fenwal Electronics UUT51J1 Thermistor. Data was stored on a Campbell Scientific datalogger and sent telemetrically. Other climate and rainfall data were collected from the NIWA Broadfields meteorological station, 2 km from the trial site. Historical data for Lincoln was downloaded from the CliFlo NIWA national climate database (NIWA 2016) and means aggregated over a 35-year period from 1980-2014.

Plate 3.5-1. Lincoln University dairy research farm climate station.

3.6 Rainfall and irrigation simulation system

The rainfall and irrigation simulation system (RISS) was used to best simulate actual rainfall and irrigation events, particularly in terms of application frequency, intensity and rate. The system is designed to generate sufficient drainage water during the winter/spring period to achieve a complete nitrate breakthrough curve and does this by setting daily average rainfall and evapotranspiration data to the 75th percentile, as defined by the base setting period (1975-1999).

The system operates on predefined daily climate parameters derived from data collected by the NIWA Broadfields weather station. The system was calibrated to apply water in 0.5 mm bursts to avoid saturated flow. The system is described in full in Appendix D.

3.7 Statistical analysis

Statistical analysis was done using both balanced and unbalanced (non-orthogonal comparisons) analysis of variance (ANOVA) within the Genstat 9.2 statistical package (Lawes Agricultural Trust 2007). Further details on specific statistical methods used are given in each chapter. Unless otherwise stated, a least significant difference (LSD) value of 95% confidence (0.05) is displayed as a bar on figures and quoted in tables.

Chapter 4 Nitrogen Balance of Applied Urine

'A field lysimeter experiment to investigate the pathways and endpoints of applied urinary-N post-winter forage grazing'.

4.1 Introduction

Increased nitrate levels in drinking water are undesirable, with implications for both human health (WHO 2011) and the environment from excessive aquatic plant growth and eutrophication within water bodies (Smith & Schindler 2009; Ministry for the Environment 2010). Agriculture is a major non-point source contributor of nitrogen, and intensive animal grazing systems in particular have been implicated in declining water quality (Jarvis 2000; Ledgard 2001; Erikson et al. 2010; Cameron et al. 2013; Burkitt 2014). Mitigating nitrate leaching losses in intensive agriculture requires technologies that increase N efficiency whilst improving environmental outcomes and ultimately, improving agricultural sustainability (Di & Cameron 2002a).

Forage crops such as kale (*Brassica oleracea* L.) are commonly grazed over the winter in New Zealand, and particularly in the South Island. Dairy farmers use winter forage crops to achieve body condition score targets for pregnant, non-lactating dairy cows prior to the start of calving in early spring (Judson et al. 2010). These forage crops are normally sown in the previous spring, reaching yields of 10–16 t DM ha^{-1} by winter (Brown et al. 2007) and are grazed for 8-10 weeks from early June. After grazing, the cropped area remains fallow until the next crop is sown in late spring (2-3 months later). Although these are effective low-cost systems that provide large quantities of feed at a time of the year when the growth of pasture, the most common feed for dairy cows, is limited by cold temperatures, they can lead to large nitrate leaching losses. Mitigation options to reduce N loss from intensively-grazed pastures in New Zealand have been reviewed by Cameron et al. (2013), Ledgard et al. (1999) and Monaghan et al. (2007a) but winter forage grazing presents a new challenge. The high stocking rates during grazing mean large volumes of urine are deposited onto bare soil with no opportunity for plant uptake of N, at a time of year when drainage rates are typically high, creating a high potential for nitrate leaching. There is also the potential for multiple urine applications on the same area because of the intensity of stock, per unit area of land.

Whilst a fast-growing catch crop has been reported as a useful mitigation strategy for reducing nitrate leaching loss in cereal cropping systems (McLenaghen et al., 1996; Francis et al., 1998; Shepherd, 1999; Di & Cameron, 2002), there is no information on the effect of using a catch crop to reduce nitrate leaching from winter forage grazing systems. With several recent New Zealand winter forage grazing studies reporting nitrate leaching losses ranging from 52-173 kg N ha^{-1}

(Shepherd et al., 2012; Smith et al., 2012; Monaghan et al., 2013; Malcolm et al., 2015) there is recognition that these need to be reduced if these open grazing systems are to continue. There are however, several difficulties that could beset the use of a catch crop to reduce nitrate leaching. Firstly, planting a catch crop in winter means germination and growth might be too slow to make any significant impact on N losses before the main part of the drainage season has passed. Second, the lag phase between the finish of winter grazing and soil physical conditions being suitable for sowing a crop may also limit its effectiveness. Selection of a suitable catch crop, therefore, relies on a plant species that is both winter-active and able to withstand less than ideal soil physical conditions. Additionally, the catch crop should be able to deal with the uneven distribution of urinary-N to the soil from no coverage to potentially multiple urine applications resulting from the high stocking rates. The selection of potential catch crops is, therefore, limited to winter-active annual grass species like Italian ryegrass *(Lolium multiflorum* L.) or forage cereals like oats (*Avena sativa* L.). Recent research by Malcolm et al. (2014) examining the use of differing pasture species to reduce nitrate leaching found It. ryegrass was particularly effective, reducing nitrate in drainage by 24-54% from an autumn (mid-May) urine application. Although there is little current research on the use of forage cereals post-winter forage grazing, oats has been used successfully in Canterbury as a catch crop in cropping sequences although the oats was sown in autumn (Francis et al. 1998). Oats also has other advantages due to its ability to tolerate low temperatures in seedling and tillering stages whilst resisting frost better than other forage cereals such as wheat and barley (Zwer 2004).

Mitigation technologies to reduce nitrate leaching might also include the use of a nitrification inhibitor such as dicyandiamide (DCD). Nitrification inhibitors could extend the period between urine application and maximal soil nitrate concentrations, thus enabling greater N uptake from the developing crop in the intervening period. However, whilst its effectiveness in reducing nitrate leaching in pastoral situations has been amply demonstrated (Di & Cameron 2002b; Di & Cameron 2003, 2004a, b; Di & Cameron 2004c; Di et al. 2007; Monaghan et al. 2009; Sprosen et al. 2009; Cameron et al. 2014), its use in winter forage grazing is more equivocal. Shepherd et al. (2012) found that application of DCD one day after winter forage grazing in a Waikato field trial reduced

nitrate leaching losses by 20-27% but conversely, Smith et al. (2012) found it less effective and not significant in a Southland field trial, where DCD was applied 1-12 days after grazing.

Ascertaining the fate of a significant N input to an agricultural system means measuring both the mass balance of N within the system, and the changes that occur to each mass component, in response to climatic, soil and plant growth factors (Allison 1966). Urine application represents a major perturbation of the cropping/pastoral system and using a labelling tool such as ^{15}N is essential to differentiate between the different N pathways (Clough et al. 2001). Differences in the ^{15}N isotopic ratios can help determine the immediate, medium and long-term effects of urine-N application on each component (i.e. soil, gas or water) and these have been important in deriving the relative importance of each N pathway in both pastoral and catch crop studies (Martinez & Guiraud 1990; Di et al. 2002).

This experiment tested three hypotheses:

1. *That oats are a more effective catch crop than an It. ryegrass catch crop to reduce nitrate leaching losses in post-winter forage grazing systems,*
2. *That nitrogen leaching losses from single and double urine applications post-winter forage grazing will be additive, and*
3. *That using a nitrification inhibitor (DCD) in conjunction with a catch crop will reduce nitrate leaching losses and increase N uptake from winter forage grazing compared with a non-DCD treated catch crop (control).*

Consequently, there were three main objectives for this experiment:

1. Compare the performance of two winter-active plant species to capture N from a simulated winter forage grazing (single and double urine applications) and quantify differences in nitrate leaching loss,
2. Obtain a nitrogen balance of ^{15}N-labelled urine to ascertain the pathways of urinary-N transfer and loss after sowing a catch crop post-winter forage grazing, and
3. Measure the effect of a single DCD application on the above and its effect in reducing nitrate leaching loss.

4.2 Methodology

4.2.1 Lysimeter preparation

Soil type and properties, collection, preparation and installation of the lysimeters in the field trench lysimeter facility located at the Field Research Centre, Lincoln University has already been outlined in Chapter 3. Once the lysimeters had been installed, the pasture was sprayed out with glyphosate herbicide and kale transplanted into the lightly worked surface of each lysimeter a month later (mid-December). Wind cloth was put up around each lysimeter and the plants irrigated to aid establishment. Lysimeters received sufficient natural rainfall prior to the trial starting to maintain soil moisture at field capacity for all treatments.

June to November constituted the winter-spring drainage period with a rain irrigation simulation system (RISS) supplementing natural rainfall. After the first urine application (June 25), the supplementary rainfall was randomly generated, through a series of irrigation events, to meet the daily target levels necessary to reach the 75th percentile of local rainfall records (c. 670 mm versus annual mean of 600 mm; NIWA Broadfield weather station data 1975-1999;). This simulated, for the winter-spring period, a realistic "wet" year and avoided the risk of a drier-than-average winter significantly affecting drainage results. (Di & Cameron 2002b).. Around October 31, RISS moved to summer irrigation mode with water applied at 15 mm every three days to meet evaporative demand. A detailed description of the RISS protocol and its operation is given in Appendix D.

Water applications were made to the lysimeters as either simulated rain or irrigation through an automated sprinkler system (Plate 4.2-1). These were applied by Tee Jet FL-5VC spray nozzles mounted directly over the top of each of the lysimeters. During drier periods of the year (approx. October-March), the lysimeters were irrigated at regulated rates and time intervals to replace moisture lost through evapotranspiration and to prevent soil moisture deficiency. The system was primarily controlled by historical and daily climate data, driven by a CR 1000 Campbell Scientific data logger (Utah, USA). Aluminium gas rings were fitted to the top of each lysimeter and sealed with a heavy-duty silicone rubber sealant to ensure an airtight fit and enable gas measurements of nitrous oxide (N_2O) and di-nitrogen (N_2) gas. These measurements are discussed in greater detail in the following sections.

Plate 4.2-1. Irrigation tee-jets and gas rings (A and B) shown in place on lysimeters and kale (C) shortly before cutting and urine application.

4.2.2 Treatments

Treatments for experiment 1 consisted of two catch crops (oats or lt. ryegrass) x 3 rates of urine-N application (control, 350 & 700 kg N ha^{-1}) x 4 replicates (24 lysimeters) in an orthogonal design (Table 4.2-1). Urine at the 700 kg N ha^{-1} rate was split over two applications, 7 days apart. In addition to the main experiment, an additional test was set up to compare two rates of DCD application (0 & 20 kg DCD ha^{-1}) applied to the lower urine-N treatment only (350 kg N ha^{-1}), across both crop types (8 lysimeters per crop type).

An isotopic ^{15}N label was added to the urine by addition of 98% ^{15}N-urea and ^{15}N-glycine in a 9:1 ratio, respectively, as used by Fraser (1992). This ratio was used to approximate the 20-30% of the N fraction in urine that is present in organic but readily mineralisable N compounds such as hippuric acid (conjugate of glycine and benzoic acid), creatine, allantoin, free amino and uric acids. These constitute the major non-urea compounds present in cattle urine with the majority readily hydrolysed and broken down to ammonium/ammonia in soil over days or weeks (Bristow et al.

1992). The addition of the 98% ^{15}N-urea/glycine solution to the collected urine raised its ^{15}N enrichment to approximately 9 atom %.

The DCD nitrification inhibitor was applied once (20 kg DCD ha^{-1}) to the It. ryegrass (IR) and oats 350 kg N ha^{-1} treatments (IR:U350+DCD & OT:U350+DCD) one day after urine application (June 27). Application was as a DCD solution, surface applied by an air-assisted sprayer at a rate of 40 ml per lysimeter.

The catch crops were sown per current recommended practice and the ability to prepare a suitable seedbed. For the larger oats seeds, this meant an ability to sow in mid-August but for much smaller It. ryegrass seeds, it was four weeks later in mid-September (Table 4.2-2). The oats (Milton cultivar; 120 kg ha^{-1}) and It. ryegrass (Moata cultivar; 25 kg ha^{-1}) were both hand sown after a light hand working of the soil surface to prepare a level seedbed. Problems were experienced in sowing and establishing the It. ryegrass control and DCD treatments for the It. ryegrass lysimeters for reasons that were not entirely clear, meaning the seed was required to be sown again three weeks later around October 7. Results from these treatments are discussed in this context.

Table 4.2-1. Treatment ID (It. ryegrass-IR; oats-OT) and urine/DCD application rates for field experiment 1.

Trial 1			
Treatment ID	**Crop**	**Urine rate (kg N ha^{-1})**	**DCD rate (kg AI ha^{-1})**
IR: Control	Italian ryegrass	0	-
IR: U350		350	0
IR: U700		700	-
IR: U350+DCD		350	20
OT: Control	Oats	0	-
OT: U350		350	0
OT: U700		700	-
OT: U350+DCD		350	20

Table 4.2-2. Major tasks and dates for field lysimeter experiment 1.

2013-14	
Task	**Date**
Lysimeters installed	15 Jan
Kale transplanted	15 Feb
Kale cut	14 Jun
Surface trampled	15 Jun
1st urine application	26 Jun
Volatilisation expt. started	26 Jun
DCD applied	27 Jun
1st leachate collection	27 Jun
2nd urine application	3 Jul
Volatilisation expt. finished	1 Aug
Oats sown	18 Aug
It. ryegrass sown	16 Sep
It. ryegrass re-sown	7 Oct
Oats harvested	22 Nov
1st ryegrass harvest	1 Dec
Kale sown & fertiliser applied	6 Dec
Kale harvested	30 May
Lysimeters deconstructed	31 May

4.2.3 Ammonia volatilisation

Ammonia volatilisation losses following urine application were measured using soil blocks collected from the field site at Ashley Dene research station. The soil blocks (23 cm diameter x 7 cm deep) were carefully transferred intact to the field measurement site at Lincoln University (Plate 4.2-2). These soil blocks were inserted into bare soil and the same rates of urinary-N (350 and 700 kg N ha^{-1}) applied as used in the lysimeter experiment, with the latter rate split between two equal applications, 7 days apart. Each treatment was replicated 3-times and the gas enclosures were then sealed with clear perspex covers. Three additional enclosures did not receive urine and were used as controls. Ambient air was continuously drawn through each enclosure at 0.41 L s^{-1} (approx. 17 air changes min^{-1}). The flow from each gas enclosure was partitioned such that 10% passed through an acid trap containing 50 ml of 0.05 M H_2S0_4 whilst the remaining flow was vented (Plate 4.2-3). Solution from each acid trap was changed daily with any evaporative loss replaced prior to collection (Black et al. 1985a). The solutions were stored in 50 ml plastic bottles and frozen until

the ammonium content of the solutions could be determined by flow injection analysis (FIA) by Analytical Services at Lincoln University. Volatilisation rates were determined over the monitoring period (~1 month) until rates approached background levels. More details of the method are provided in Appendix C.

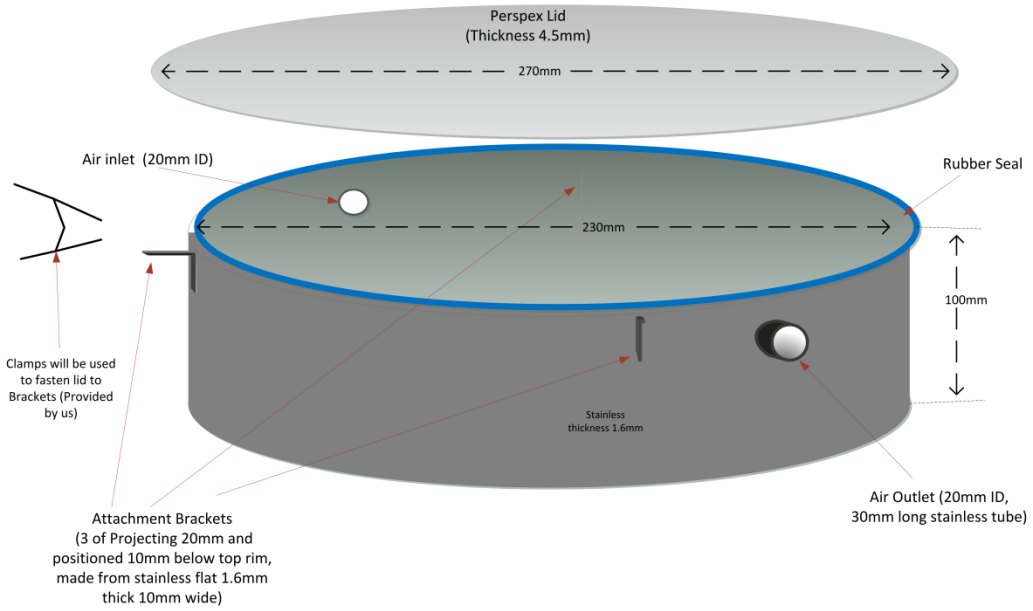


Plate 4.2-2. Diagrammatic representation of enclosure used for volatilisation measurements of ammonia from applied urine.

Total volatilisation losses were calculated (4.2-1) as follows:

$$Volat_{NH_3} = \sum \frac{Am_N \times CF}{SA \times AF_{\%}}$$

4.2-1

where: $Volat_{NH_3}$ = the sum of the daily volatilisation N loss (kg N ha^{-1}), Am_N = the ammonium (NH_4^+) concentration in the trap solution (mg N L^{-1}), CF = conversion factor (5 x10^{-8}) for mg N L^{-1} to kg N (x10^{-6}) x total volume (L) of solution (0.05), SA = surface area of enclosure (m^2) and $AF_{\%}$ = the fraction (10%) of airflow passed through the solution.

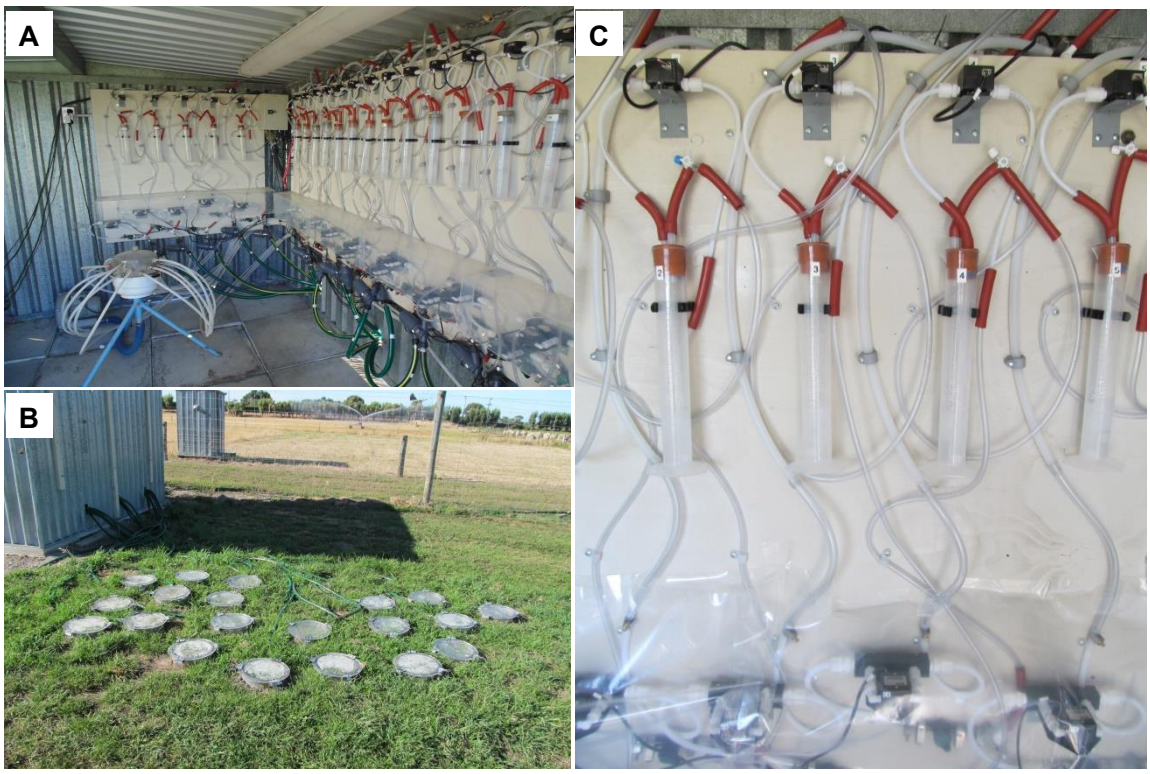


Plate 4.2-3. Volatilisation experiment showing A) collection room, B) field enclosures in place and C) collection system.

4.2.4 Nitrous oxide measurements

Collection and measurement of N_2O emissions was carried out using a steady state chamber method (Denmead 1979) involving an estimate of the hourly flux of N_2O using gas samples taken during a period of chamber enclosure over the soil surface (Hutchinson & Mosier 1981), followed by analysis using gas chromatography (GC) . A number of recommendations have been made for a standard chamber methodology and these were adopted in the procedure used (Rochette 2011).

The apparatus consisted of a chamber placed over the surface of each lysimeter, fitted into an aluminium ring attached to the upper part of the lysimeter with heavy duty silicone rubber to provide a gas tight fit. Water poured into the gas ring trough provided an air tight seal for the period of enclosure (40 minutes per sampling) (Figure 4.2-1). The chamber was 100 mm in height, 500 mm in diameter and the sides were constructed using 0.4 mm galvanised steel rolled in a circular shape to fit into the gas ring and the top sealed with a polystyrene foam cover (25 mm thick) with heavy duty silicone rubber that was both light to handle and insulated the sealed volume from exterior temperature change. The chamber dimensions allowed sufficient headspace volume for N_2O

accumulation over the duration of the enclosure period and sufficient height for catch crop growth. The distance to the soil surface (*x*) between the inside of the top cover and the soil surface was averaged over 30 readings for each lysimeter to enable calculation of an internal volume for each lysimeter when the cover was in place. A rubber septum was inserted into one of two holes in the top of each chamber which provided a seal against the outside atmosphere and a port through which N_2O samples could be taken from the chamber. The other hole provided a lidded port that equalised pressure initially between the internal and external atmospheres prior to measurements commencing (Plate 4.2-4).

Measurement of N_2O emissions was carried out the day following urine application and thereafter, twice a week, for approximately 6 months (28 June 2013-24 Jan 2014) till N_2O emissions were negligible. On each sampling day, measurements were carried out between 11 am and 3 pm. Temperature was monitored and measured over the period using an extra enclosure with a thermometer probe fitted. After chamber placement, the first sample was taken immediately (t0, time equals zero minutes), followed by another after 20 minutes (t20) and after 40 minutes (t40). A gas chamber was sampled each minute, in order, beginning at lysimeter one for the first 16 lysimeters. The second sequence of 16 lysimeters (17-32) was usually done simultaneously. Chambers were sampled by inserting a hypodermic needle attached to a 50-mL plastic syringe through the rubber septum into the chamber headspace. Twenty mL of headspace air was then primed twice in the syringe before 20 mL was extracted using the syringe and transferred to a pre-evacuated 7 mL septum-sealed glass exetainer (Labco Ltd, UK) The exetainer was over-pressurised to prevent any leakage in of ambient air into the vial. Samples were analysed within seven days to reduce potential leakage or contamination.

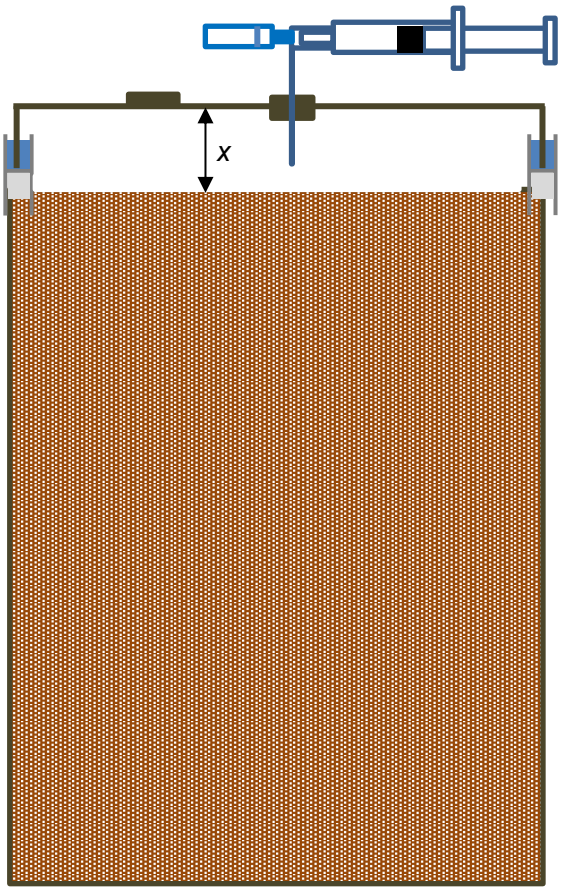


Figure 4.2-1. Lysimeter schematic showing gas ring with water seal, and headspace enclosure and gas sampling equipment in place.

Nitrous oxide concentration was analysed using a gas chromatograph (GC) (Model 8610C, SRI Instruments, California) with an automated Gilson GX-271 auto sampler (Gilson Inc., Michigan, USA) coupled to an electron capture detector (ECD). The radioactive ^{63}Ni source sealed inside the detector emits beta particles (electrons) that collide and ionise the make-up gas (10% methane in argon) and carrier gas (nitrogen) molecules. Detection limit for N_2O is 0.07 ppm with a quantification range up to 1000 ppm v/v. The results were expressed as a chromatogram (Simplepeak software, version 4.32, SRI Instruments, California) where areas under the peaks were measured against a calibration curve of N_2O concentration from 0.2-10 ppm v/v (Figure 4.2-2) (Mosier & Mack 1980).

Plate 4.2-4. Enclosure in place over lysimeter and gas ring with syringe and evacutainer.

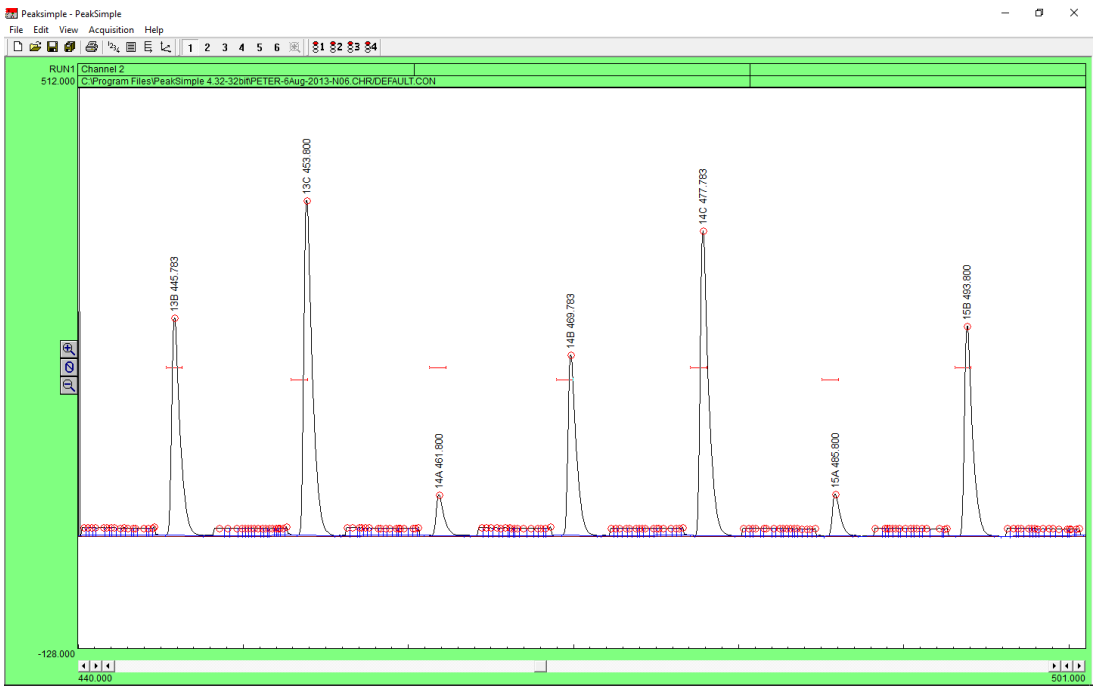


Figure 4.2-2. Sample chromatogram for N_2O detection and measurement.

The increase in N_2O accumulation in the chamber during sampling was approximately linear for the concentration range encountered. Accordingly, a linear regression calculation was used to estimate the N_2O flux (4.2-2):

$$N_2O\ flux = \frac{[(C_1 - C_0) + (C_2 - C_1)] \times V \times P \times CF \times MW_N}{[(T_1 - T_0) + (T_2 - T_1)] \times R \times T \times SA}$$

4.2-2

where:

N_2O *flux* = hourly N_2O emission (N_2O-N m^{-2} h^{-1}),

C_0, C_1, C_2 = N_2O concentration at times T_0, T_1, T_2, respectively (μL/L),

T_0, T_1, T_2 = measurement times at 0, 0.33 and 0.67 hours, respectively,

V = enclosure headspace volume (L), *P* = atmospheric pressure (nominally assumed at 1 atm.),

CF = conversion factor (10^{-4}) for μL to L (μL/10^6) N_2O and dm^2 to m^2 (dm^2 x10^2),

MW_N = molecular weight of N in N_2O (28.0 g mol^{-1}),

R = the universal gas constant (0.0821 L atm. mol^{-1} K^{-1}),

T = temperature (K) at midday-to-2 pm for each measurement time, and *SA* = surface area (m^2).

Daily emissions were then calculated using the hourly flux, assuming it represented the average hourly flux of the day (de Klein et al. 2003). Cumulative N_2O emissions were calculated by integrating the calculated daily N_2O fluxes and linearly interpolating between each subsequent measurement for each lysimeter to calculate weekly and total N_2O emissions over the sampling period.

Emission factors (EF_3) for each treatment were calculated using the following equation (4.2-3) (de Klein et al. 2003):

$$EF_3 = \frac{N_2O_{Urine} - N_2O_{Control}}{Urine - N_{applied}} \times 100$$

4.2-3

where: EF_3 = N_2O emitted as % of urine-N applied, N_2O_{Urine} = nitrous oxide emitted from the urine treatment or patch (kg N ha^{-1}), $N_2O_{control}$ = nitrous oxide emitted from the control or non-urine area (kg N ha^{-1}), and $Urine\text{-}N_{applied}$ = the amount of N applied in the urine (kg N ha^{-1}).

4.2.5 Nitrogen leaching

Drainage water was collected from the lysimeters after every major rainfall event or when there was more than 0.5 L in the drainage collectors. A 50-ml sample was collected from each lysimeter for chemical analysis and frozen at -18°C until required. Accumulation was approximately linear except where there was intervening rainfall in which case the time interval between simulated rainfall or irrigation applications using the RISS protocol was extended to remain on track with the climate accumulation line. Drainage and N leaching loss was considered over two periods; winter-spring (Jun 26 - Nov 30) and "annual" (Jun 26 – May 31). The first took in the main Canterbury drainage season and the latter, the duration of the study up to soil deconstruction.

Drainage water samples were analysed by flow injection analysis (FIA) for NH_4^+-N and NO_3^--N by Analytical Services at Lincoln University, Canterbury (Gal et al. 2004). From the analyses, ammonium and nitrate leaching losses from each lysimeter treatment were determined. A detailed explanation of the procedure for ammonium and nitrate analysis is outlined in Appendix C. Nitrogen leaching losses were calculated using the following equation (4.2-4):

$$N_{drn} = \sum \frac{N_{conc} \times Vol \times CF}{SA}$$

4.2-4

where: N_{drn} = total nitrogen leaching loss (kg N ha^{-1}) over the drainage period, N_{conc} = leachate nitrogen concentration (mg N L^{-1}), v*ol* = litres collected (L), *CF* = conversion factor ($x10^{-2}$) for mg N to kg N ($x\ 10^{-6}$) and m^2 to ha ($x\ 10^4$) and *SA* = surface area of the lysimeter (m^2).

Plate 4.2-5. Stages of soil deconstruction from clockwise top left; A) removal of base plate, B) lysimeter installed on stand, C) exposure of first 10 cm increment, D) second subsoil increment and attached winches, E) exposure of final 20 cm increment.

4.2.6 Lysimeter harvests, kale establishment and deconstruction

Pasture on the It. ryegrass lysimeters was harvested at regular intervals, from Dec 1 till May 30 whilst the oats were harvested once only in mid-November (Nov 22). The oats treatment lysimeters were sprayed with glyphosate to kill any remaining oats activity and then the surface of each was lightly reworked to prepare a seed bed for the sowing of the second kale crop (Regal cultivar; 4 kg ha^{-1}). An application of DAP at 200 kg ha^{-1} was applied (36 kg N ha^{-1}) at sowing to help establish the plants (Table 4.2-2). Kale that was grown after the oats harvest was cut on May 30 just prior to deconstruction of the lysimeters. All harvest material was place in a fan-forced oven and dried at 60°C. This material was stored until ground for analysis using a Retsch (Hann, Germany) cyclonic mill (<0.5 mm mesh).

After the end of the experiment in May 2014, the lysimeters were uplifted and a process of deconstruction of the soil within the lysimeters was begun on 31 May to sample soil, plant roots and tops to complete the ^{15}N balance study. The lysimeters were inverted again using the hydraulic lift on a tractor, the drainage base and gravel layer removed before a base plate that fitted completely within the lysimeter and with positions for legs was fitted and held in position by two cross-sectional steel bars. The lysimeter was then returned to its original position and transferred to the Field Research Centre where it was placed on a frame and the bars removed. A winch was attached to each side of the lysimeter and the frame, and then tightened progressively to force the casing down the side of the soil monolith in 10 cm increments to 40 cm, with the final increment, from 40-60 cm, collected as a single increment (Plate 4.2-5).

Soil and root mass samples were extracted and stored at 4°C for up to two weeks prior to further cleaning of the roots, drying and grinding (plant) or pulverising (soil) for analysis of total-N and ^{15}N content. Soil mineral-N was extracted using 2 M KCL as outlined in Blakemore et al. (1987). The exact process for the removing, weighing, drying and recording of plant, soil and stone weights is described in Appendix C.

4.2.7 ^{15}N balance measurements

Recovery of the N from the ^{15}N-labelled urine was measured in four major components: N in drainage water, gaseous N emissions ($NH_3/N_2O/N_2$), plant N uptake (tops and roots) and soil

immobilised-N (organic and inorganic N). The total mass (m) of N recovery can be represented by the following equation (4.2-5):

$$^{15}N\ recovery\ = \sum Gaseous\ ^{15}N_m + Drain\ ^{15}N_m + ^{15}N\ uptake_m + Soil\ ^{15}N_m$$

4.2-5

where: *Gaseous N* is the mass of N recovered as volatilised ammonia (NH_3), emitted dinitrogen (N_2) and nitrous oxide (N_2O), *Drain ^{15}N* is the ^{15}N mass leached in drainage water (ammonium, nitrate and organic-N), *^{15}N uptake* is the ^{15}N mass held in plant harvests and retained vegetation and roots, and *soil ^{15}N* is the ^{15}N mass still retained or immobilised in the bulk soil. The percentage recovery of the ^{15}N applied in urine for the four mass components was calculated as follows (4.2-6):

$$^{15}N\%\ recovery\ = \sum \frac{Gas\ ^{15}N_m + Drain\ ^{15}N_m + ^{15}N\ uptake_m + Soil\ ^{15}N_m}{^{15}N\ applied} \times 100$$

4.2-6

where: *^{15}N% recovery* is the proportion of ^{15}N recovered in the four major components (gaseous, liquid, plant and soil forms) from the applied ^{15}N-labelled urine. All other terms are the same as those in equation 4.2-5.

The ^{15}N content of nitrous oxide ($^{15}N_2O$) and di-nitrogen was determined by taking an additional gas sample (12 ml exetainer vial), 2 hours after placing the enclosure over the gas ring and once all the T_2 (40 min.) N_2O samples had been taken. These samples were taken in the same manner as described in section 4.2.4. The samples were analysed using a PDZ Europa 20-22 continuous flow isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) enabling measurement of stable isotopes of gases at both enriched and natural abundance levels. Gas samples were prepared using a TGII trace gas system using cryo-trapping and focusing to isolate the N_2O/N_2 species.

Concentrations of ^{15}N%-N_2O were interpolated between weeks and this allowed calculation of the proportion of N_2O derived from the applied urine for the unmeasured as well as measured collection of ^{15}N%-N_2O. Total ^{15}N%-N_2O evolved between the twice weekly samplings was derived from summation of the estimated daily totals over the sampling period of 136 days (when N_2O levels

had declined to negligible levels) and calculated using the formula from Cabrera and Kissel (1989) to produce the following equation (4.2-7):

$$ {}^{15}_{Tot}N_2O - N \; = \sum \frac{({}^{15}_{\%}N_a - {}^{15}_{\%}N_b) \times N_2O_{flux} \times CF}{{}^{15}_{\%}N_c - {}^{15}_{\%}N_b} $$

4.2-7

where: ${}^{15}_{Tot}N_2O - N$ = the total nitrous oxide loss derived from the applied ^{15}N-enriched urine over the sampling period (kg N_2O-N ha^{-1}), ${}^{15}_{\%}N_a$ is the daily average atom% ^{15}N abundance of nitrous oxide, ${}^{15}_{\%}N_b$ is the atom% ^{15}N natural abundance of nitrous oxide (0.3663%), N_2O_{flux} is the hourly nitrous oxide emission (mg N-N_2O $h^{-1}m^{-2}$) (from equation 4.2-7), *CF* is the conversion factor (0.24) to convert units for mg N_2O-N m^2 h^{-1} to kg N_2O-N ha^{-1} day^{-1}, and ${}^{15}_{\%}N_c$ is the ^{15}N-enriched content (%) of the applied urine or water (controls).

Calculations of the ^{15}N enrichment in di-nitrogen gas (N_2) were carried out using essentially the same equations as those for ^{15}N-N_2O determination but were more problematic due to the relatively small differences created by a high background of N_2 concentration (i.e. N_2 =~80% of atmospheric gases) and a relatively low initial ^{15}N enrichment (~9%). More comprehensive measurements of N_2 denitrification losses would require increasing the ^{15}N enrichment to ~40% (Clough et al. 2001) but the cost of doing so in this study was prohibitive. Measurements were linearly interpolated between weeks and the rate of $^{15}N_2$ evolved was also assumed to be linear (Clough et al. 2001). Total ^{15}N-N_2 was measured over 15 weeks and calculated using the following equation (4.2-8):

$$ {}^{15}_{Tot}N_2 \; = \sum \frac{N_2^{\%} \times ({}^{15}_{\%}N_a - {}^{15}_{\%}N_b) \times V \times P \times MW_N \times CF}{\left({}^{15}_{\%}N_c - {}^{15}_{\%}N_b\right) \times R \times T \times SA} $$

4.2-8

where:

${}^{15}_{Tot}N_2$ = total N loss over monitoring period as di-nitrogen gas derived from the ^{15}N-enriched labelled urine (kg N ha^{-1}),

$N_2^{\%}$ = Percentage of air in enclosure present as di-nitrogen,

${}^{15}_{\%}N_a$ = is the weekly average atom% ^{15}N abundance of di-nitrogen,

$^{15}_{\%}N_b$ = is the atom% ^{15}N natural abundance of di-nitrogen (0.3663%),

$^{15}_{\%}N_c$ = is the atom% ^{15}N abundance of the applied urine,

V = enclosure headspace volume (L),

P = atmospheric pressure (nominally assumed at 1 atmosphere),

CF = conversion factor (7 x 10^{-4}) for days to weeks (days x7) μL to L (μL/10^6) N_2 and dm^2 to m^2 (dm^2 x10^2),

MW_N = molecular weight of N in N_2 (28.0 g mol^{-1}),

R = universal gas constant (0.0821 L atm mol^{-1} K^{-1}),

T = temperature (K) at midday-to-2 pm for each measurement time, and

SA = surface area (m^2) of the lysimeter.

Measurements of the ^{15}N proportion of the ammonium and nitrate content of the leachates was undertaken using a selection of samples covering the breakthrough curve of concentration of both ions after FIA results were obtained. The ^{15}N present in the samples was concentrated on 7 mm glass fibre disks using the method described by Brooks et al. (1989) before combustion at 1000°C using a PDZ Europa 20-20 stable isotope mass spectrometer (Sercon Ltd., Cheshire, UK). Further details are outlined in Appendix C. The ^{15}N content in organic-N was determined in a selected range of drainage water samples after oxidation to nitrate using the method as described by Cabrera and Beare (1993). The proportion of ^{15}N present as NH_4^+ and NO_3^- in the leachate was interpolated between sampling points and N drainage losses calculated using the following equation (4.2-9):

$$_{Tot}^{15}N = \sum {}^{15}_{\%}N \times N_{drn}$$

4.2-9

where: $_{Tot}^{15}N$ = The total-^{15}N leaching loss as mineral-N, ammonium or nitrate summed over the drainage period, $^{15}_{\%}N$ is the actual or interpolated ^{15}N fraction of the ammonium and/or nitrate ion concentration, and N_{dm} is the N leaching loss for each drainage collection (equation 4.2-4).

4.2.8 Statistical analysis

Statistical analysis of the data was conducted in two stages: crop-by-N-rate interactions between treatments was conducted using an orthogonal non-block ANOVA procedure in Genstat 9.0 (Lawes Agricultural Trust 2007), whilst the testing of the effect of DCD on both crop types was done using a standard t-test. Residuals were tested for normalisation of data.

4.3 Results

4.3.1 Climate data

Average winter weekly soil and air temperatures were both about one degree warmer over 2013-14 compared to the recent long-term means (2000-2016) (Figures 4.3-1 & 4.3-2; Table 4.3-1) whilst mean spring, summer and autumn weekly soil and air temperatures were only slightly above average (~0.3°C; Table 4.3-1). Weekly mean seasonal solar radiation and potential evapotranspiration (Penman) values for 2013-14 were, overall, similar to the 2000-16 means (Table 4.3-1).

Table 4.3-1. Winter (Jun-Aug), spring (Sep-Nov), summer (Dec-Feb) and autumn (Mar-May) soil and air temperatures, solar radiation and evapotranspiration mean values for 2013-14 compared with recent long-term (LT; 2000-16) means.

Climate variable	Winter		Spring		Summer		Autumn	
	2013-14	LT	2013-14	LT	2013-14	LT	2013-14	LT
Soil temperature (°C)	5.9	4.8	10.6	10.3	16.0	16.3	11.1	10.8
Air temperature (°C)	6.6	5.6	11.7	11.4	15.8	15.6	11.2	11.4
Solar radiation (MJ ha^{-1})	5.8	5.8	17.1	17.5	22.3	21.6	9.3	10.1
Evapotranspiration (mm)	0.8	0.8	3.0	3.2	4.7	4.5	1.6	1.9

Daily rainfall and simulated rain/irrigation to the 75th percentile totalled 1374 mm over the study period (25 June, 2013 to May 30, 2014) with natural rainfall comprising 734 mm or 53% of the total (Figure 4.3-3). In the main winter/spring period from June 25 to November 31, however, only 211 mm or 38% of the 560-mm total fell as natural rain so the remainder was applied using the RISS protocol. Total cumulative drainage at ~710 mm comprised half of the total rainfall for the period with more than 50% of this accumulated in the first five months to the end of spring.

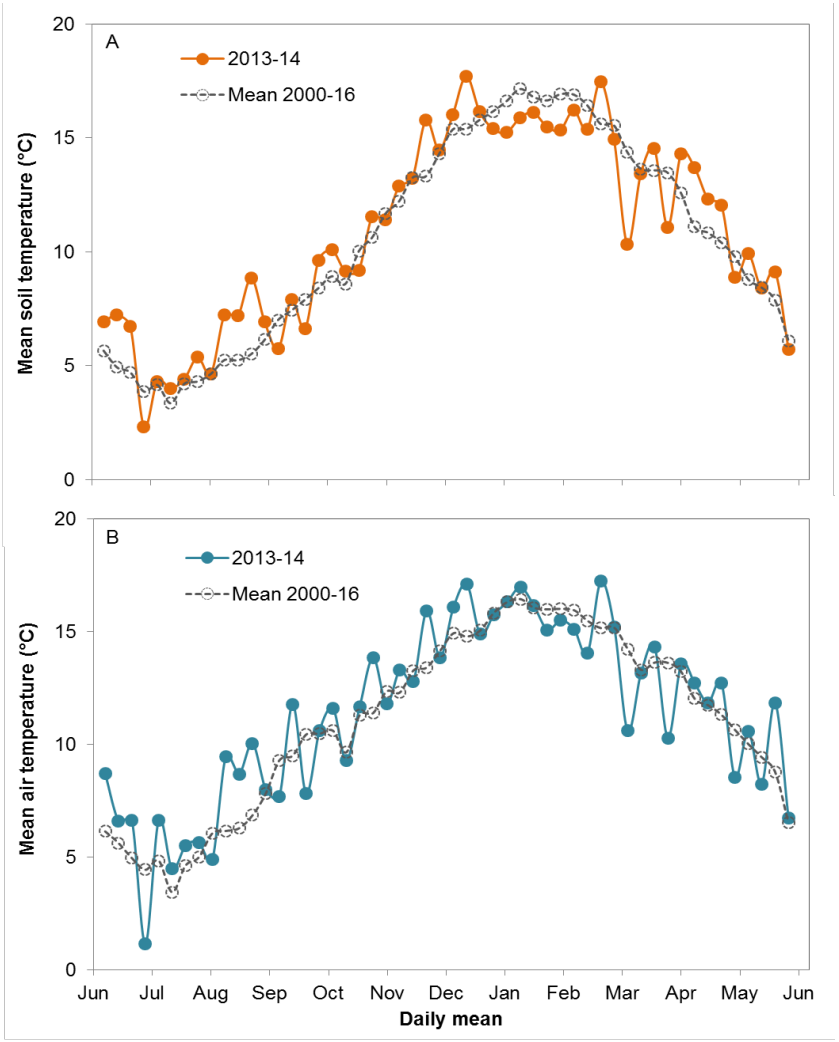


Figure 4.3-1. Mean soil (A) and air (B) temperatures for 2013-14 following treatment application (data points represent the weekly average) compared with 2000-16 means.

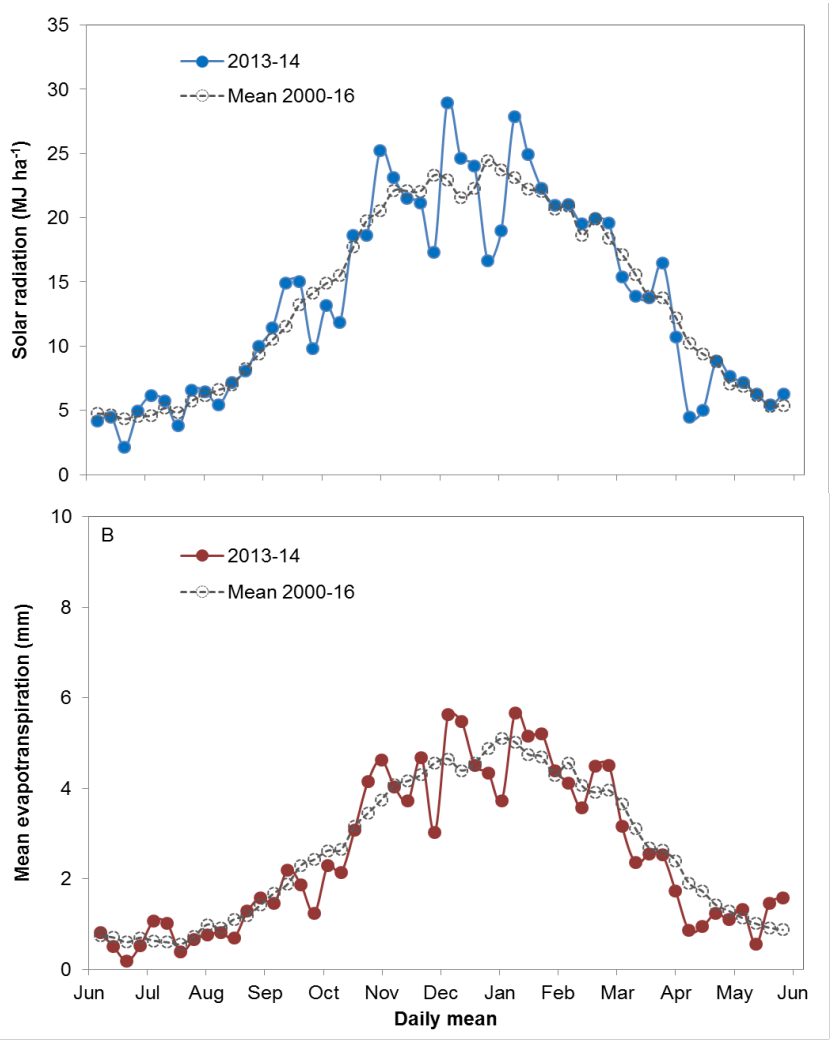


Figure 4.3-2. Mean solar radiation (A) and evapotranspiration (B) (Penman) values for 2013-14 following treatment application (data points represent the weekly average) compared with 2000-16 means.

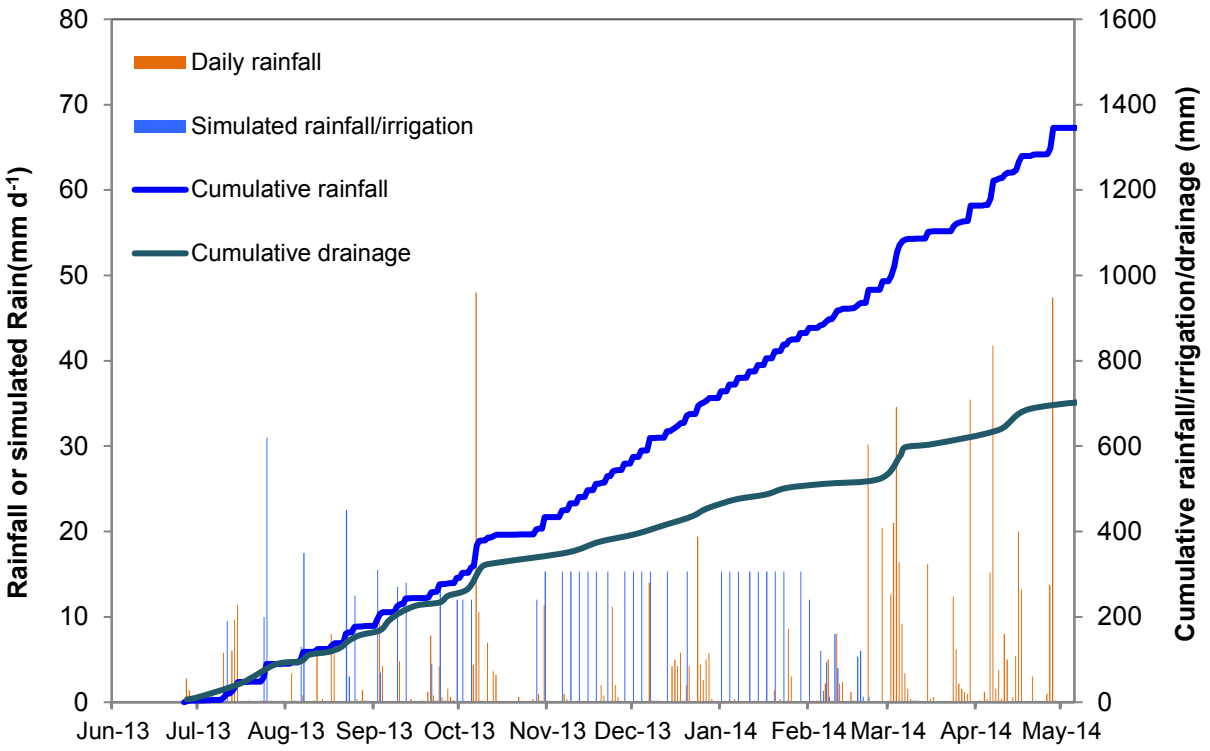


Figure 4.3-3. Daily rainfall, simulated rainfall/irrigation and cumulative rainfall and drainage for 2013-14 (from commencement of treatment application;(lysimeter experiment 1).

4.3.2 Crop establishment

Oats, despite its earlier sowing date in August, generally established well and plants initially thrived. However, signs of N deficiency started to show in Control, U350 and some U700 treatments by October (Plate 4.3-1A & B), and into November. Oats U350+DCD treatments (Plate 4.3-1C) grew better and remained greener, for longer, than the U350-urine only treatment, before also starting to show signs of N deficiency by November.

Although Italian ryegrass treatments established well in most lysimeters (Plate 4.3-1D) during September, there were problems with some lysimeters, requiring re-sowing, particularly the Control and U350+DCD treatments. This seemed related to underlying compacted soil conditions brought about by the simulated treading and reduced DM production as well as N uptake (section 4.3.3).

4.3.3 Winter-spring nitrogen leaching losses

Nitrogen leaching losses were initially considered over the winter-spring period (26 June-30 November) as this was considered the critical drainage interval for nitrate leaching. Cumulative

drainage over this period was 22% less (P<0.001) for oats U350 & U700 treatments compared to lt. ryegrass treatments (313 vs. 401 mm, respectively) (Table 4.3-2). Drainage nitrate concentration for both the lt. ryegrass and the oats 350 (U350) and 700 (U700) kg urine-N ha^{-1} treatments peaked at ~100 and ~150 mg N L^{-1}, respectively, with the latter peak arriving later (~140 mm vs ~200 mm), (Figures 4.3-4 & 4.3-5). Nitrate peaks for the control treatments (0 kg urine-N ha^{-1}) generally remained below 10 mg N L^{-1}. Ammonium concentrations in the leachate varied greatly between lysimeters but on average peaked at approximately ~25 and ~45 mg N L^{-1} for both lt. ryegrass and oats U350 and U700 treatments, respectively, after ~50 and ~200 mm drainage, respectively (Figures 4.3-6 & 4.3-7). Ammonium in the drainage of the U700 treatments persisted to the end of the winter-spring period. Negligible ammonium was recovered in the drainage of the control treatments.

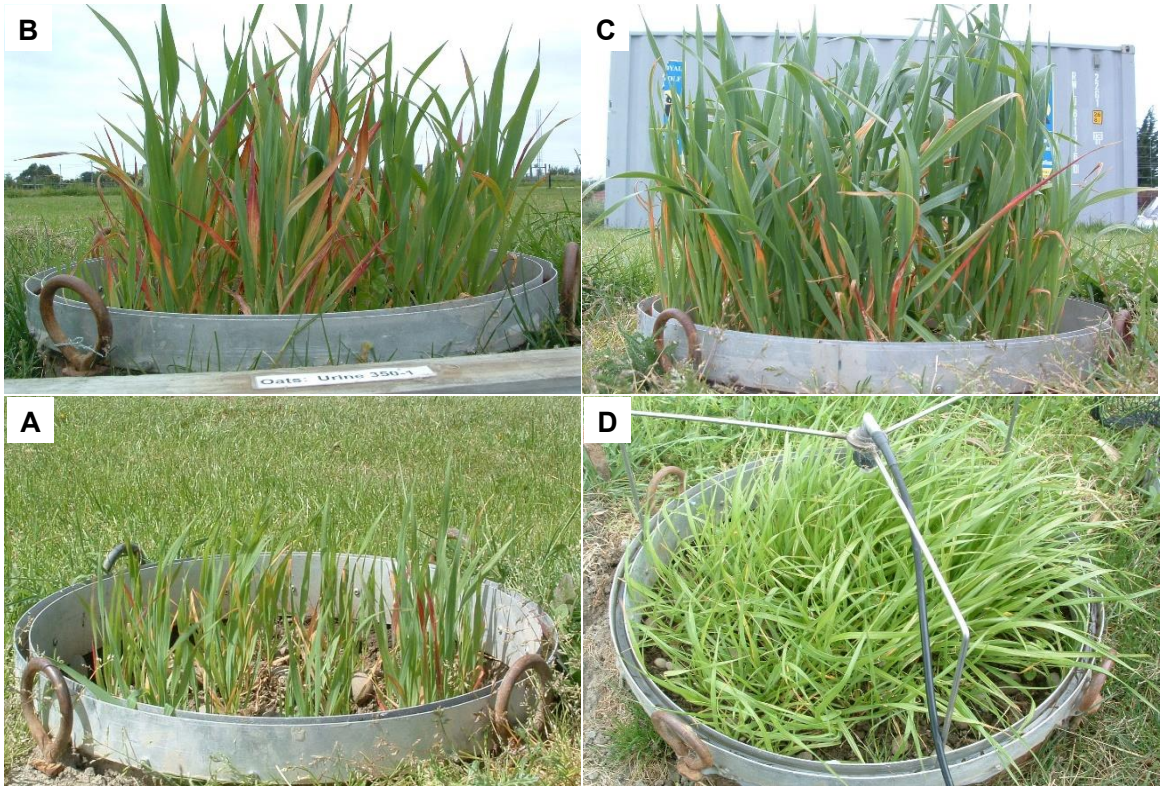


Plate 4.3-1. Sample lysimeters showing oats (A- control, B- U350 and C- U350+DCD) and lt. ryegrass (D- U350) treatments in November 2013.

Sowing oats in the urinary-N treatments (350 and 700 kg N ha^{-1}) significantly reduced total nitrate loss (P<0.01) and total-N leached (P<0.05) overall by 25% and 21%, respectively, compared to the equivalent lt. ryegrass treatments (Table 4.3-2). This effectively reduced nitrate loss from 167 and

279 kg N ha^{-1} to 131 and 193 kg N ha^{-1} for the U350 and U700 treatments, respectively, the majority in the U700 treatments. Nitrate and inorganic-N losses for both U700 over U350 treatments were about 55% (range 47-59%) and 72% (range 63-82%) greater overall, respectively. Nitrogen drainage losses for control treatments were also lower for oats but not significantly. Approximately 60% of the nitrate leaching loss (range 55-65%) to the end of the winter-spring period was directly attributable to the applied ^{15}N-labelled urine with trends similar between treatments. Total-^{15}N losses for the U350 treatments were not dissimilar between crops due to more ^{15}N-ammonium being leached in the oats treatments for reasons unknown. Nevertheless, sowing oats was still more effective at reducing nitrate leaching than sowing lt. ryegrass.

Ammonium loss in drainage was not different between catch crops but increased significantly with urinary-N rate (P<0.001), leaching relatively large amounts at ~30 and ~80 kg N ha^{-1} for the U350 and U700 treatments, respectively. This represented, on average, 9% (range 7–13%) of the total urinary-N applied for the U350 and U700 treatments, respectively. (P<0.001; Table 4.3-2). Control treatments leached negligible ammonium. The ^{15}N-ammonium fraction made up more than three-quarters of total ammonium loss (range 76-78%) and followed similar trends to nitrate losses.

Table 4.3-2. Total inorganic-N leaching (NO_3^--N, NH_4^+-N and total-N), drainage and inorganic-^{15}N leaching losses for lt. ryegrass and oats urine treatments (control, 350 and 700 kg N ha^{-1}) to the end of the winter-spring drainage period.

Treatment		*[a] Inorganic-N leached (kg N ha^{-1})*			*Drainage (mm)*	*Inorganic-^{15}N leached (kg N ha^{-1})*		
Crop	**Urinary-N**	**NO_3^--N**	**NH_4^+-N**	**Total-N**	**Win/Spr**	**NO_3^--^{15}N**	**NH_4^+-^{15}N**	**Total-^{15}N**
Italian ryegrass	Control	46 a	2 a	48 a	351 b	6 a	0 a	6 a
	350	167 bc	26 ab	192 b	369 b	93 b	19 b	112 b
	700	264 d	85 c	349 d	433 c	167 d	66 c	233 d
Oats	Control	11 a	0 a	11 a	321 ab	3 a	0 a	3 a
	350	131 b	41 b	172 b	332 ab	78 b	31 b	109 b
	700	193 c	81 c	280 c	295 a	126 c	68 c	194 c
Crop		**	ns	*	***	*	ns	ns
Urinary-N		***	***	***	ns	***	***	***
Crop x urinary-N		ns	ns	ns	***	ns	ns	ns
[b] LSD (5%)		52	28	62	41	32	24	43

[a] Means followed by a letter in common are not significantly different according to Duncan's multiple range test (P < 0.05), [b] LSD: least significant difference (P < 0.05) at crop x urine rate level.

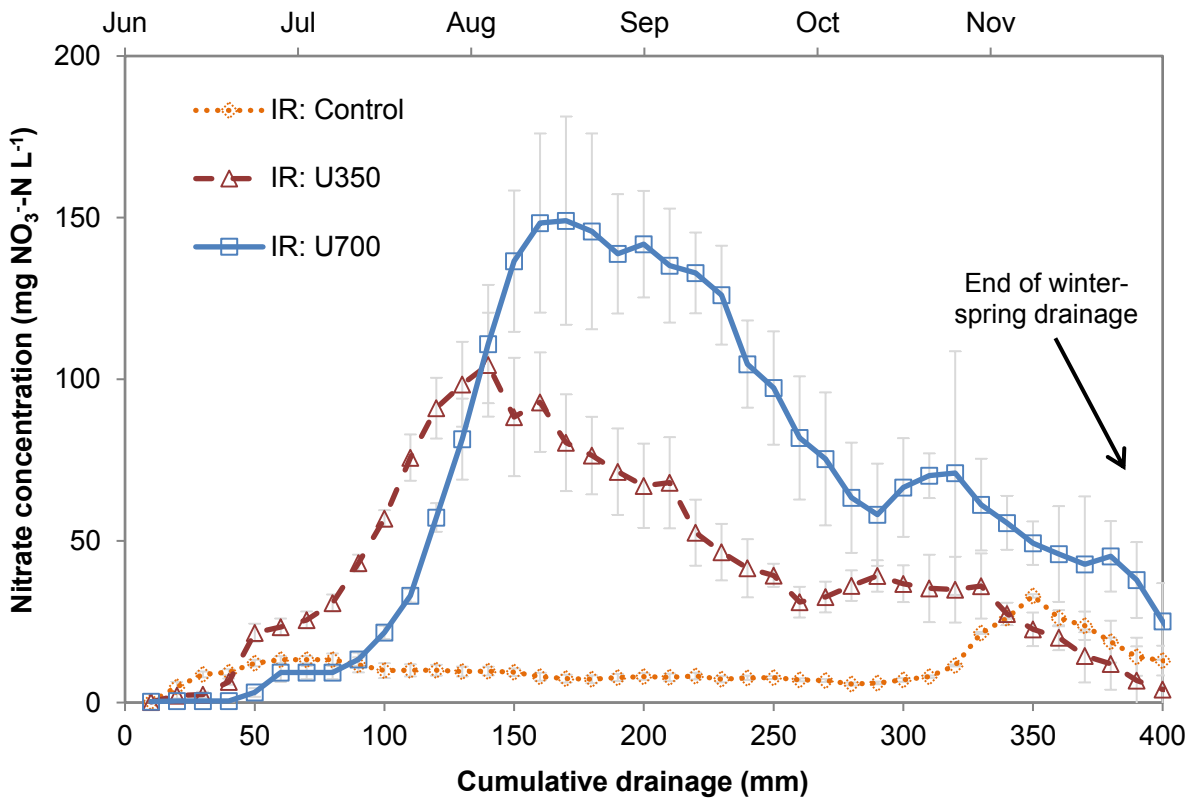


Figure 4.3-4. **Nitrate concentrations vs. cumulative drainage following the June/July urine applications (0, 350 & 700 kg N ha^{-1}) to the lt. ryegrass lysimeters. Time scale indicative only.**

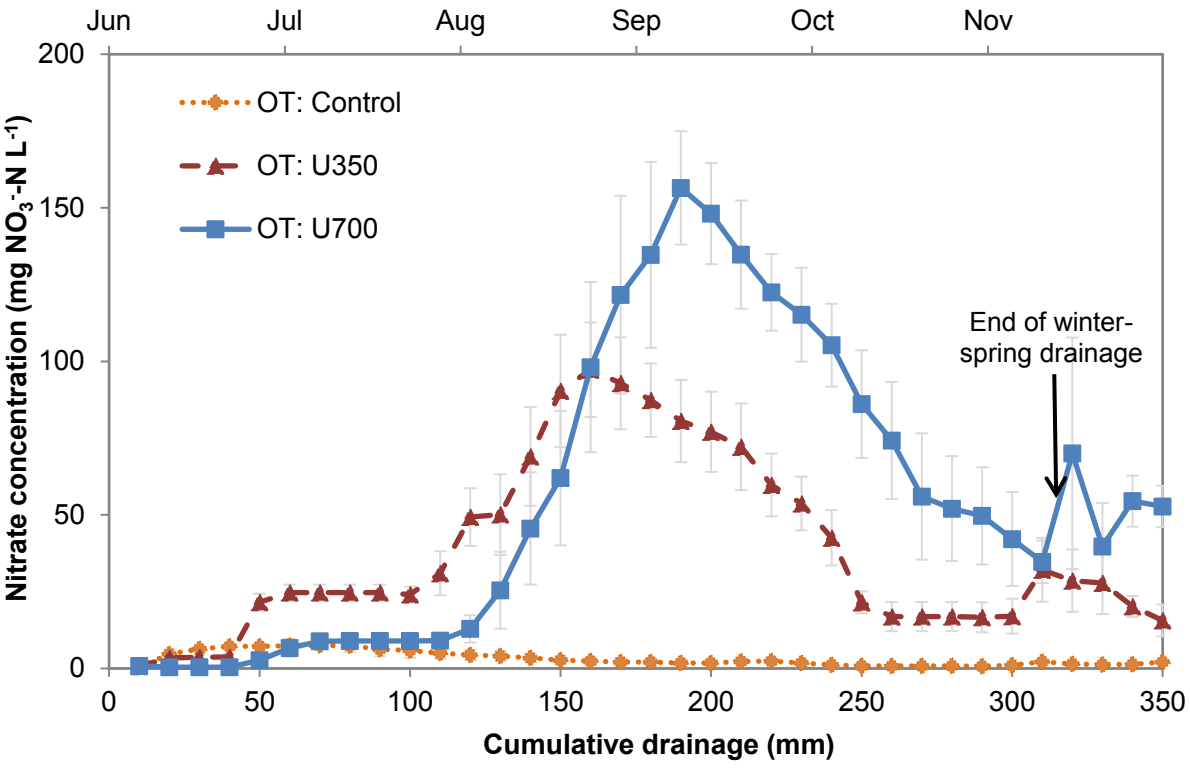


Figure 4.3-5. **Nitrate concentrations vs. cumulative drainage following the June/July urine applications (0, 350 & 700 kg N ha^{-1}) to the oats lysimeters. Time scale approximate.**

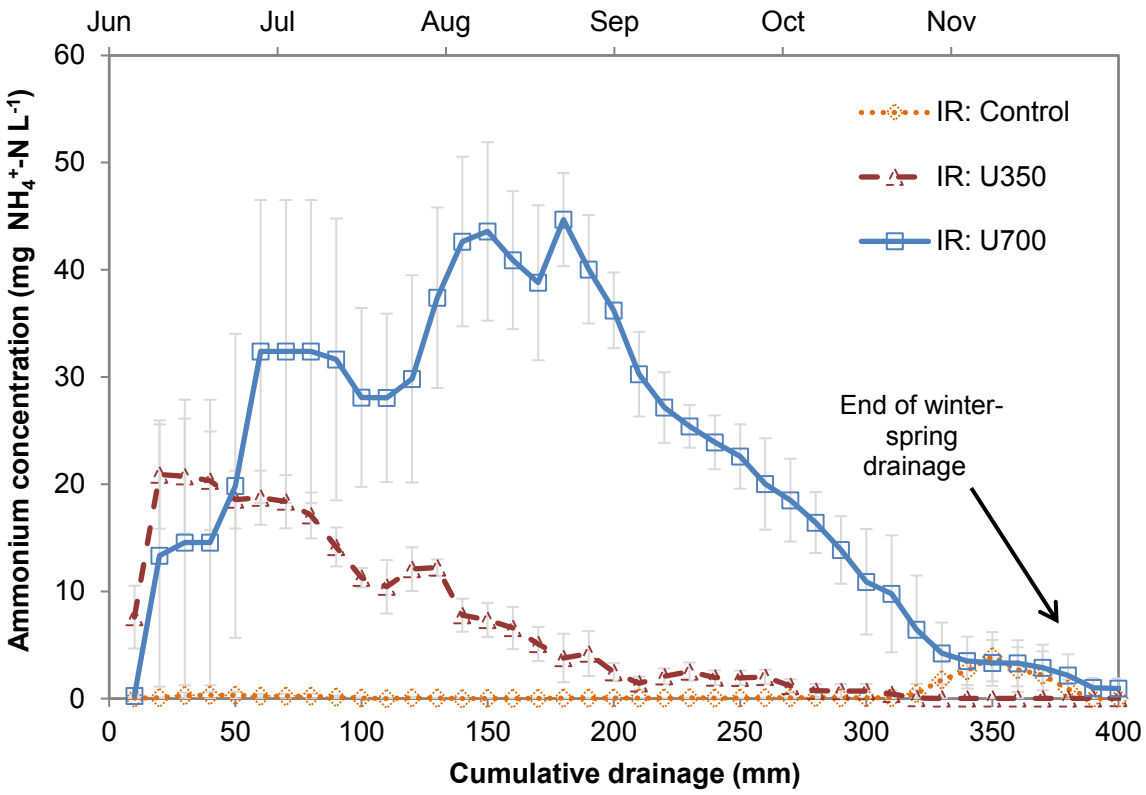


Figure 4.3-6. Ammonium concentrations vs. cumulative drainage following the June/July urine applications (0, 350 & 700 kg N ha^{-1}) to the lt. ryegrass lysimeters. Time scale approximate.

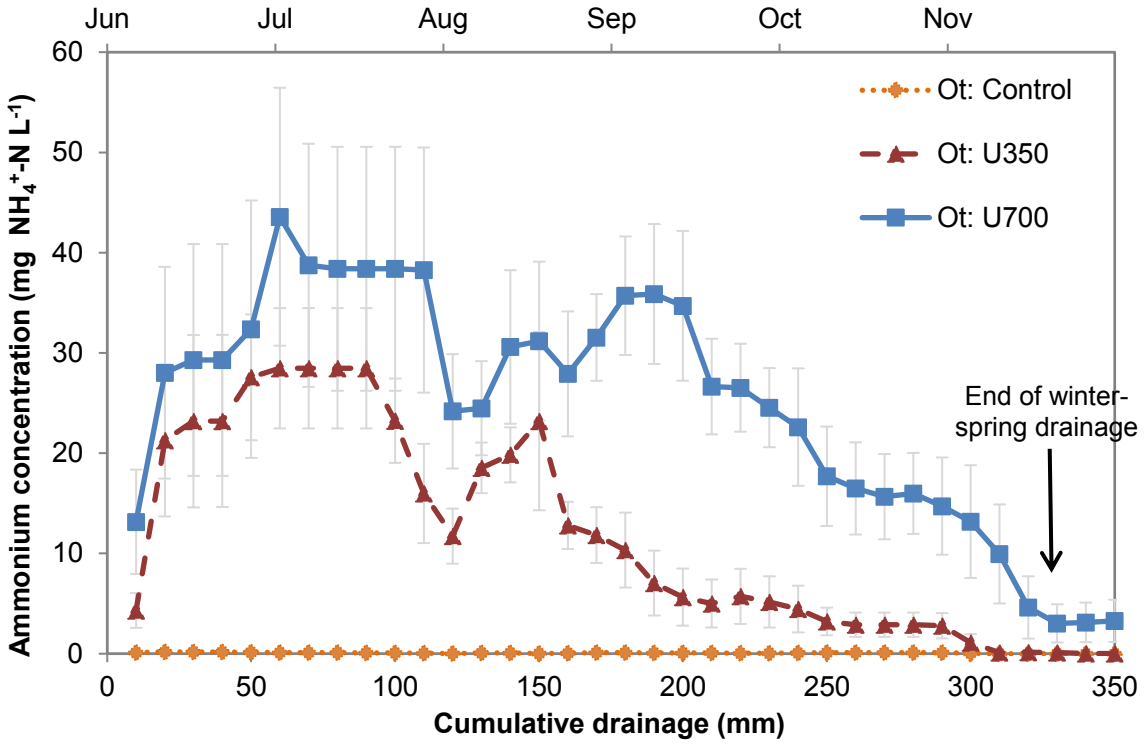


Figure 4.3-7. Ammonium concentrations vs. cumulative drainage following the June/July urine applications (0, 350 & 700 kg N ha^{-1}) to the oats lysimeters. Time scale approximate.

The application of DCD one day following urine application (350 kg N ha^{-1}) reduced peak nitrate concentration for both crops by more than 50% (Figures 4.3-8 & 4.3-9) over the drainage period. DCD application had no effect on peak ammonium concentrations for either crop (Figures 4.3-10 & 4.3-11). Total nitrate leached over the winter-spring drainage period was significantly reduced for both crop DCD treatments (P<0.001) but especially for the oats (P<0.01) where there was a 55% reduction (33% for lt. ryegrass; Table 4.3-3). There was no significant difference between DCD treatments for ammonium leaching loss (~30% of total nitrate leached to the end of the winter-spring drainage period) but there was a difference between breakthough curves with DCD application reducing peak ammonium concentrations but prolonging its presence in drainage (Figures 4.3-10 & 4.3-11). Differences between crop (P<0.01) and DCD treatments (P<0.001) for total inorganic-N leaching losses between DCD treatments were similar as for nitrate (Table 4.3-3).

Approximately 45-55% of the total nitrate leaching loss for the DCD treatments was directly attributable to the ^{15}N-labelled urine and 69-74% for ammonium loss (Table 4.3-3). Drainage for each crop was not significantly different between DCD treatments although drainage overall was ~21% lower for the oats treatments (P<0.01).

Table 4.3-3. Total inorganic-N leaching (NO_3^--N, NH_4^+-N and total-N), drainage and inorganic-^{15}N leaching losses for lt. ryegrass and oats DCD treatments (350 kg N ha^{-1}; 0 or 20 kg DCD ha^{-1}) to the end of the winter-spring drainage period.

Treatment		*[b] Inorganic-N leached (kg N ha^{-1})*			*Drainage (mm)*	*Inorganic-^{15}N leached (kg N ha^{-1})*		
Crop	**Inhibitor**	**NO_3^--N**	**NH_4^+-N**	**Total-N**	**Spring**	**NO_3^--^{15}N**	**NH_4^+-^{15}N**	**Total-^{15}N**
Italian ryegrass	None	167 c	26 a	192 b	369 ab	93 c	19 a	112 b
	DCD	112 b	46 a	158 b	423 b	59 b	34 a	94 b
Oats	None	131 bc	41 a	172 b	332 a	78 bc	31 a	109 b
	DCD	50 a	32 a	82 a	296 a	23 a	22 a	45 a
Crop		**	ns	**	**	*	ns	*
Inhibitor		***	ns	***	ns	***	ns	**
Crop x Inhibitor		ns	ns	ns	ns	ns	ns	*
[a] LSD (5%)		41	26	41	71	27	23	33

[a] LSD: least significant difference (P < 0.05) at crop x inhibitor level. [b] Means followed by a letter in common are not significantly different according to Duncan's multiple range test (P < 0.05).

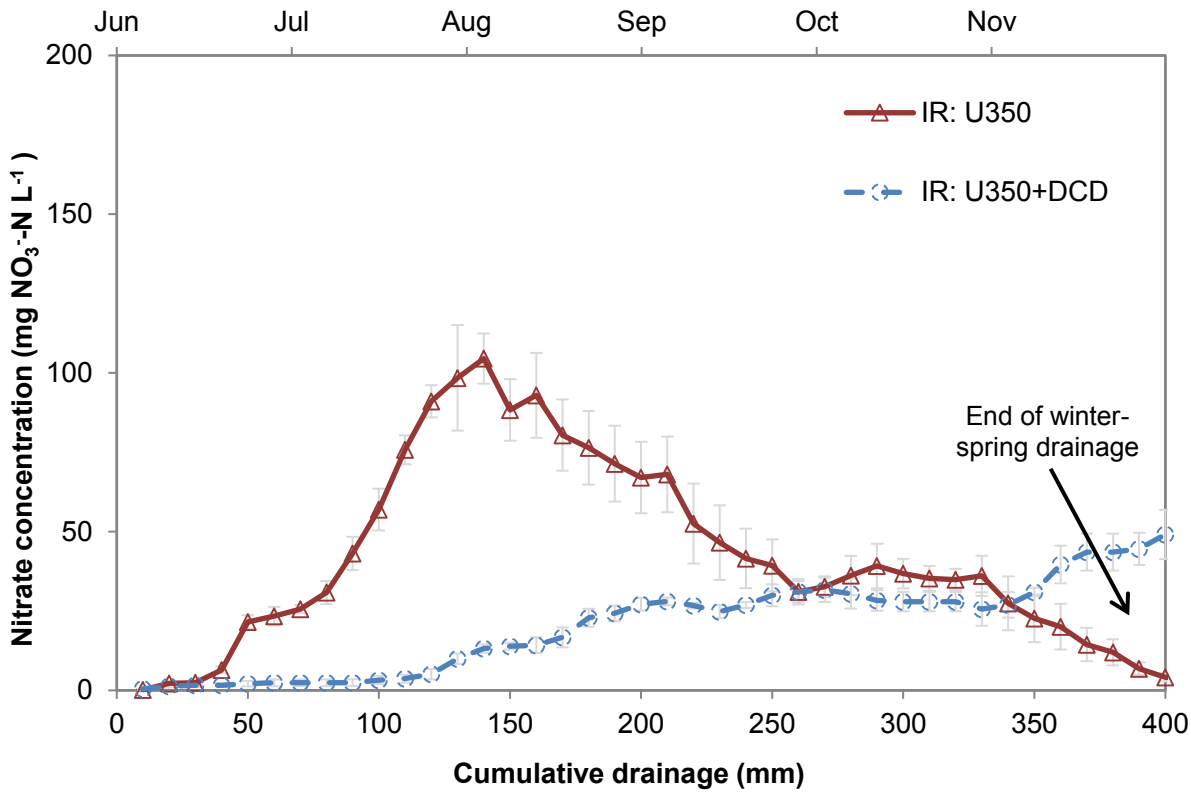


Figure 4.3-8. Nitrate concentrations vs. cumulative drainage following urine (U350) ±DCD application (0 & 20 kg DCD ha^{-1}) to the lt. ryegrass lysimeters. Time scale approximate.

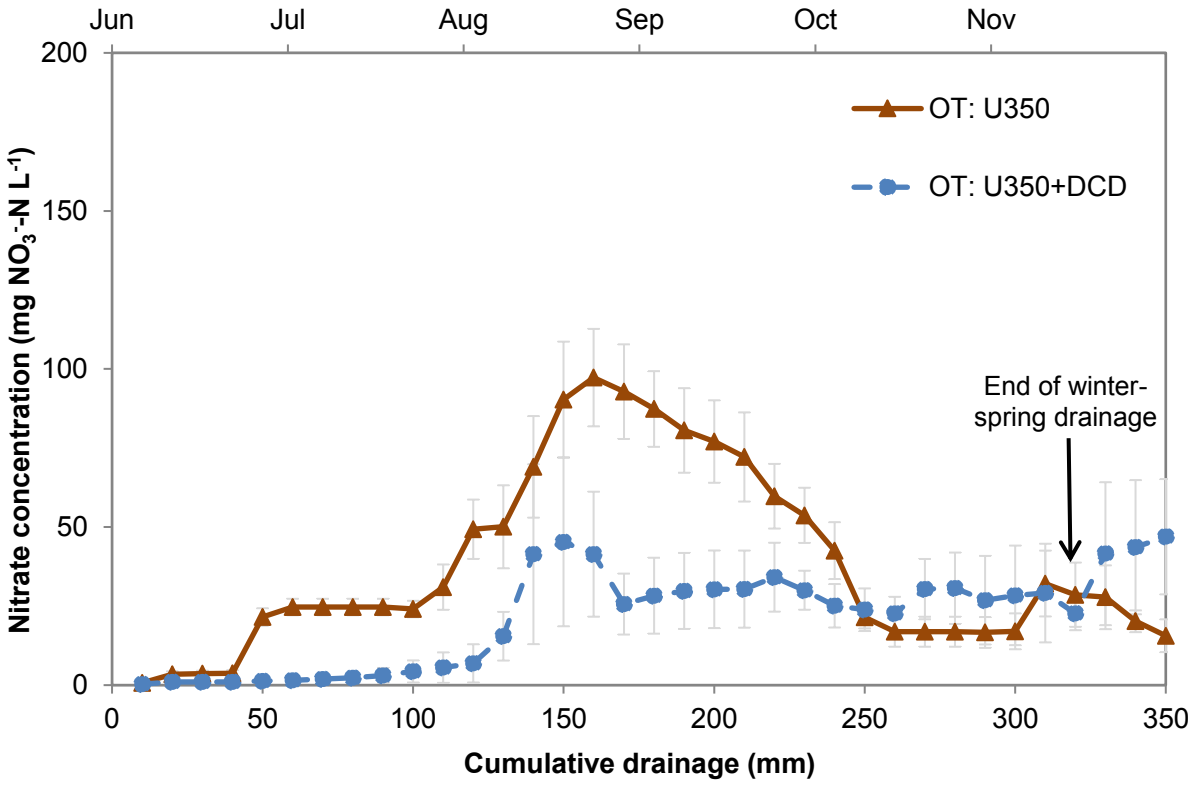


Figure 4.3-9. Nitrate concentrations vs. cumulative drainage following urine (U350) ±DCD application (0 & 20 kg DCD ha^{-1}) to the oats lysimeters. Time scale approximate.

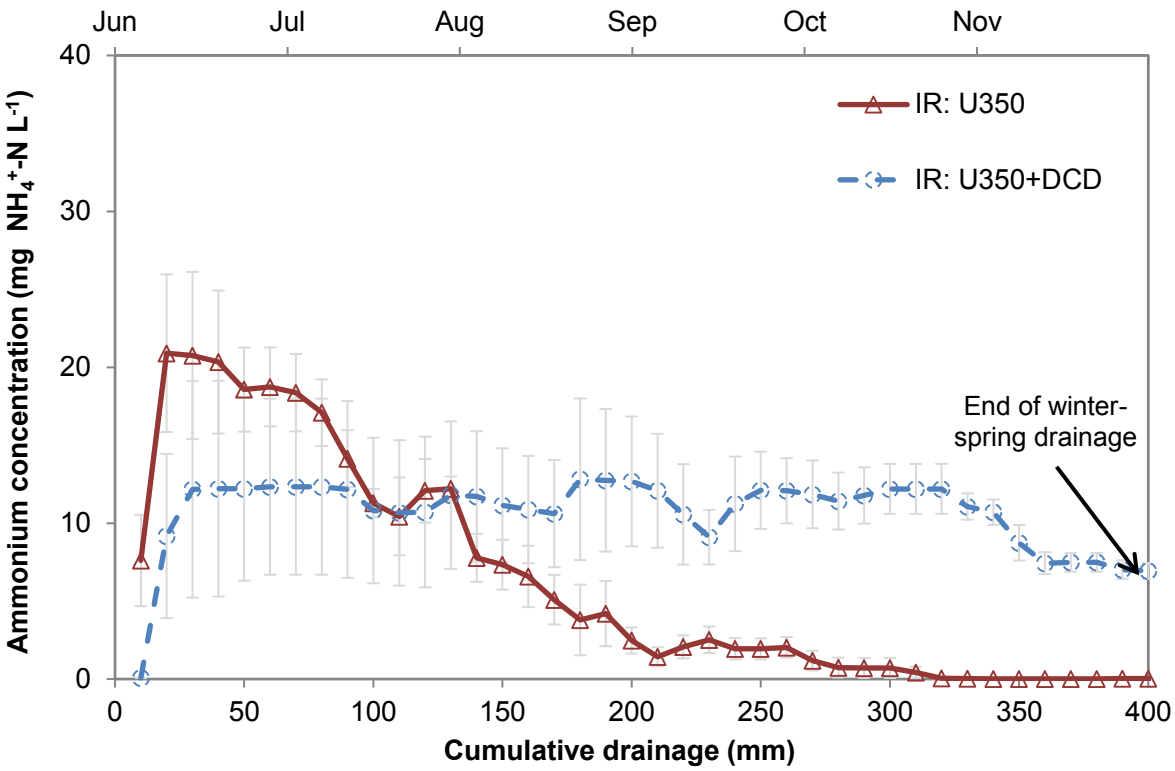


Figure 4.3-10. Ammonium concentrations vs. cumulative drainage following urine (U350) ±DCD application (0 & 20 kg DCD ha^{-1}) to the lt. ryegrass lysimeters. Time scale approximate.

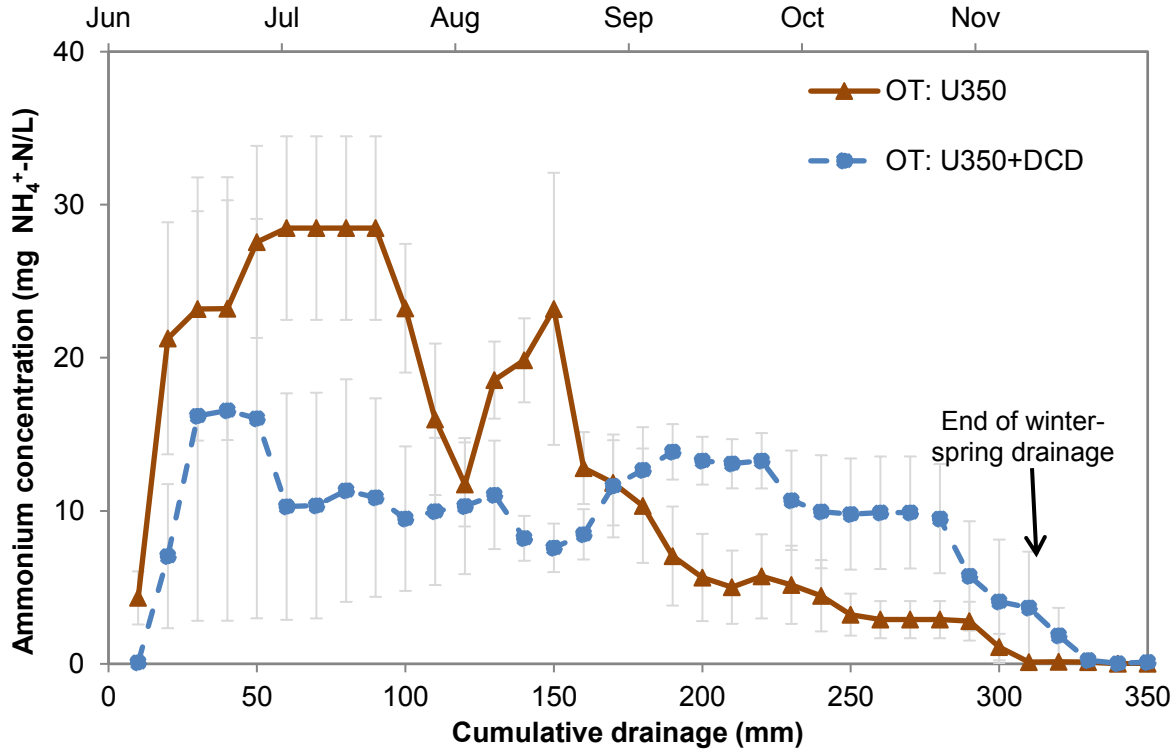


Figure 4.3-11. Ammonium concentrations vs. cumulative drainage following urine (U350) ±DCD applications (0 & 20 kg DCD ha^{-1}) to the oats lysimeters. Time scale approximate.

4.3.4 Winter-spring dry-matter production and N uptake

Dry-matter production and ^{15}N uptake for the It. ryegrass and oats treatments to Dec. 2013 increased with urinary-N rate ($P<0.001$) but the It. ryegrass treatments grew only half the DM, and with half the ^{15}N uptake, of the oats U350 and U700 treatments by the same stage (Figure 4.3-12).

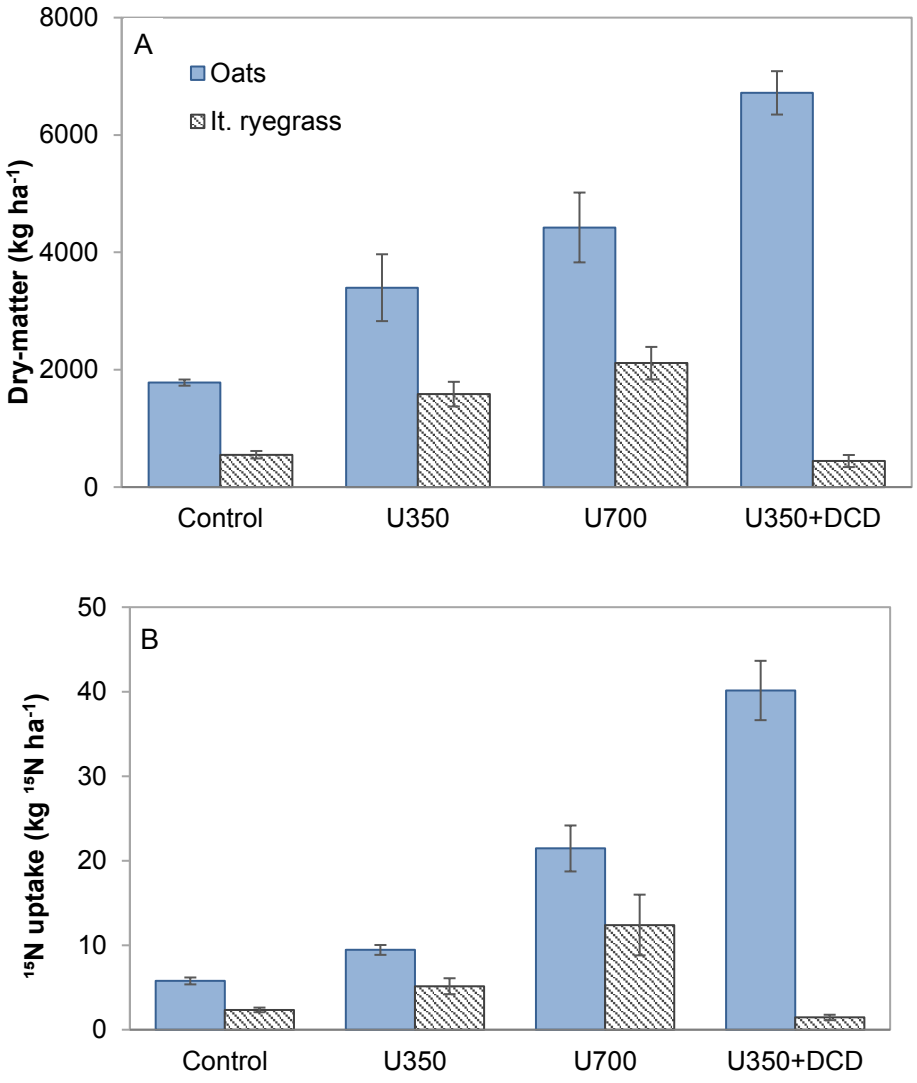


Figure 4.3-12. Oats and It. ryegrass dry-matter production (A) and ^{15}N uptake (B) to the end of the winter-spring period (Dec. 2013) after winter (Jun. 2013) application of ^{15}N-labelled urine.

However, even for the oats treatments, the proportion of ^{15}N captured from the total ^{15}N applied was small, 2-3%, increasing to 3-4% when the second kale crop was harvested at the end of the study, the same as the It. ryegrass treatments after all harvests (Table 4.3-5). DCD application had a significant effect ($P<0.001$) on both oats DM production and ^{15}N uptake, doubling and tripling (11% of the urinary-^{15}N applied) each, respectively, compared with the standard U350 treatment.

This was not the case for the ryegrass control and DCD treatments where establishment problems after sowing meant lower growth, and a lower ^{15}N recovery at the same stage.

4.3.5 Annual nitrogen recovery

Nitrogen recovery (not N balance) was calculated as the sum of the amount of N measured over the entire duration of the study (26 June 2013-30 May 2014) in drainage, gaseous-N emissions (NH_3 & N_2O) plant N uptake or remaining as soil mineral-N. Total-N recovery ranged from ~90-550 kg N ha^{-1} (control to U700 treatments; Figure 4.3-14) with nitrate loss being the single biggest component. The annual nitrate leaching loss in drainage water was not significantly different between catch crops, but at ~170 and ~260 kg N ha^{-1} for both 350 (U350) and 700 (U700) kg N ha^{-1} urine rates, respectively, it represented 37-48% of the urinary-N applied (Table 4.3-4). Control treatments had a relatively high 30-52 kg N ha^{-1} nitrate leaching loss over the annual period. Total inorganic-N leaching loss in drainage water, including ammonium, for both catch crops was ~200 and ~350 kg N ha^{-1} for U350 and U700 treatments, respectively, which represented around 50% of the total N applied. The fraction of nitrate-N and total inorganic-N (NO_3^--N+NH_4^+-N) in drainage loss directly attributable to the applied urinary-^{15}N ranged from 50-62% (Table 4.3-5). Generally, ^{15}N-nitrate concentrations followed the main nitrate leaching breakthrough curve for both lt. ryegrass and oats treatments (control, 350 and 700 kg N ha^{-1}) but after ~300 mm of drainage the proportion of ^{15}N decreased to less than half and continued to decrease thereafter (Figures 4.3-15 & 4.3-16). However, all treatments experienced a spike in nitrate concentration over the second half of the drainage curve that was mirrored in the ^{15}N-nitrate fraction, before declining again in subsequent drainage.

Volatilisation ($P<0.01$) and N_2O ($P<0.001$) losses both increased linearly with urinary-N rate, with no difference in N_2O loss between crops (Table 4.3-4). Ammonia volatilisation losses on average were 1, 11 and 28 kg N ha^{-1} for control, U350 and U700 treatments, respectively, and mainly occurred in the first week after urine application (and essentially completed within two; Figure 4.3-17). Nitrous oxide losses totalled 1.6, 7.6 and 11.7 kg N ha^{-1} for control, U350 and U700 treatments, respectively, but emission factors (EF_3 range: 0.13-0.21) weren't significantly different between treatments for either crop or urinary-N rate (Table 4.3-4). Peak N_2O emissions for U350

and U700 treatments were ~230 and ~280 g N_2O-N $ha^{-1}d^{-1}$, respectively with approximately 50% of the N_2O loss directly attributable to the ^{15}N-labelled urine (Table 4.3-5).

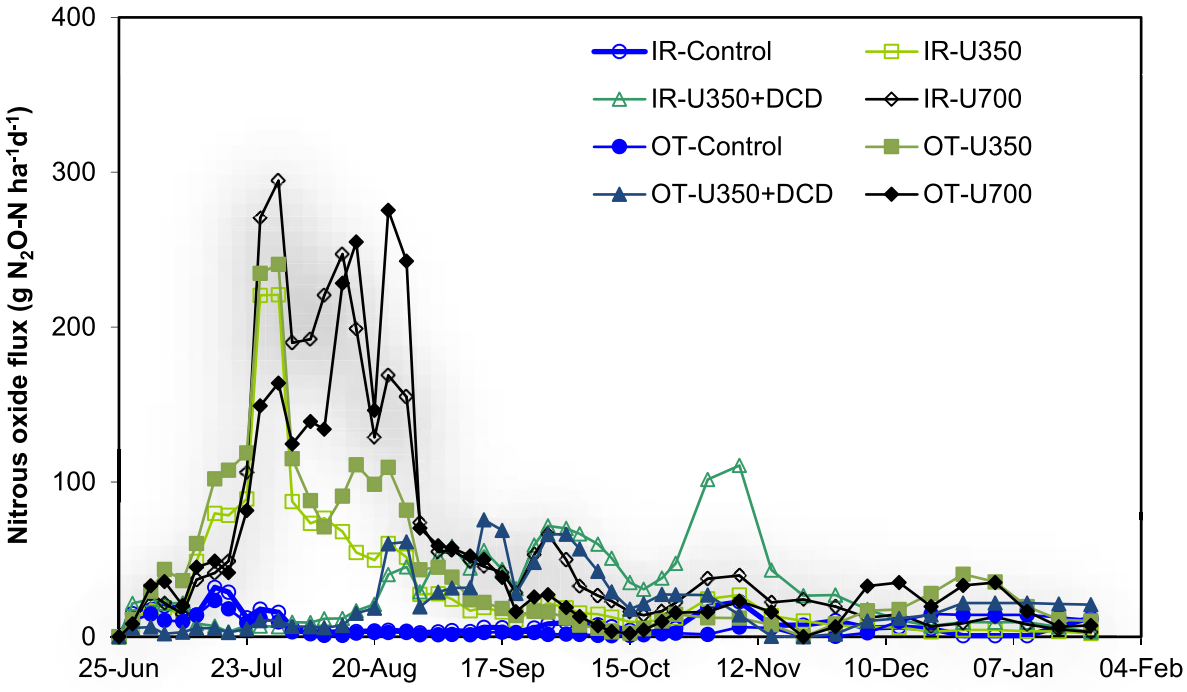


Figure 4.3-13. Nitrous oxide emissions for lt. ryegrass and oats urine treatments after urine application in June 2013.

Table 4.3-4. Annual total inorganic-N leaching losses (NO_3^--N, NH_4^+-N and total-N), gaseous-N losses, N_2O emission factors (*EF_3*), dry-matter production and N contents for lt. ryegrass and oats urine treatments (control, 350 and 700 kg N ha^{-1}).

Treatment		*[b] Inorganic-N leached (kg N ha^{-1})*			*Gaseous-N loss (kg N ha^{-1})*		*N_2O (%)*	*[c] Dry-matter (kg ha^{-1})*		*N content (kg N ha^{-1})*	
Crop	**Urinary-N rate**	**NO_3^--N**	**NH_4^+-N**	**Total-N**	**NH_3**	**N_2O**	**EF_3**	**Tops**	**Roots**	**Tops**	**Roots**
Italian ryegrass	0	52 a	2 a	53 a	0 a	1.9 a	-	1782 a	2574 b	34 b	31 b
	350	171 b	26 ab	197 b	11 b	6.4 b	1.3 a	4180 bc	3143 c	83 c	41 c
	700	274 c	85 c	359 c	28 c	12.0 d	1.5 ab	4995 c	2768 bc	100 c	40 c
Oats	0	30 a	0 a	30 a	-	1.4 a	-	1818 a	1705 a	16 a	18 a
	350	172 b	41 b	213 b	-	8.7 c	2.1 b	3468 b	2103 ab	88 b	21 a
	700	251 c	86 c	337 c	-	11.3 d	1.4 ab	4516 bc	2407 ab	98 b	23 a
Crop		ns	ns	ns	-	ns	ns	ns	***	ns	***
Urine rate		***	***	***	**	***	ns	***	ns	***	**
Crop x urine rate		ns	ns	ns	-	ns	ns	ns	ns	ns	ns
[a] LSD (5%)		49	28	61	10	2.4	0.7	1208	711	16	7

[a] LSD: least significant difference ($P < 0.05$) at crop x urine rate level. [b] Means followed by a letter in common are not significantly different according to Duncan's multiple range test ($P < 0.05$); [c] Oats treatments includes both oats and kale harvests.

Table 4.3-5. Annual total inorganic-^{15}N leaching loss (NO_3^--^{15}N, NH_4^+-^{15}N and total-^{15}N), gaseous-^{15}N loss, ^{15}N content of dry-matter and soil-^{15}N for lt. ryegrass and oats urine treatments (control, 350 and 700 kg N ha^{-1}).

Treatment		*Inorganic-^{15}N leached (kg N ha^{-1})*			*Gaseous-^{15}N loss (kg ^{15}N ha^{-1})*		*^{15}N content (kg ^{15}N ha^{-1})*		*Soil-^{15}N (kg ^{15}N ha^{-1})*
Crop	**Urinary-N rate**	**NO_3^--N**	**NH_4^+-N**	**Total-N**	**N_2O**	**N_2**	**Tops**	**[c] Roots**	**[d] Total-N**
Italian ryegrass	0	6 a	0 a	6 a	0.1 a	5 a	1 a	0.4 a	11 a
	350	94 b	19 ab	114 b	3.1 b	43 b	12 b	4.1 d	110 b
	700	171 c	66 c	237 c	6.7 c	56 c	25 c	6.3 e	137 c
Oats	0	3 a	0 a	3 a	0.1 a	5 a	11 b	0.2 a	12 a
	350	85 b	31 b	116 b	3.8 b	52 c	15 b	2.0 b	93 b
	700	142 c	68 c	210 c	5.6 c	65 c	30 c	3.0 c	158 c
Crop		ns	ns	ns	ns	ns	**	**	ns
Urine rate		***	***	***	***	***	***	***	***
Crop x urine rate		ns	ns	ns	ns	ns	ns	ns	ns
[a] LSD (5%)		29	24	40	1.3	12	8	0.9	23

[a] LSD: least significant difference ($P < 0.05$) at crop x urine rate level. [b] Means followed by a letter in common are not significantly different according to Duncan's multiple range test ($P < 0.05$); [c] Oats roots refers to subsequent kale crop; [d] Total-N includes organic and inorganic N.

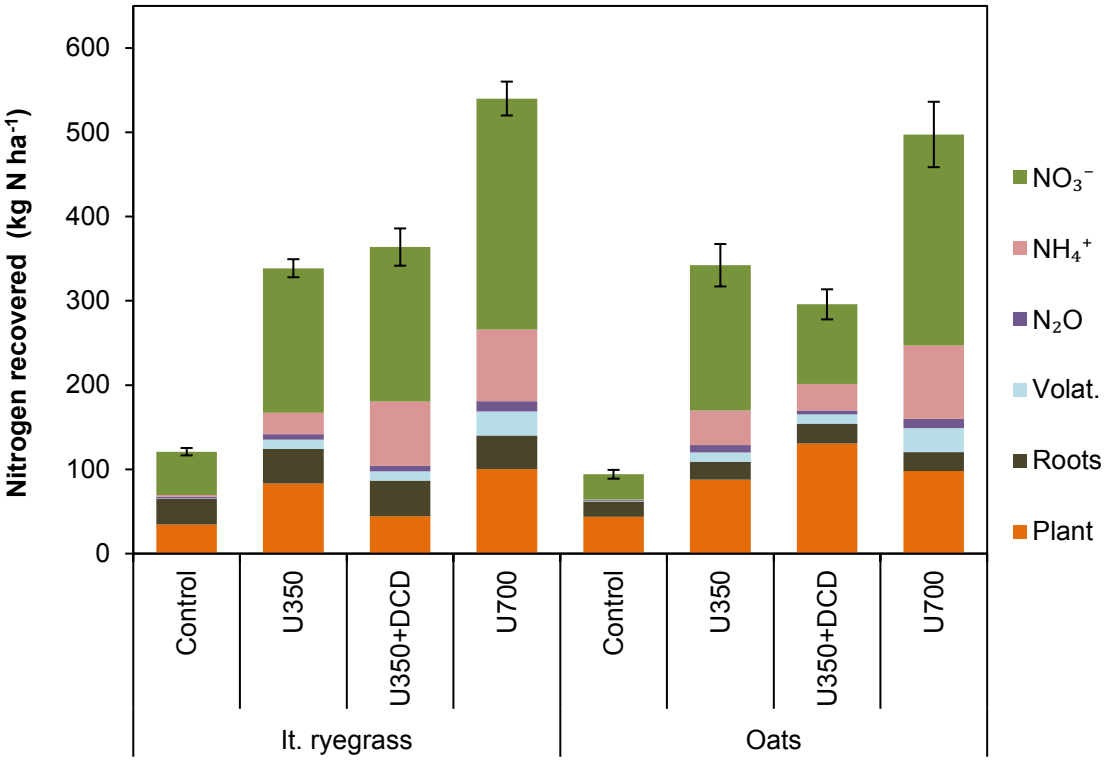


Figure 4.3-14. Annual losses of nitrogen from applied urine recovered in plant dry-matter, gas products and leachate for all crop treatments.

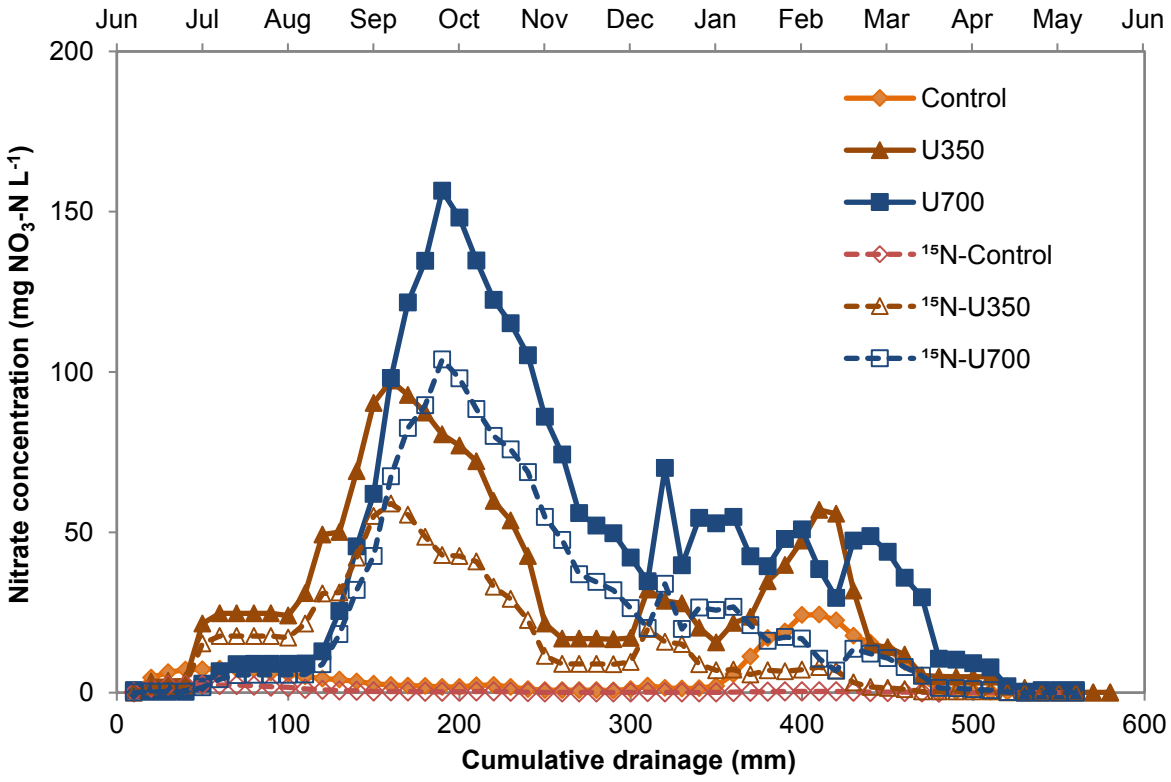


Figure 4.3-15. Nitrate concentration and ^{15}N-fraction for lt. ryegrass urine treatments (control, 350 and 700 kg N ha^{-1}) over the full year. Timeline indicative only.

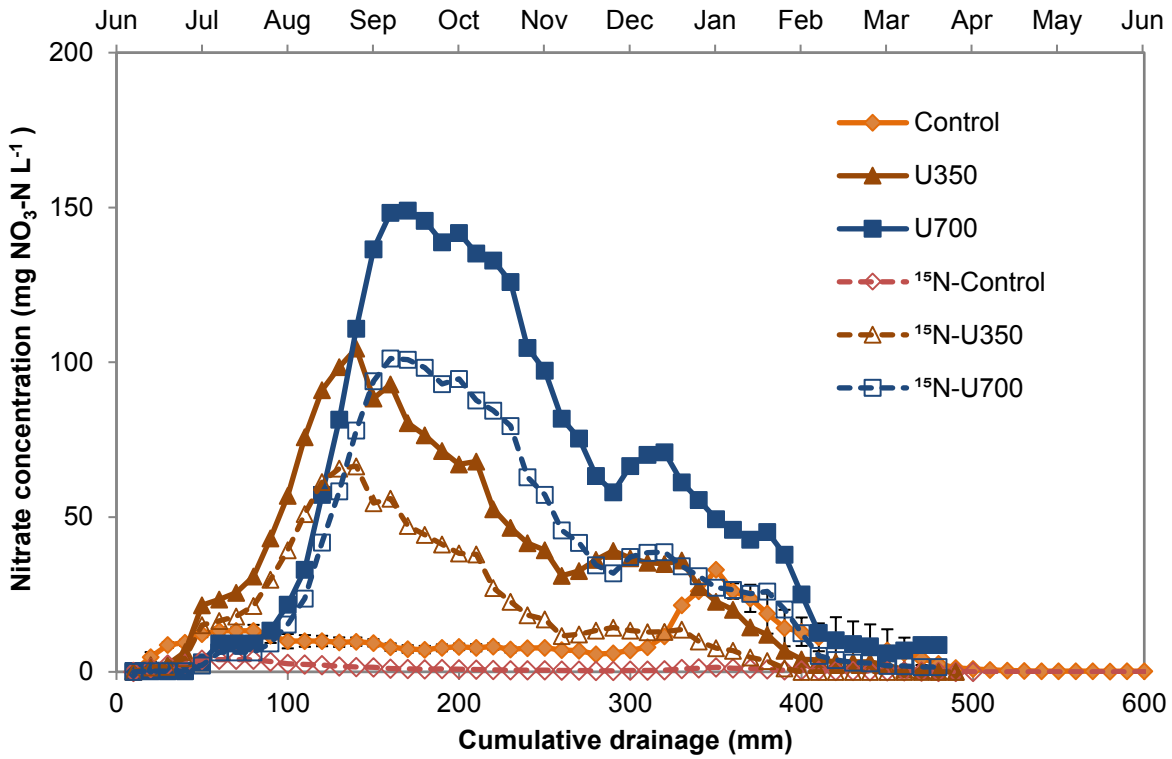


Figure 4.3-16. Nitrate concentration and ^{15}N-fraction for the oats urine treatments (control, 350 and 700 kg N ha^{-1}) over the full year. Timeline indicative only.

Annual DM production increased with urinary-N rate (P<0.001; Table 4.3-4) but the quantities harvested for the U350 and U700 treatments were not large overall at 3500-5000 kg DM. Nitrogen uptake between respective catch crop treatments (including the subsequent kale crop for the oats' treatments) was similar overall (Figure 4.3-14) at ~40, ~85 and ~100 kg N ha^{-1} for control, U350 and U700 treatments, respectively. However, the proportion of N captured attributable to the ^{15}N-labelled urine was similar between crops at 3-4% of that applied (Table 4.3-5).

The application of DCD reduced annual nitrate leaching losses overall for both catch-crops but especially for the oats where the total nitrate drainage loss was reduced by 45% compared with no DCD (Table 4.3-6). The nitrate loss directly attributable to the ^{15}N-labelled urine from the oats DCD treatment was lower still with a 59% reduction over the non-DCD treatment; but only 21% for the It. ryegrass DCD treatment (Table 4.3-7). Nitrate breakthough curves for the DCD treatments, and the ^{15}N proportion for each, differed between crops. For oats, the peak nitrate concentration and overall fraction of ^{15}N (<50%) was considerably less than for the non-DCD treatment, and the breakthrough curve less dominated by a single pronounced peak at ~150 mm drainage (Figure

4.3-18). For the It. ryegrass DCD treatment, however, there was a slower build-up in nitrate concentrations, with an initial peak at ~270 mm drainage but a larger one at ~410 mm (Figure 4.3-19). The ^{15}N-nitrate curve for the It. ryegrass DCD treatment largely remained around half that for the full nitrate-N curve.

DCD application lowered N_2O emissions on the oats U350 lysimeters, where it was approximately halved (Table 4.3-6), and where the loss directly attributable to the ^{15}N fraction was only around 25% of that applied (Table 4.3-7). However, this was not the case for the It. ryegrass U350+DCD treatments, where the seed had initially failed to flourish and there had been limited N uptake over the critical winter-spring drainage period. For these treatments a higher proportion of N_2O loss was directly attributable to the ^{15}N-labelled urine with a second peak of N_2O emission in November 2013 that coincided with a peak in nitrate concentration at ~410 mm drainage compared with that for the non-DCD treatment at 140 mm (Figure 4.3-20).

Emission factors (EF_3) were significantly different ($P<0.05$) between oats DCD and non-DCD treatments (0.9% vs. 2.1%, respectively) but not between It. ryegrass DCD treatments. DCD application to the oats U350 lysimeters more than doubled both DM production and N uptake but not on the It. ryegrass U350 treatments, with a subsequent effect on N uptake. N uptake attributable to the ^{15}N-labelled urine was even more pronounced for the oats DCD U350 treatment where it was almost three-times that of the non-DCD treatment (Table 4.3-7).

Table 4.3-6. Effect of DCD (350 kg N ha^{-1}; 0 or 20 kg DCD ha^{-1}) on the annual total inorganic-N leaching loss (NO_3^--N, NH_4^+-N and total-N), N_2O loss, N_2O emission factor (EF_3), dry-matter production and N content for lt. ryegrass and oats treatments.

Treatment		*[b] Inorganic-N leached (kg N ha^{-1})*			*N_2O loss (kg N ha^{-1}; %)*		*Dry matter (kg ha^{-1})*		*N content (kg N ha^{-1})*	
Crop	**Inhibitor**	**NO_3^--N**	**NH_4^+-N**	**Total-N**	**N_2O**	**[c] EF_3**	**Tops**	**[d] Roots**	**[e] Tops**	**Roots**
Italian ryegrass	None	171 b	26 a	197 b	6.4 ab	1.3 ab	4180 b	3143 b	83 b	41 b
	DCD	138 ab	46 a	185 b	6.8 ab	1.4 ab	2259 a	3450 b	44 a	42 b
Oats	None	172 b	41 a	213 b	8.7 b	2.1 b	3468 ab	2103 a	37 a	22 a
	DCD	94 a	31 a	126 a	4.6 a	0.9 a	6857 c	2268 a	92 b	23 a
Crop		ns	ns	ns	ns	-	***	***	ns	***
Inhibitor		***	ns	***	ns	*	ns	ns	ns	ns
Crop x inhibitor		ns	ns	*	*	*	***	ns	***	ns
[a] LSD (5%)		45	26	45	2.9	0.8	1276	798	25	7

[a] LSD: least significant difference ($P < 0.05$) at crop x urine rate level. [b] Means followed by a letter in common are not significantly different according to Duncan's multiple range test ($P < 0.05$); [c] Emission factors are calculated from net difference after control subtracted; [d] Roots for oats treatments relates to subsequent kale crop at time of harvest; [e] Oats tops refers to actual harvest not subsequent kale crop.

Table 4.3-7. Effect of DCD (350 kg N ha^{-1}; 0 or 20 kg DCD ha^{-1}) on the annual total inorganic-^{15}N leaching loss (NO_3^--^{15}N, NH_4^+-^{15}N and total-^{15}N), gaseous-^{15}N loss, ^{15}N content of dry-matter and soil-^{15}N for the lt. ryegrass and oats treatments.

Treatment		*[b] Inorganic-^{15}N leached (kg N ha^{-1})*			*Gaseous- ^{15}N loss (kg N ha^{-1})*		*^{15}N content (kg N ha^{-1})*		*Soil-^{15}N (kg N ha^{-1})*
Crop	**Inhibitor**	**NO_3^--N**	**NH_4^+-N**	**Total-N**	**N_2O**	**N_2**	**Tops**	**[c] Roots**	**[d] Total-N**
Italian ryegrass	None	94 b	19 a	113 b	3.1 bc	43 a	12 a	4.1 ab	110 b
	DCD	74 b	34 c	108 b	2.0 ab	53 a	9 a	5.7 c	69 a
Oats	None	85 b	31 a	116 b	3.8 c	47 a	15 a	2.0 a	93 ab
	DCD	35 a	22 c	57 a	1.0 a	52 a	41 b	2.7 a	102 b
Crop		*	ns	*	ns	ns	***	***	ns
Inhibitor		**	ns	*	***	ns	***	*	ns
Crop x inhibitor		ns	ns	*	ns	ns	***	ns	*
[a] LSD (5%)		29	23	35	1.2	10	7	1.4	32

[a] LSD: least significant difference ($P < 0.05$) at crop x urine rate level. [b] Means followed by a letter in common are not significantly different according to Duncan's multiple range test ($P < 0.05$); [c] Oats roots refers to subsequent kale crop; [d] Total-N includes organic and inorganic N.

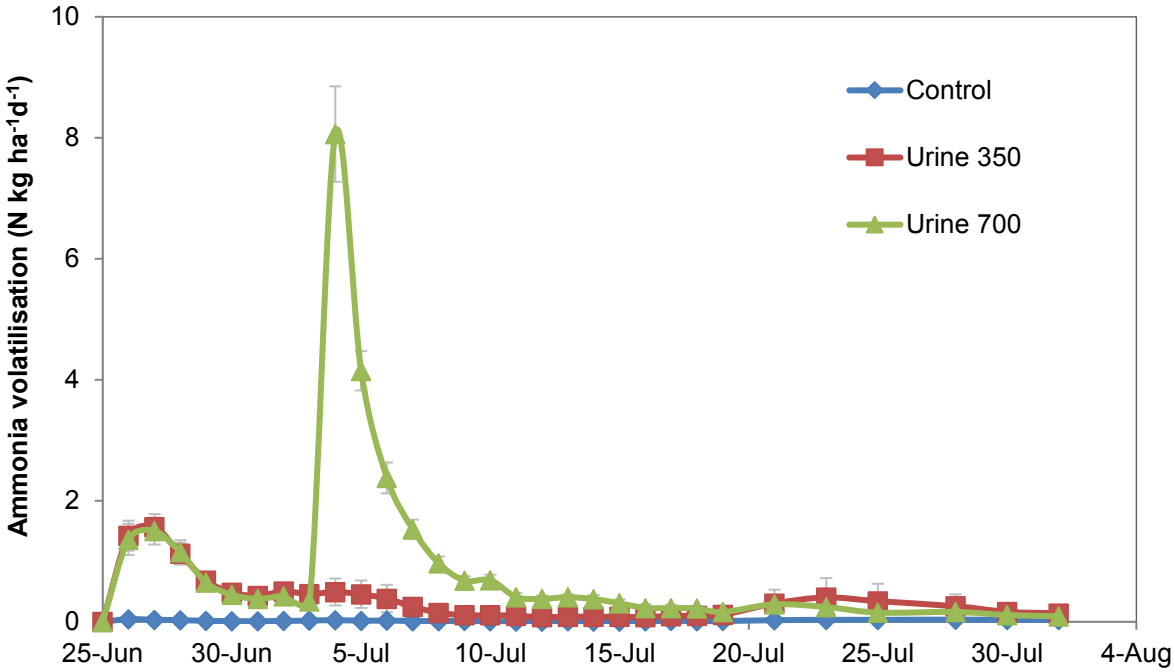


Figure 4.3-17. Daily ammonia volatilisation losses from urine application to fallow soils at 0, 350 and 700 kg N ha^{-1}.

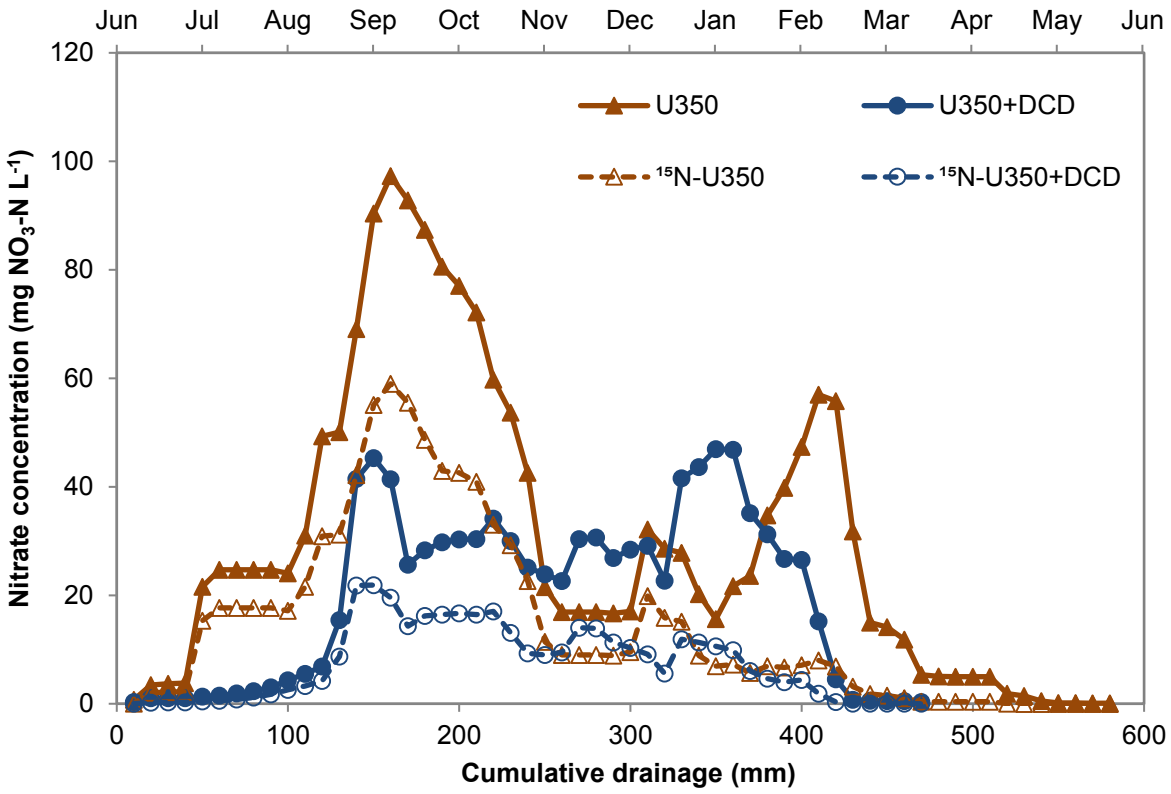


Figure 4.3-18. Effect of DCD on nitrate concentrations and ^{15}N-fraction for oats U350 urine treatments ±DCD (0 & 20 kg DCD ha^{-1}) for the full year. Timeline indicative only.

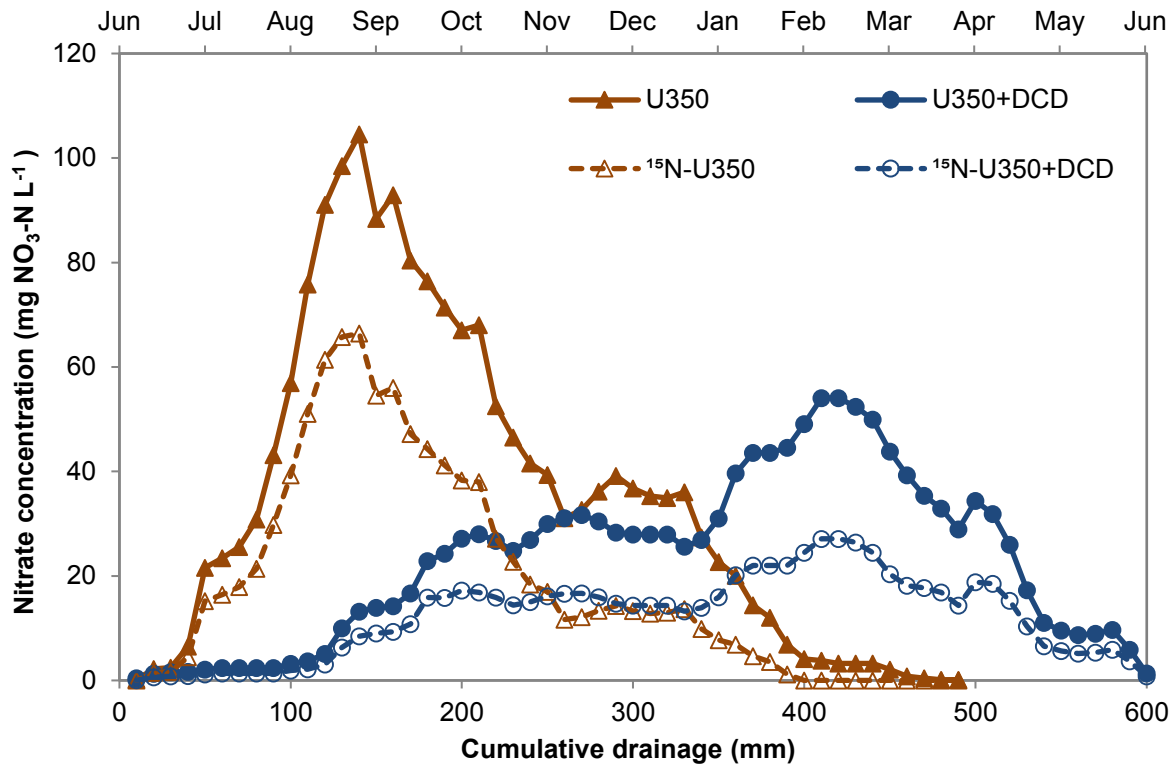


Figure 4.3-19. Nitrate concentration and ^{15}N-fraction for lt. ryegrass urine treatments U350 ±DCD (0 & 20 kg DCD ha^{-1}) over the full year. Timeline indicative only.

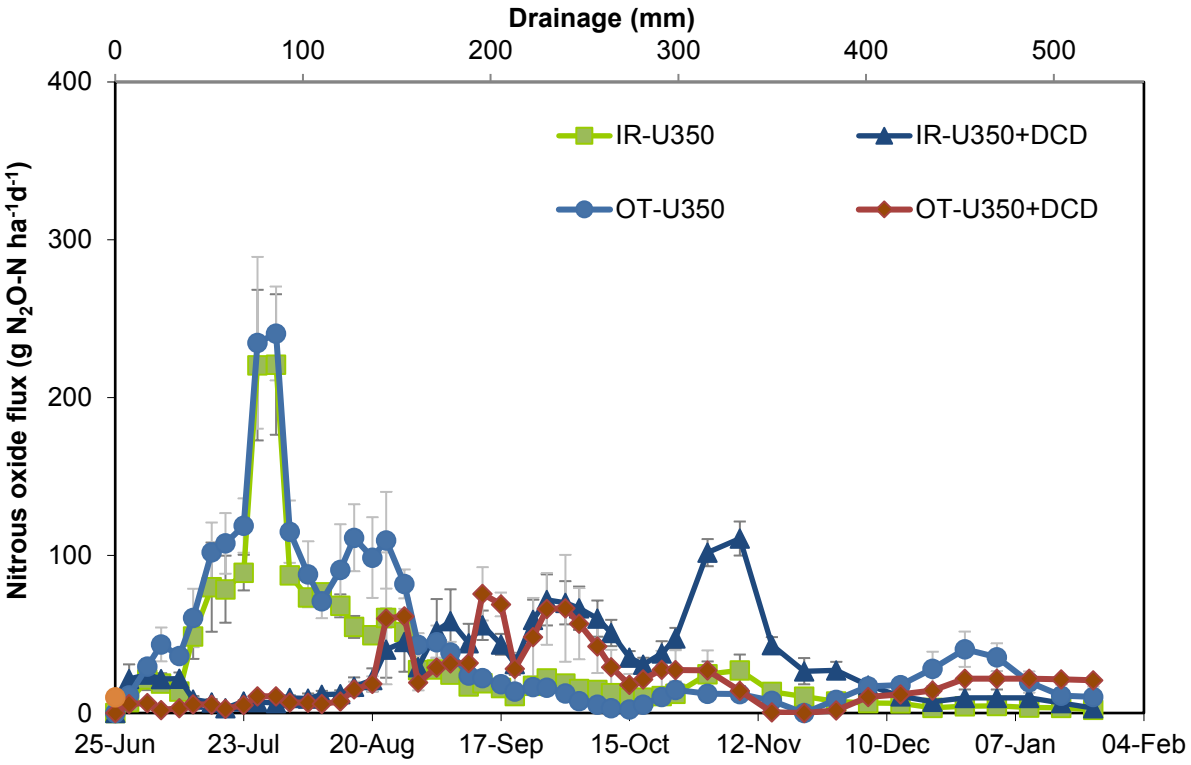


Figure 4.3-20. Effect of DCD on daily N_2O emission for lt. ryegrass and oats urine ±DCD treatments (350 kg N ha^{-1}; 0 & 20 kg DCD ha^{-1}). Drainage axis approximate only.

4.3.6 ^{15}N balance

Total ^{15}N recovery ranged from ~67-91% with average recoveries for the oats slightly higher than the lt. ryegrass treatments (79% vs 73%). Apart from the lt. ryegrass control treatment, total ^{15}N recoveries were higher at the lower urinary-N rate (Figure 4.3-21). Approximately one-third of the labelled urinary-N in the 350 and 700 kg N ha^{-1} treatments was recovered in drainage, with the next largest component, soil, accounting for a further 30% (range 23-37%). Doubling the urinary-N application rate from 350 to 700 kg N ha^{-1} increased nitrate- and inorganic-^{15}N leaching losses by 75% (range 68-81%) and 90% (range 81-108%), respectively.

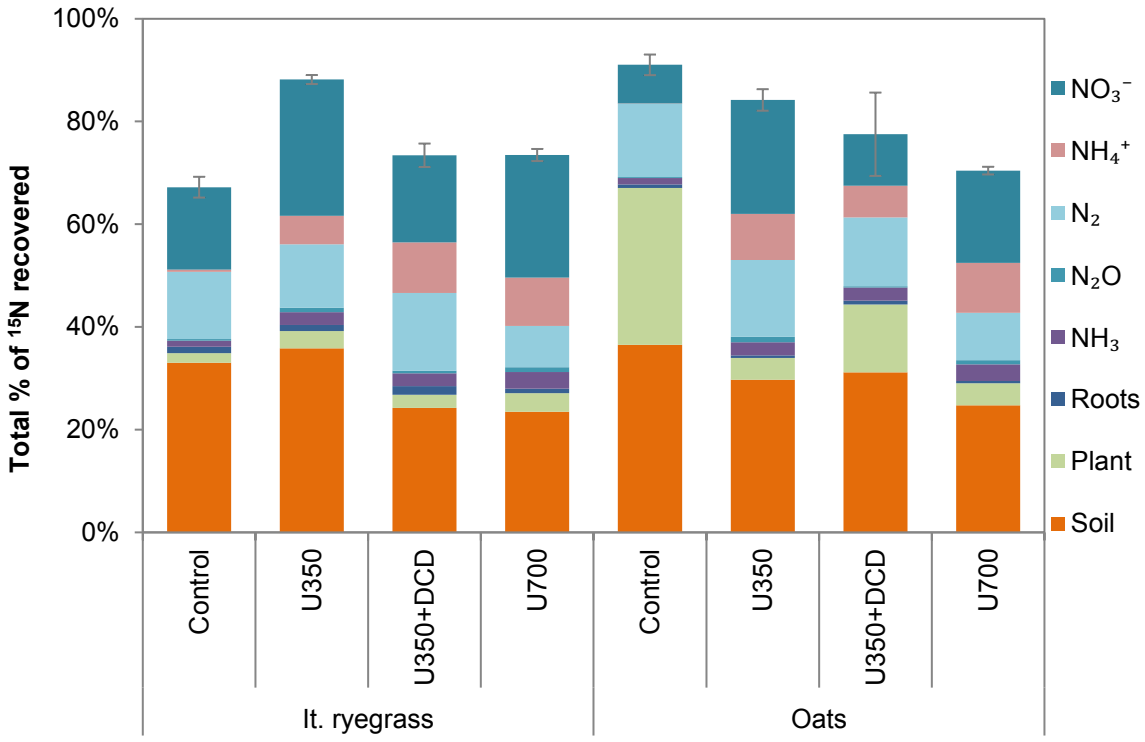


Figure 4.3-21. ^{15}N recovery (% of applied) in drainage, soil, plant and gaseous-N fractions from applied urine or water. Standard errors for total ^{15}N recovery shown.

The soil-^{15}N fraction decreased with increasing N application rate (~35%, ~33% and ~24%, on average, for control, U350 and U700 treatments, respectively). Half-to-two-thirds of the ^{15}N recovered in the soil component was, on average, located in the 0-10 cm soil depth with the remainder distributed down the profile (Figure 4.3-22). Therefore, the ^{15}N detected was mostly located within the organic material (because of immobilisation). Soil mineral-N analysis for each depth increment revealed negligible quantities of N present (<1 kg N ha^{-1}) as either NH_4^+ or NO_3^- at the time of lysimeter deconstruction. Ammonium-^{15}N in drainage water was a significant

proportion of the ^{15}N recovered ranging from 6-10% of the total urinary-^{15}N applied for both U350 (±DCD) and U700 treatments (negligible NH_4^+-N was recovered in controls).

DCD application reduced ^{15}N-nitrate drainage losses for the oats compared to the non-DCD treatment by more than half (10% vs 22%, respectively). It. ryegrass DCD treatments also leached less nitrate than the equivalent non-DCD treatment (17% vs 27%, respectively). DCD application increased the proportion of N retained in the harvested oats (13% vs 4% of N applied, respectively) compared with the oats U350 non-DCD treatment but not for the It. ryegrass. Generally, however, the fraction of ^{15}N retained in harvested plant material for both catch-crop in the 350 and 700 kg N ha^{-1} treatments was small and comprised only 3-4% of the N applied (Figure 4.3-21). ^{15}N recovery in roots was small at only 1-3% and made up almost entirely of the roots collected in the top 10 cm. Dinitrogen-^{15}N loss comprised around 10-15% of the total-N applied but was only able to be measured in the first 3 months after urine application due to emissions falling below measurable levels. Nitrous oxide loss, as a proportion of urinary-^{15}N applied, was small (<1.0%). The proportion of volatilisation loss as ^{15}N-NH_3 was estimated at 90% of the measured values (Lee et al. 2011).

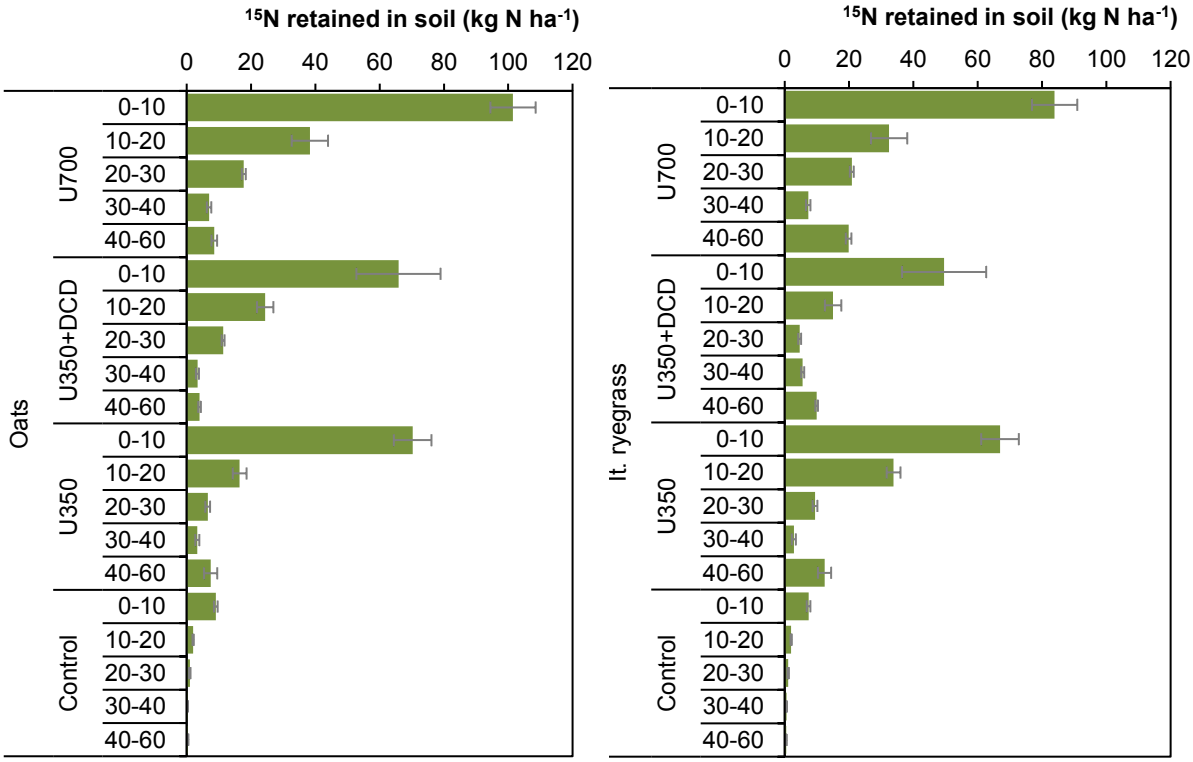


Figure 4.3-22. Soil ^{15}N recovery by depth for all oats and It. ryegrass lysimeter treatments.

4.4 Discussion

4.4.1 Drainage and nitrogen leaching

Nitrogen leaching losses in drainage were considered over two periods, from the time of urine application (26 June) to the end of spring (30 Nov), termed the winter-spring drainage period, and from the time of urine application to lysimeter deconstruction, termed the annual nitrogen recovery period. The critical leaching period for nitrogen loss is the winter-spring period because, while excess drainage might still occur post-spring, it is less likely given the water demands of a crop are starting to reach a peak by mid-December. The annual nitrogen recovery period includes post-spring drainage from natural rainfall but also any irrigation used to maintain soil moisture. Drainage from irrigation was aided because 15 mm was applied in a single event on a 3-day return as opposed to a daily 5 mm irrigation event that might have been in better balance with daily demand. However, this was considered necessary as irrigation post-spring was done in part to obtain a complete nitrate breakthrough curve as well as providing plants with sufficient soil moisture.

Sowing oats reduced nitrate leaching losses over the winter-spring drainage period by between 21-27% (av. 25%) for urine 350 (U350) and 700 (U700) kg N ha^{-1} treatments compared with their comparative It. ryegrass treatments. However, this was not due to differences in N uptake alone between the catch crops (<2% of urinary-N for both) but principally because of the smaller amount of drainage (~22% less) under the oats compared with the It. ryegrass over the winter-spring period (Table 4.3-2). Oats have an ability to grow under cool, moist soil conditions and have a higher water use per unit DM than other cereals (Zwer 2004) meaning oats can establish in conditions where even winter-active ryegrass species might struggle. In doing so, they will use more soil water, especially over the latter part of the critical winter-spring drainage period. However, why this resident nitrate was not taken up by the oats treatments' lysimeters is unclear. The main period of N uptake for cereals is in the tillering, stem elongation, booting, heading and grain filling phases (Wendling et al. 2016) so it seems unlikely that if this N was accessible, the oats would not have taken it up. However, Carey et al. (2016) found that earlier sowing of oats, following urine application, did not actually increase N uptake appreciably over the later sowings (in a drier-than-average winter) despite the former having up to 3-times as much DM. This obviously indicates

some N dilution within the plant but whether this is a plant physiology, N availability or an accessibility issue, is difficult to say.

The small proportion and few differences in plant N uptake between catch crops underlined that the sowing of both catch crops 7-11 weeks after urine application was too late to reduce the soil mineral-N pool sufficiently before extensive nitrate leaching took place, especially under the monthly 75th percentile rainfall regime used. Although the soil was trampled and compacted prior to urine application, drainage rates did not appear to appreciably slow and, indeed, the amount of ammonium recovered in drainage would also indicate that leaching was relatively rapid. Although the soil was trampled and compacted prior to urine application, this did not appear to substantially slow drainage flow rates and indeed, the amount of ammonium recovered in drainage would also indicate that leaching was relatively rapid. By the time the crops were sown, 100-200 mm of drainage had already been collected and the BTCs for both crops show nitrate concentrations by this stage were at, or nearing, maximums (Figures 4.3-4 & 4.3-5). If a catch crop is to be effective in reducing soil mineral-N to reduce nitrate leaching then earlier establishment should be considered (Carey et al. 2016). Some recent field research has shown this to be a practical proposition (Malcolm et al. 2016).

Although It. ryegrass is known to be winter active and able to take up and utilise N at a time when an equivalent perennial ryegrass pasture would not (Malcolm et al. 2014), establishing a small seed ryegrass species on a heavily compacted soil post-winter forage grazing may be problematic at times (as occurred in this experiment). Cereals like oats, with their larger seed size and sowing rates, are able to get a head start on smaller-seeded crucifers and grasses in establishing their root systems, especially in the early growth stages where roots can grow to 40-50 cm depth after only 400 degree (C°) days (Thorup-Kristensen 2001). Although winter cereals are not necessarily better at taking up nitrate at low soil temperatures compared with, for example, winter rape (Laine et al. 1994), their ability to translocate N to shoots and to the root system whilst remaining active probably assists in retaining some N against leaching during this period. Indeed, dicot species like forage radish or winter rape, for example, are superior in terms of DM production and N uptake compared with winter cereals, but on average they require 600 degree days before their root systems reach the same level of development as cereals (Thorup-Kristensen 2001). A key to catch-crop success

is the rate of root growth and, therefore, deeper rooting species like winter cereals have an immediate advantage (Thorup-Kristensen et al. 2003). Although from 600 degree days onwards other catch-crop species might have growth characteristics superior to cereals in terms of N uptake, the window to act as an effective catch crop post-winter forage grazing is small. Consequently, the list of suitable catch crops that might germinate and establish an effective deep root system at an initial soil temperature range of ~5-6°C is small, and winter-active cereals like oats have, in theory, an advantage.

4.4.2 Annual N losses

Annually, the total N leaching loss was similar between catch crops at ~40, ~200 and ~350 kg N ha^{-1} for the control, U350 and U700 treatments, respectively, comprising around 50% of the N applied. Nitrate comprised ~75-88% of the total-N leaching loss with ammonium leaching comprising the remainder (12-25%). Frequent irrigation events after the end of the winter-spring period (end of November) largely leached any remaining nitrate present within the lysimeters but it differed significantly between catch crops. Breakthrough curves for the oats saw larger and repeated spikes in nitrate concentration in drainage immediately following the post-oats harvest period, but for the It. ryegrass there was only a single smaller peak in nitrate concentration before declining to baseline. This suggests that while the oats did not take up any more N than the ryegrass, it could lower drainage sufficiently to retain the nitrate within the lysimeter, albeit to lose it in drainage again in the post-harvest period. Consequently, nitrate losses for the It. ryegrass U350 and U700 treatments were largely completed by the end of the winter-spring period but for the oats a further 23-24% of the annual nitrate leached was captured in that subsequent period. Under a winter-forage/catch-crop system, the normal management plan would be to harvest the oats by early-to-mid November and immediately prepare a seed bed for another winter forage crop or new pasture. This suggests that with careful management, much of this N could be captured by a following crop or pasture if excess drainage could be avoided.

The relatively large proportion of ammonium recovered in drainage for both U350 and U700 treatments (13-26% of total-N leached) was surprising but similar to the value of 25% reported by Malcolm et al. (2015) for a urine-N leaching study on the same Balmoral soil. Reports of significant

ammonium leaching are rare but most N leaching studies are typically conducted under less-free draining and/or deeper soil profiles e.g. Fraser (1992) and Sprosen et al. (2009) and/or at more optimal times for nitrification and plant-N uptake (Di & Cameron 2002b). The significant quantity of ammonium in drainage water can largely be attributed to a combination of preferential leaching and saturation of the soil's cation exchange complex. Cichota et al. (2016) has recently reported a study examining preferential leaching under a Canterbury stony Lismore soil under two levels of irrigation intensity and reported in both cases that the lower soil water fraction (f_t) (boundary) involved in solute transport for a moist soil was ~0.35. The instantaneous winter applications of urine (10 mm at a time) occurring on the U350 (1 application) and U700 (2 applications) lysimeters, a week apart, would not be dissimilar to an intense irrigation event. This means, given the Balmoral soil's relatively high stone content (around 50% at 20-30 cm) and reduced pore water capacity, a greater potential for rapid solute transport. Although the soil surface on each lysimeter was pugged by the artificial hoof prior to urine application, some preferential channels likely remained open for rapid transfer of the urine to occur. It is also highly likely that with potassium concentrations in urine comparable with those for N (Williams et al. 1990), there was competition between K^+ and NH_4^+ ions for the soil's cation exchange sites, but these diminish rapidly with depth. Once below the A horizon (>15 cm) there is a reduced presence of nitrifying bacteria and with soil temperatures remaining cool, ammonium concentrations might decline relatively slowly. As shown in the drainage of the U700 treatments, once at depth, this ammonium can apparently persist for some time.

The range of N_2O emission factors (EF_3 range 1.3-2.1%) for our treatments were relatively high compared to values of ~1% given by de Klein et al. (2003) but peak daily N_2O emissions were similar to those reported by de Klein (2014) when the urinary-N rates were similar. The high EF_3 values arise because of measurements directly from a urine patch involving a single or double urine application to an already compacted wet soil surface, with reduced aeration, minimal plant growth and a high water filled pore space (WFPS) (Ball et al. 2008). Nitrate, under these soil conditions, is highly favoured to undergo denitrification (Bolan et al. 2004) and since these conditions are often prevalent in winter forage grazing, N_2O emission factors will likely tend towards the higher end of the range (Ball et al. 2012).

The calculation of the proportion of denitrification contributed from di-nitrogen emissions was partly compromised by the difficulties in obtaining accurate values at low rates of emissions due to the relatively low ^{15}N enrichment (~9%) of the labelled urine. It is likely that further $^{15}N_2$ loss continued after the first three months of measurement and the reported $^{15}N_2$ loss of 8-12% is probably an underestimate. Other New Zealand studies quantitatively reporting N_2 loss directly or indirectly from pasture-applied ^{15}N-labelled urine have suggested values from 23-37% (Clough et al. 2001; Di et al. 2002; Buckthought et al. 2015).

Overall, N uptake in oats and lt. ryegrass treatments was similar but only increased by about 25% between U350 (24% of total) and U700 (14% of total) treatments. This would appear to indicate a greater proportionate loss from the U700 treatments but this is not reflected in the plant ^{15}N fraction which was a similarly-sized (3-4%), if considerably smaller, proportion for both crop U350 and U700 treatments. Thus, it is more likely that the larger non-labelled N fraction retained in the plant material is from subsequent SOM mineralisation and/or applied fertiliser, rather than from the urine application directly.

4.4.3 Effect of DCD

The application of the nitrification inhibitor, DCD, proved a successful strategy in reducing both NO_3^- leaching and N_2O emissions. In this experiment its use at 20 kg a.i. ha^{-1}, 1 day after urine application, achieved a 3-fold increase in N uptake over the non-DCD treated oats crop, whilst reducing direct nitrate leaching loss by ~60%. The effectiveness of DCD in reducing nitrate leaching and N_2O emissions from pastures has been well reported (Di & Cameron 2002b; Di & Cameron 2003; Sprosen et al. 2009; Cameron et al. 2014) but its use under cropping, and particularly winter forage grazing, is more equivocal (Smith et al. 2012; Abalos et al. 2014). DCD's effectiveness in reducing both nitrate leaching loss and N_2O emissions is enhanced the sooner it is applied after grazing (Monaghan et al. 2013), and goes someway to explain its ineffectiveness in a Southland winter forage grazing trial where it was applied up to twelve days after grazing. However, as our results show, DCD merely postpones nitrification; if there is no crop or pasture to take up the N it will eventually be lost via leaching or denitrification. Issues with establishing the lt. ryegrass seed in some of the DCD (and control) lysimeters demonstrated this as, with little N uptake occurring in

the first 4-8 weeks after sowing, the effectiveness of the inhibitor declined and the ammonium eventually began to nitrify again. This manifested as a peak in nitrate concentration (~54 mg N L^{-1}) at around 400 mm drainage. Nevertheless, although soil type and drainage conditions will undoubtedly be factors in why DCD is effective in some situations and less so in others, DCD demonstrates useful potential in winter forage grazing situations.

The reduction in N_2O emissions from the application of DCD (~50%) was similar to the range of reductions reported by Di et al. (2007) (65-73%) and de Klein (2011) (61-70%) on pastures and demonstrates DCD's potential effectiveness in reducing N_2O emissions under winter forage grazing. However, the postponed build-up in nitrate concentration in the ryegrass DCD treatment meant a noticeable second peak in N_2O emissions was observed and N_2O losses were, at the end, not noticeably different between ryegrass treatments.

4.4.4 Fate of urinary-^{15}N and ^{15}N balance

Total ^{15}N recovery was as high as 90% (oats control) but generally, recoveries declined with increasing urinary-N rate to between 65-70% for the U700 treatments. The lower recoveries probably stem from undetectable N_2 denitrification losses below the level of ^{15}N detection. Given that soil and climatic conditions under winter forage grazing favour an accumulation of nitrate, then prolonged low-level denitrification loss is probably not surprising. The lower N recovery in the lt. ryegrass control treatment probably stemmed from the poorer initial establishment producing a similar, albeit smaller, pool of nitrate subject to the same denitrification processes but at a greater enrichment level (98% ^{15}N; 35 kg N ha^{-1}). If it is assumed the unaccounted ^{15}N proportion is due to dinitrogen loss then the total denitrification loss under winter forage grazing, and as a proportion of urinary-N applied at 350-700 kg N ha^{-1}, can probably be estimated at 30-35%. This is similar to that reported by Fraser et al. (1994) (~28%) but high compared with the range of ^{15}N recoveries published for other New Zealand studies where N_2 loss was measured or implied by difference (Clough et al. 2001; Di et al. 2002; Buckthought et al. 2015). However, soil temperatures (>9°C), WFPS, labile-C and nitrate supply conditions in this study were probably near optimal for denitrification in the A horizon by late August/early September (Haynes & Williams 1993; Di et al. 2014).

Nitrate-^{15}N leaching losses for U350 and U700 treatments were about 25% of the urinary-N applied but this was only 52-60% of the total nitrate leaching loss although doubling the urinary-N application rate had a largely additive effect on nitrate- and inorganic-^{15}N drainage losses. Di et al. (2002) and Fraser et al. (1994), who measured ^{15}N-nitrate leaching losses under a urine patch in two pasture trials on the same deep Templeton silt-on-sandy loam, found 83% and 69% of nitrate leaching losses occurred from autumn- (May; 1000 kg N ha^{-1}) and winter-applied (July; 500 kg N ha^{-1})^{15}N-labelled urine applications, respectively. The reason for a relatively lower percentage of ^{15}N recovered in drainage losses in this study is a little unclear but may be due to the history of the field site from which the lysimeters were taken. The lysimeters were collected 6 months before the trial began and came out of established pasture but only modest drainage occurred prior to the trial's start. It is possible some SOM mineralisation occurred over this period, diluting the NO_3^--^{15}N pool. Indeed, the It. ryegrass control lysimeters (where establishment problems were encountered) had an annual nitrate loss of 52 kg NO_3^--N ha^{-1}, but only a small proportion (<12%) of this was derived from the ^{15}N-labelled pool i.e. there was a large background contribution.

Ammonium-^{15}N leaching constituted between 6-10% of the ^{15}N loss for U350 (±DCD) and U700 treatments of both catch crops, that in turn made up ~40% of the total N leaching loss. These are high values from what has been reported previously in similar lysimeter studies. Di and Cameron (2002b) in a ^{15}N-labelled urine (1000 kg N ha^{-1}) leaching experiment on a Lismore stony pasture soil reported negligible leaching of NH_4^+-N but this was a spring (November) application where nitrification was likely rapid. The ^{15}N proportion of ammonia loss from volatilisation was not measured directly but was assumed to be the majority (i.e. ~90%) of the 3% and 4% recorded for U350 and U700 treatments, respectively (Lee et al. 2011).

The proportion of the ^{15}N-labelled urine retained in the soil fraction was reasonably high (approximately 33% and 24% on average for U350 and U700 treatments, respectively, for both catch-crops) but similar to that reported by Di et al. (2002) and Fraser et al. (1994). However, their studies were on pastures whereas soils in this study were essentially fallow at the time of urine application (apart from the harvested kale plant roots) and thus little of the urinary-N applied could be recycled in root or plant residues prior to the establishment of the crops. In addition, the urinary-N rates used in this study were considerably lower in this study and thus the absolute amount of N

retained from the application ranged between ~115-169 kg N ha^{-1} as opposed to the 200-250 kg N ha^{-1} cited in the other two studies. This ^{15}N fraction has presumably been retained by a mix of immobilisation, root uptake and ammonium fixation but as there is little other ^{15}N recovery data for winter urine applications, let alone under winter forage grazing conditions, it is difficult to infer much. Indeed, comparisons can only be made with cropping where the N is usually applied as inorganic fertilisers, but these applications are normally in spring or autumn, not mid-winter. Nevertheless, Gardner and Drinkwater (2009) found in a meta-analysis of cropping ^{15}N studies that soil accounted for ~29% of the ^{15}N applied.

By the end of the study there was relatively little ^{15}N recovered in the roots of the remaining vegetation (~1%) and this is similar to Fraser et al. (1992), who found that live roots also only accounted for 1% of the ^{15}N recovered. The oats, having been harvested six months prior to soil deconstruction, meant whatever ^{15}N was retained in the roots would have been recycled back into the soil with only a small proportion recovered in the subsequent kale crop. The ryegrass treatments had five harvests over the growing season; thus, little ^{15}N remained in the root mass by the time of lysimeter deconstruction.

4.5 Conclusions

The main conclusions from this experiment are:

- The earlier sowing and establishment of an oats catch-crop reduced nitrate leaching losses over the winter-spring period by approximately 25% ($P<0.01$) compared to the later sowing of It. ryegrass (both sown at recommended dates) but this was mainly due to lower drainage (22% less, $P<0.001$) rather than from differences in N uptake between crops.
  - Catch crops were sown between 7 (oats) and 11 (It. ryegrass) weeks after urine application but this proved too late to reduce the soil mineral-N pool sufficiently to avoid substantial nitrate leaching. Annual N leaching losses were similar between catch crop treatments but only 3-4% of the urinary-N was recovered (as ^{15}N) in either catch crop, indicating the lower initial nitrate loss for the oats' treatments was due to nitrate being retained within the soil monolith because of the oats' greater evapotranspiration loss and subsequent lower drainage.

  - Nitrate leaching was reduced from 46, 167 and 279 kg N ha^{-1} to 11 (76% reduction), 131 (22% reduction) and 193 (31% reduction) kg N ha^{-1} for control, 350 and 700 kg N ha^{-1} urine treatments under oats, respectively.
  - Annual nitrate leaching losses were considerably larger than winter-spring losses but not significantly different between catch crops indicating the lower initial nitrate loss for the oats' treatments was due to nitrate being retained within the soil monolith rather than differences in N uptake *per se*.
  - Ammonium-N loss in drainage was significant, representing between 13-26% of the total N leached and indicates that rapid leaching during winter is a feature of the Balmoral soil.
  - The fraction of ^{15}N in both nitrate and total N in drainage loss directly attributable to the ^{15}N-labelled urine was less than 60% at the end of the winter-spring period indicating that there was mineralisation of soil organic-N from the previous pasture.
- The effect of doubling the rate of urinary-N application from 350 to 700 kg N ha^{-1}, seven days apart, on winter-spring period nitrate and inorganic-N drainage losses was largely additive.
  - Doubling the urinary-N rate increased winter-spring nitrate and inorganic-N losses by 55% and 72%, respectively.
  - Nitrate- and inorganic-^{15}N leaching losses increased by 75% (range 68-81%) and 90% (range 81-108%), respectively.
- The application of DCD one day after urine application reduced inorganic-N leaching losses in the oats and lt. ryegrass catch-crops by 55% and 33%, respectively, and improved N efficiency by increasing N uptake in the oats 3-fold.
- The ^{15}N recovery from the labelled urine ranged from 90-65% with values declining with increasing urinary-N rate.
  - For the U350 and U700 treatments, approximately 23% of the ^{15}N applied was recovered in drainage water from nitrate leaching whilst a further 6-10% was recovered in ammonium leaching.
  - A relatively high proportion (33%) of the ^{15}N loss was attributed to denitrification.

– ^{15}N retained in the soil and roots was approximately 25% and 1%, respectively, whilst 3-4% was lost via volatilisation.

Questions raised by the study

This study has established that oats appears to have the potential to be a more effective catch crop in reducing nitrate leaching under winter forage grazing situations but not inorganic-N leaching. Several specific questions were raised regarding this study:

- What is the sensitivity between winter urine application date and oats sowing date on nitrate leaching and N uptake?
- What are the actual nitrate leaching losses under a fallow urine patch deposited when grazing winter forage crop?

Chapter 5

Effect of Timing of Winter Urine Application and Sowing Date of an Oats Catch Crop on Nitrate Leaching Loss and N Uptake

"A field lysimeter study examining the timing of winter urine application and sowing date on the effectiveness of an oats catch crop to capture nitrogen and reduce nitrate leaching."

5.1 Introduction

Sowing a catch crop in winter to mitigate N leaching from winter forage grazing is problematic given that urine deposition occurs at a time of year when plant growth is minimal. Findings from the first-year winter urine application experiment (Chapter 4) showed that although sowing oats (*Avena sativa* L.) reduced nitrate leaching compared with It. ryegrass (*Lolium multiflorum* L.), this reduction was mainly due to lower drainage losses, rather than urinary-N uptake. Indeed, the actual proportion of the labelled urinary-N accounted for by both catch crops was similar but small (3-4%). In that experiment, urine was applied 1-2 months before sowing of either catch crop meaning nitrification had already largely occurred, and with drainage from rainfall (both natural and simulated) set to the local 75th percentile meant that, by mid-September, over 200 mm of drainage had already been collected on average. This represented a nitrate leaching loss over the period of ~90-130 kg N ha^{-1}, about half of the total annual nitrate leaching loss. Thus, this delay in sowing the catch crop meant that the opportunity to capture significant urinary-N had largely been lost by the time the plants emerged.

Determining the timing of sowing a catch crop is of course dependent on whether it will grow under the prevailing temperatures and light levels and whether an adequate seed bed can be prepared in advance. Usually the latter is not attempted until late winter but the window between urine deposition and sowing a catch crop needs to be as short as possible to maximise the possibility of increasing N uptake and reducing nitrate leaching losses. Results from Chapter 4 suggested that oats was a better plant to sow (rather than Italian ryegrass) in winter and could be a viable catch crop for use after winter forage grazing.

This chapter reports findings from a field-based lysimeter experiment designed to quantify the effectiveness of sowing oats as a catch crop to reduce nitrate leaching losses over a range of winter urine application dates and sowing times after simulated winter grazing of a kale crop. The experiment compared plant N uptake and nitrate leaching losses from a urine patch under several combinations of timing of urine deposition and the interval between urine deposition and sowing of the catch crop.

This study tested three hypotheses:

1. Sowing oats as a catch crop after simulated winter forage grazing will reduce nitrate leaching losses compared with a bare fallow treatment,
2. Nitrogen leaching will be greater from an early winter urine application compared to a later application, and
3. Nitrogen uptake will increase, and nitrate leaching decrease, the sooner the oats catch crop is sown after winter forage grazing.

Consequently, there were two main objectives for the experiment:

1. Measure nitrate leaching losses under bare fallow and catch crop treatments over a complete Canterbury winter, and
2. Quantify the interaction between date-of-urine-application with catch-crop sowing date (post-winter forage grazing) on nitrate leaching losses.

5.2 Materials and Methods

5.2.1 Soil and lysimeter collection

Many of the details of the soil and lysimeter collection have already been outlined in Chapters 3 and 4 but are briefly presented here. The study was conducted using the same Balmoral stony silt loam soil (Typic Dystrudept, USDA) collected from Lincoln University's Ashley Dene research station situated near Springston (NZGD2000: 43 38 42S 172 20 33E) on the Canterbury Plains of New Zealand. Soil texture in the first 15 cm of each lysimeter profile was silt loam, but the soil profile became increasingly stony below this with ~50% of the soil volume occupied by stone below a depth of 30 cm. Below 30 cm, the remaining volume not taken up by stone was increasingly interspersed with fine-to-coarse sands. The lysimeters were collected from established pasture consisting of a mixture of perennial ryegrass (*Lolium perenne* L., cultivar Grasslands Nui) and white clover (*Trifolium repens* L., cultivar Grasslands Huia). Forty-eight monolith lysimeters, 50 cm in diameter and 70 cm deep, were collected from the site in February 2014 (late summer). Key soil chemical and physical properties are presented in Table 5.2-1. Lysimeter collection followed the procedure described by Cameron et al. (1992). Briefly, each lysimeter casing was placed upon the

soil surface and a small trench was dug around it to expose a 10-cm depth of soil. The lysimeter casing was progressively pushed down in several steps over the repeatedly exposed depth of the soil monolith until the soil inside the lysimeter casing was a few cm from the top. An internal cutting ring in the casing created a 5-mm gap between the side of the casing and the soil monolith. The gap between the soil monolith and casing was filled with liquefied (50°C) petroleum jelly. Once cool the petroleum jelly prevented edge flow of drainage water. At a depth of 70 cm, a hydraulically-assisted cutting plate was used to separate the soil column from the subsoil. Each lysimeter was uplifted and inverted and c. 50 mm of soil removed from the base and replaced with a layer of coarse gravel before attaching a drainage base plate to collect leachate. The installation of a gravel layer at the base of each lysimeter reproduced a situation common to the Canterbury Plains where soils often overlie coarse gravels. The gravel layer also allowed a free drainage system to be used in the lysimeter study, as matric potential in this layer was assumed to be zero (Clothier et al. 1977). The lysimeters were moved to a purpose-built field trench facility at Lincoln University where the top of each lysimeter was flush with the surface of the soil surrounding it. This ensured that the lysimeters were exposed to the same environmental conditions as the rest of the field.

Table 5.2-1. Key soil fertility (0-7.5 cm) and physical properties of the Balmoral soil.

Soil analysis	Value	Soil depth (cm)	Soil bulk density (g cm^{-3})	Stone volume (%)	Soil porosity (%)	Soil texture[1]
pH	6.1	0-10	1.14	11	58	stony ZL
Olsen-P	33 µg ml^{-1}	10-20	1.57	30	42	stony ZL
Exch-Ca	8.1 $cmol^{+}$ kg^{-1}	20-30	1.90	51	29	stony SL
Exch-Mg	0.5 $cmol^{+}$ kg^{-1}	30-40	2.05	54	24	stony S
Exch-K	0.4 $cmol^{+}$ kg^{-1}	40-60	1.96	51	27	gravelly S
Exch-Na	0.2 $cmol^{+}$ kg^{-1}					
CEC	16 cmol kg^{-1}					
Reserve-K	3.6 cmol kg^{-1}					
Sulphate-S	4 µg g^{-1}					
Organic-C	45 g kg^{-1}					
Total-N	4.0 g kg^{-1}					
Organic-S	5 µg g^{-1}					
Base saturation	65%					

[1]ZL -silt loam; SL -sandy loam; S –sand

5.2.2 Treatments

After the lysimeters had been installed, the pasture vegetation was sprayed out with herbicide, and the soil surface was lightly cultivated by hand a fortnight later. Three, 3-month old forage kale (cultivar Regal) plants were transplanted into each lysimeter in early February, giving a plant population of ~16 plants m^{-2}. Basal nitrogen (equivalent to 40 kg N ha^{-1}, supplied as urea), phosphorus (15.4 kg P ha^{-1}; supplied as 15% potassic superphosphate), potassium (15 kg K ha^{-1}) and sulphur (18.4 kg S ha^{-1}) were applied after transplantation to help establish the plants. The kale grew until winter when it was harvested to simulate grazing. Once the kale plants had been cut, the soil surface was trampled using a manually operated trampling device, designed to provide c. 200 kPa downward pressure, similar to that of the mechanical hoof described by Di *et al.* (2001). This simulated the impact of animal grazing on the soil surface under typical winter forage grazing conditions.

Treatments were based on different combinations of the timing of winter urine application, and the interval between urine deposition and the sowing of the catch crop. Three different urine application times spanning early- to mid-winter (early June, early July and late July) were chosen to represent the eight-week period over which winter forage grazing typically occurs in Canterbury. Oats (cultivar Milton) catch crop treatments were sown on randomly selected lysimeters within 1-3 days after each urine application event, and then nominally at 21-day intervals (Table 5.2-2). Sowing dates were reduced from four, for the first urine application (nominally 1, 21, 42 and 63 days), to two occasions (nominally 1 and 21 days), by the third urine application date so that the final sowing occurred at approximately the same time (mid-August) for each urine application date treatment. Each urine application treatment combination was replicated 4 times, including a control treatment that received urine but was left fallow; (Table 5.2-2). Problems with rodent damage to seeds and germinating seedlings caused the 2nd urine application (early July) 21-day sowing treatment combination to be abandoned and removed from the analysis.

Urine was collected from non-lactating cows grazing on forage kale at the Ashley Dene research station and was applied to the lysimeters at a rate equivalent to 350 kg N ha^{-1}. This rate was based on the analysis of the total-N content of urine from winter kale-fed cows where the urinary-N loading is lower than urine from cows fed on pasture (Edwards et al. 2014a).

The urine was applied to each lysimeter in 2 L volumes, similar to the typical urination volumes of cows grazing on winter forage crops (Ravera et al. 2015). The urine was analysed for total-N content immediately following collection and refrigerated overnight. The urine was poured rapidly on to the surface of each lysimeter to simulate a urination event.

Table 5.2-2 Urine application dates to lysimeters and post-application sowing dates of oats crops. Fallow treatments received urine only.

Treatments	Urine application date		
	June 5	July 5	July 25
Oat crop sowing dates	Fallow	Fallow	Fallow
	1 day (Jun 6)	-	-
	22 days (Jun 27)	2 days (Jul 7)	-
	43 days (Jul 18)	[a] 25 days (Jul 21)	3 days (Jul 28)
	64 days (Aug 8)	44 days (Aug 11)	24 days (Aug 18)

[a] Removed due to rodent damage.

Prior to sowing the oats, the soil surface was lightly worked to a depth of 5 cm and the seed sown by hand at a rate equivalent to 130 kg ha^{-1} (2.5 g/lysimeter). Lysimeters were left exposed to natural rainfall over the 2014 winter with no artificial watering through this period until the oats were harvested in late spring (November 3). Irrigation water was applied from November 7 to mid-December to bridge the rainfall deficit until the breakthrough curves for each fallow treatment were essentially completed and an average annual amount of drainage produced. For a Canterbury well-drained stony soil under irrigation, ~260 mm was considered representative and was based on 10 years data from the Lincoln University dairy farm and drainage model estimates covering a range of soil textures, from extremely light to heavy (Lilburne et al. 2010). Irrigation was automated to apply water in 0.5 mm increments over several hours to ensure leaching was largely conducted under unsaturated conditions. The oats were all harvested on November 3 (2014).

5.2.3 Measurements and data analysis

Climate and rainfall data were collected from the Broadfields meteorological station 2 km from the trial site and compared with long-term means (1980-2014). Leachate from each lysimeter was collected and the volume measured after every significant rainfall event and/or once a volume of more than 0.2 L was present. A sub-sample of leachate was frozen until it was analysed for nitrate

and ammonium (NH_4^+-N and NO_3^--N) concentrations by flow injection analysis (FIA) (Tecator, Sweden). Analysis of leachates showed they contained little or no organic-N (<4% of total-N on average) and thus no data is reported. After the oats were harvested they were dried in a fan-forced oven at 60° C before weighing and ground to pass through a Retsch cyclonic mill (<0.5 mm mesh). A sub-sample was analysed and measured for plant N content using an Elementar Vario-Max CN Elemental Analyser (Germany).

Total NH_4^+-N and NO_3^--N leaching losses were calculated as the product of their concentration in the leachate and the volume of leachate. Average annual leaching losses were then calculated using values from the four replicates. Statistical analysis was done using both balanced (sowing dates within an application) and unbalanced (across all application dates) non-randomized block ANOVA within the Genstat 9.2 statistical package (VSN International Ltd. 2005). Duncan's multiple range test was used for comparisons between sowing days for individual urine application dates. Least significant differences (LSD) were calculated for multi-treatment comparisons.

Plate 5.2-1. Treaded lysimeter prior to urine application (A), post-application (B) and at 21 days after sowing of oats in early winter (June), one day after urine application (C).

5.3 Results

5.3.1 Climate and drainage

Air and soil temperatures during June 2014 were higher than the long-term average, but only slightly so in July and close to the average thereafter (Figure 5.3-1). Daily solar radiation and evapotranspiration rates were also both higher than average for the period from mid-June to early-December (Figure 5.3-2). Consequently, rainfall in spring was below the long-term average, especially in the period from August to October (Figure 5.3-1), and the amounts of drainage for the same period also low at 75, 42 and 19 mm for the early, mid and late urine applications, respectively. More than half of the drainage for the early urine application was in the six-week period following application. A further ~200 mm drainage was collected in the simulated rainfall period after November 7, bringing the total drainage for each treatment closer to the district average by the end of spring (December 1) when it was realised that there was a significant drainage deficit.

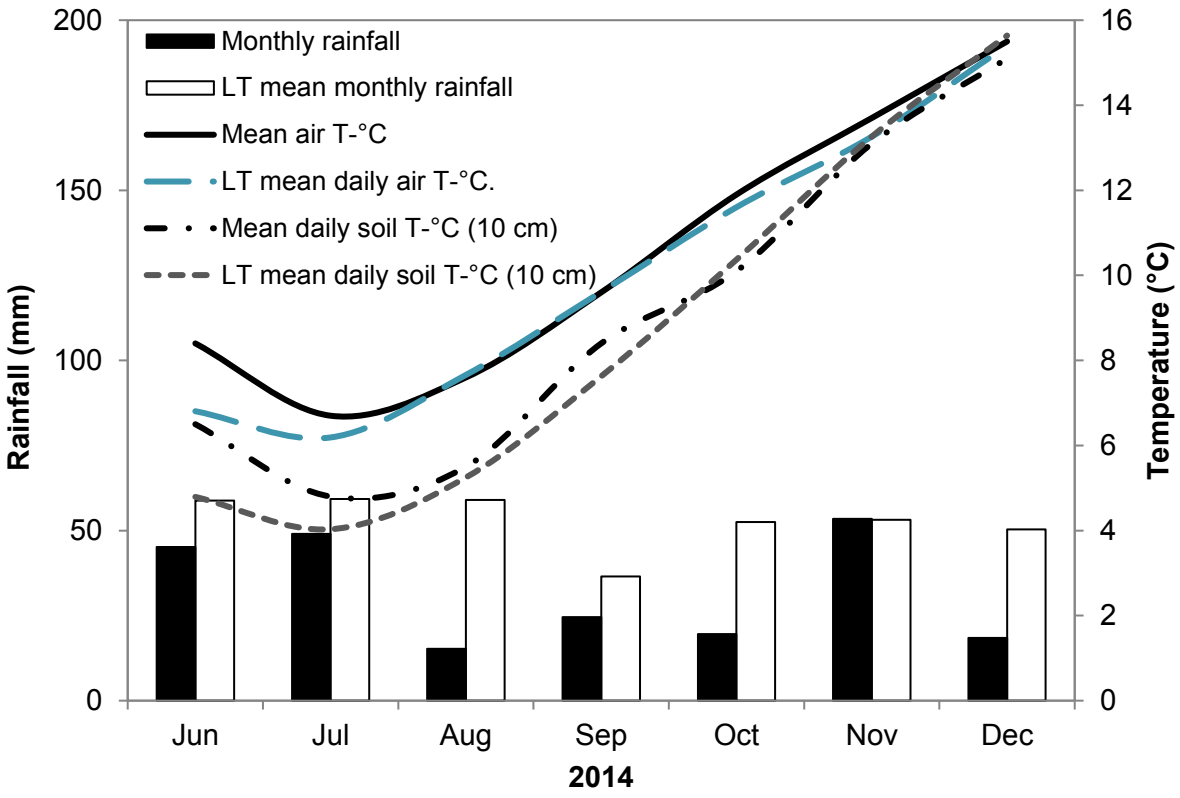


Figure 5.3-1. Mean Lincoln monthly rainfall and air and soil (10 cm) temperatures for the 2014 winter-spring period, compared with long-term (LT) means (1980-2014).

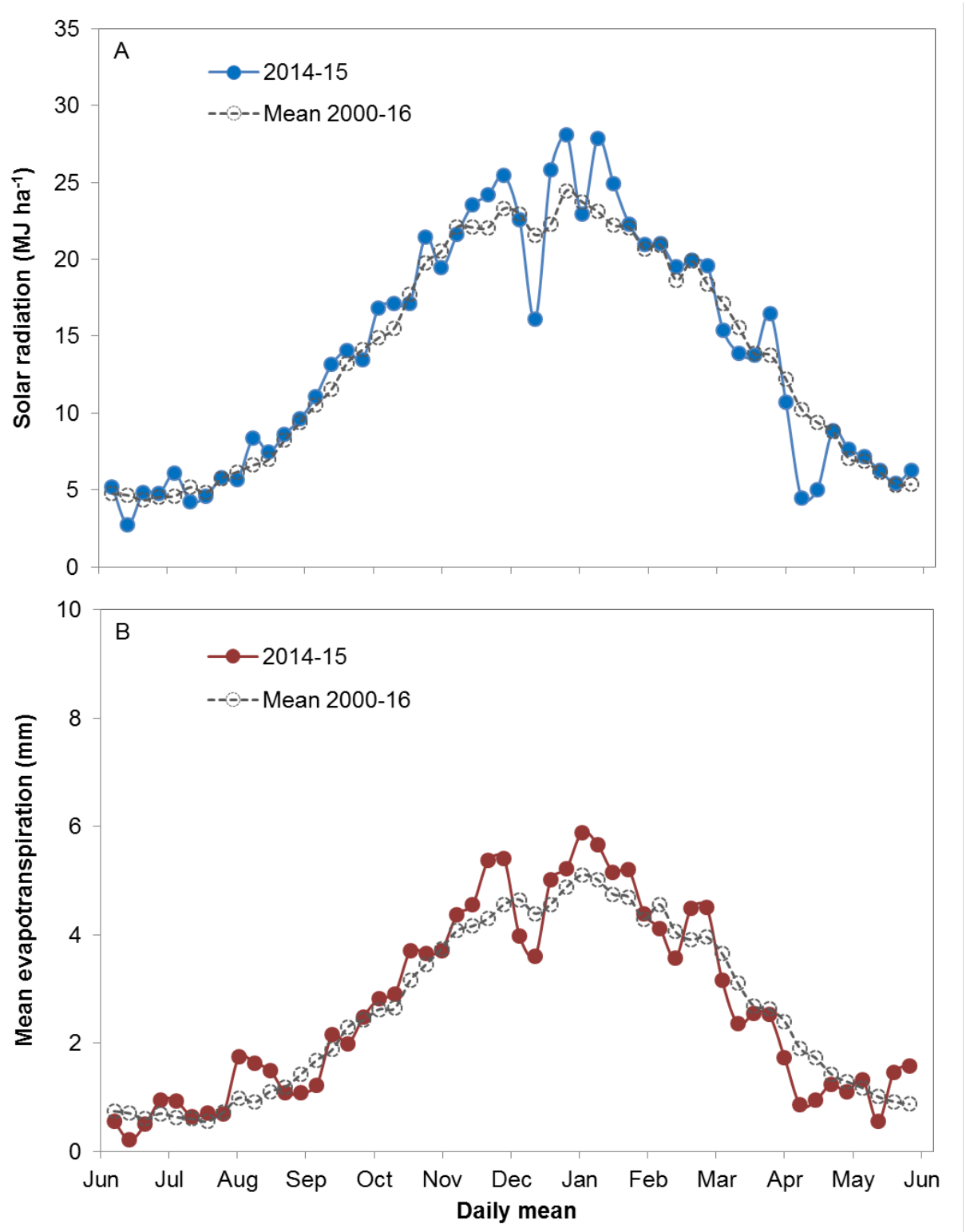


Figure 5.3-2. Daily solar radiation and evapotranspiration (weekly average) at Lincoln over the 2014 winter and spring periods (Broadfields NIWA).

5.3.2 Nitrate leaching loss and crop N uptake

Breakthrough curves of NO_3^--N concentrations for the fallow treatments of the early, mid and late urine application timing treatments peaked at around 230, 180 and 130 mg N L^{-1} respectively, declining below 30 mg N L^{-1} after 260 mm of drainage had been collected (Figure 5.3-3). Nitrate comprised almost the entire amount of N leached, apart from a small amount of ammonium leached initially after the early urine application (<3% of total-N leached). Nitrogen leaching losses for the annual drainage estimate were significantly different between urine application dates (P<0.001) at 272, 224 and 172 kg N ha^{-1} for early, mid and late fallow treatments, respectively (Table 5.3-1).

Table 5.3-1. Inorganic-N leaching loss, % N leaching decrease (from respective fallow treatment), oats crop yield, N uptake, N content and drainage depth for each urine application and sowing date treatment.

Treatment		*Inorganic-N leached (kg N ha^{-1})*		*Catch crop (kg ha^{-1})*		*N content*
Urine application	**Oats sowing day**	**[a] Total**	**% diff**	**Dry matter**	**N uptake**	**N%**
Early June 5th	Fallow	272 b	0	-	-	-
	1	160 a	-41%	4062 a	80 a	2.0 a
	22	162 a	-40%	3595 a	73 ab	2.0 a
	43	198 a	-27%	2092 b	61 b	2.9 b
	64	198 a	-27%	2024 b	65 ab	3.2 c
Mid July 5th	Fallow	224 b	0	-	-	-
	2	142 ab	-37%	2735 a	60 a	2.2 a
	44	115 a	-49%	2374 a	73 a	3.2 b
Late July 25th	Fallow	172 b	0	-	-	-
	3	117 a	-32%	2268 a	72 a	3.2 a
	24	138 ab	-19%	1922 a	65 a	3.4 a
	Urine	***		***	ns	***
	Sowing day	***		***	ns	***
	Urine x Sowing day	ns		**	*	ns
	[b] LSD (5%)	47		570	16	0.3

[a] Means within one urine application date followed by a letter in common are not significantly different according to Duncan's multiple range test (P < 0.05); [b] Least significant difference (average) from unbalanced ANOVA and applicable between individual means; * p<0.05, ** p<0.01, *** p<0.001.

Sowing a catch crop reduced N concentrations in the leachate by 20-60% (Figure 5.3-3) compared with the fallow treatments. The catch crop significantly reduced (P<0.001) nitrate leaching loss by between 19-49% and by ~34% on average for all treatments over the drainage period (Table 5.3-1). When the catch crop was sown between 42 and 63 days after urine application in early winter, N losses were reduced on average by 30%. In the early urine treatments, the nitrate leaching

losses generally increased with later sowing of the oats although differences between early and later sowing dates were not significant overall (Figure 5.3-4 & Table 5.3-1).

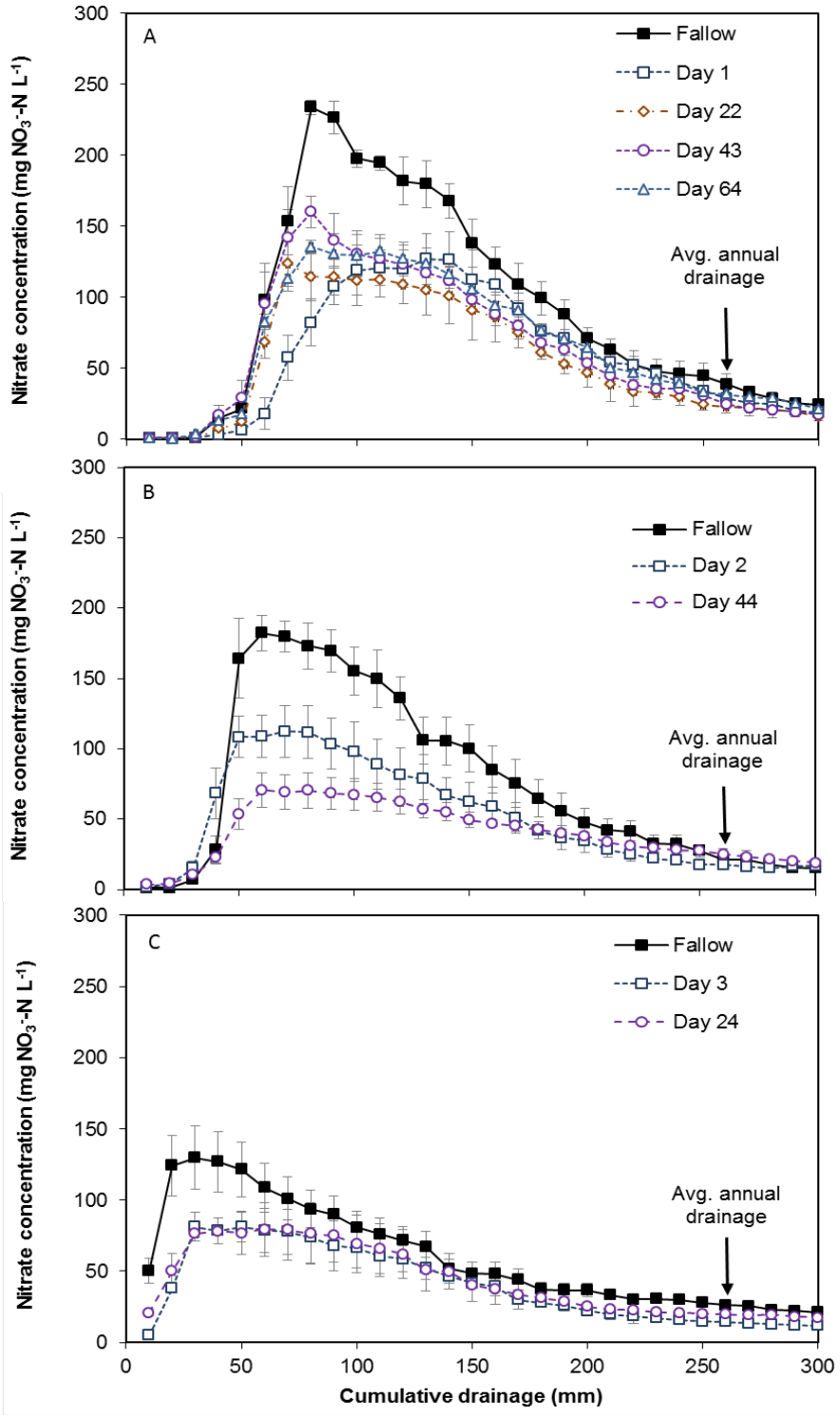


Figure 5.3-3. Nitrate leaching breakthrough curves for fallow and catch crop treatments for A) early, B) mid and C) late urine applications (350 kg N ha^{-1}). Crops were sown approximately 2, 23, 44 or 64 days after urine application. Standard error bars (±1 SE) shown. Annual regional average drainage indicated for an irrigated Balmoral soil (Lilburne et al. 2010).

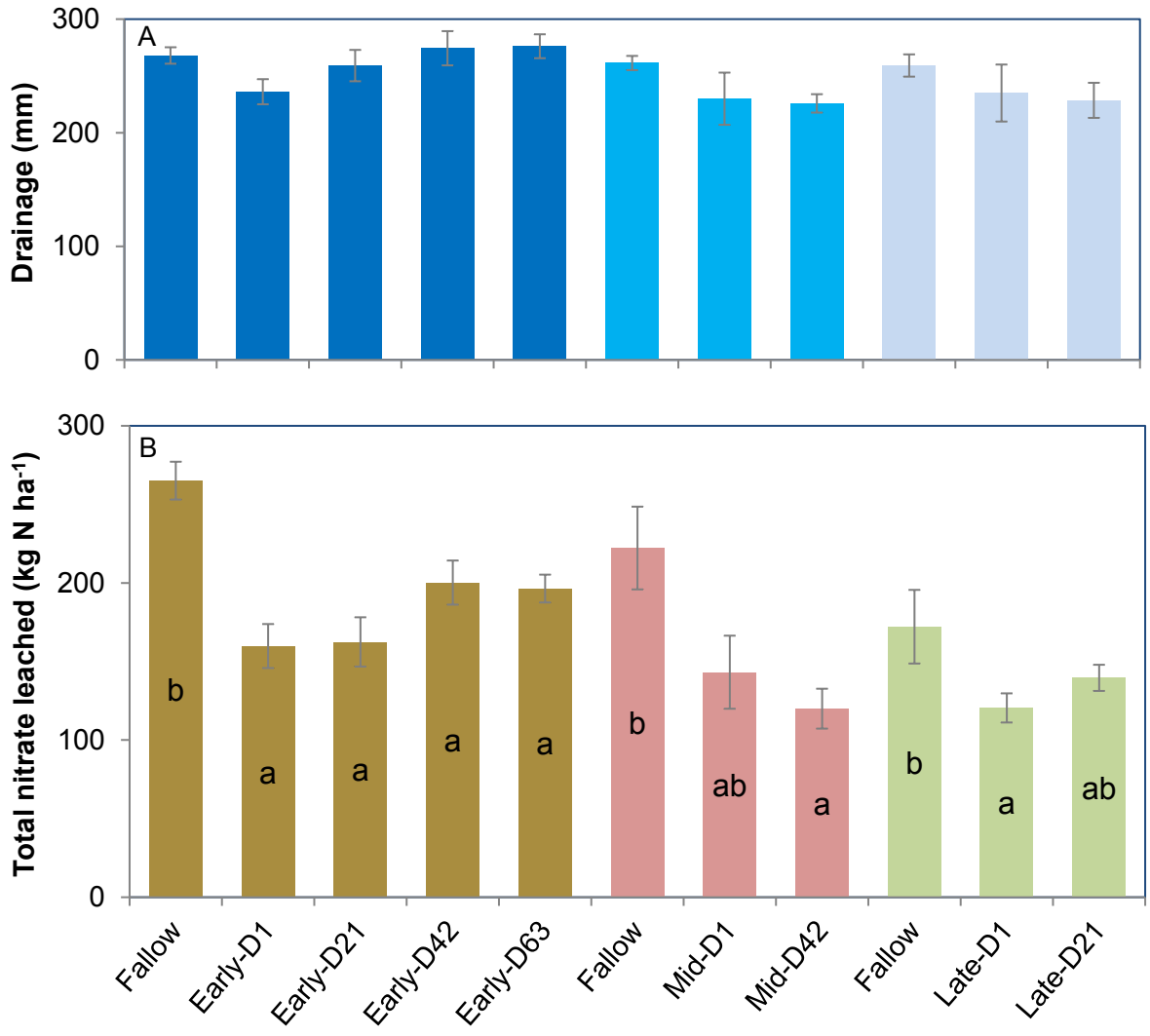


Figure 5.3-4. Total winter-spring drainage (A) and total nitrate leached (B) for urine-by-sowing application dates. An uncommon letter indicates a significant difference (P<0.05) within a urine application date. Standard error bars (±1 SE) shown.

For the early urine application, there was a statistically significant effect of the interval between urine deposition and sowing of the catch crop on total oats DM harvested (P<0.001), and on total plant N uptake by the oats (P<0.05; Table 5.3-1). In general, the sooner the oats were sown after the urine was applied, the greater the amount of DM harvested and N taken up by the crop. There was a strong inverse relationship between DM harvested and the N concentration of the DM (r^2= 0.71, Figure 5.3-5). Crops sown within the first 21 days or so of the early urine deposition event contained only 2% N in the DM, whereas crops sown 21 days after the late urine N event contained more than 3% N but grew less than half the total DM of the former treatments (Figure 5.3-5). Consequently, differences in catch crop N uptake were only significant between the day 1 and day

42 sowing dates for the early urine application and were similar overall (~70 kg N ha^{-1}) between early, mid and late urine applications (Table 5.3-1). Drainage volumes were generally least for the earliest oats sowing dates for each of the urine applications compared to the corresponding fallow treatment (all approximately 260 mm) and/or later sown treatments but it was difficult to compare as much of the drainage occurred after the oats were harvested and were affected by the regenerating oats.

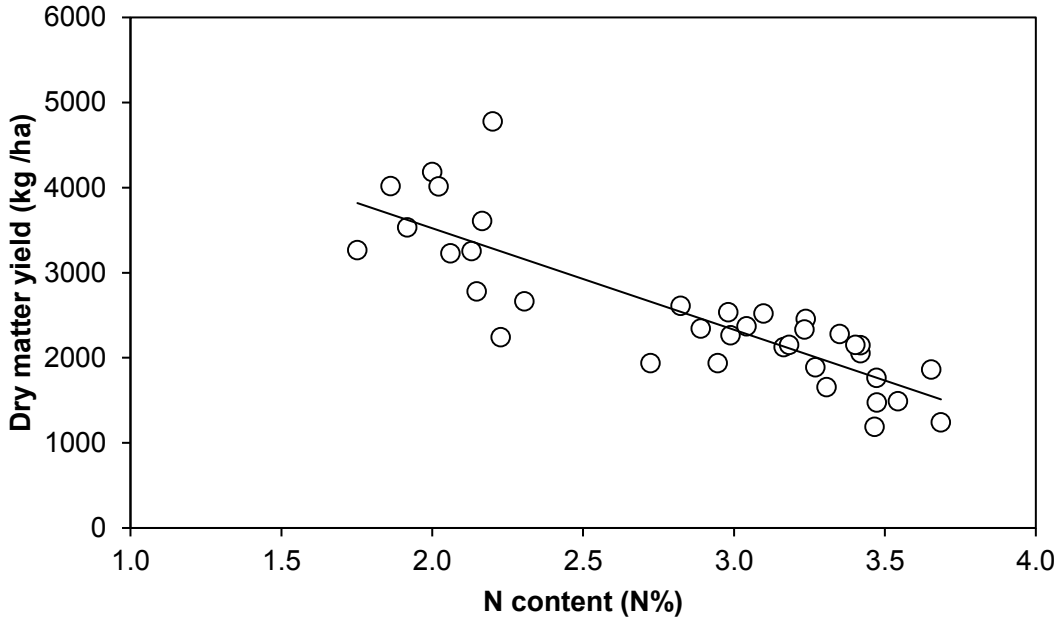


Figure 5.3-5. Relationship between oats dry matter production and N concentration in the catch crop (*y*= -1193*x*+5908*;* r^2=0.72, P<0.001).

5.4 Discussion

5.4.1 Nitrogen leaching and N uptake

Sowing oats as a catch crop following urine application after simulated winter forage grazing reduced nitrate leaching losses by ~34% over all treatments compared with the fallow treatments (range 19-49%). In the critical 42-63-days period after winter forage grazing, when soil conditions were more favourable to sowing a catch crop, N loss was reduced by ~30%. Earlier sowing of the oats catch crop produced up to 25% less nitrate leaching than later sowings. The overall reduction is similar to that reported by Francis *et al.* (1995) who found an average 30% reduction in N loss from autumn-sown green feed oats following ploughing-in of a 4-year old Canterbury ryegrass/white clover pasture.

There was little ammonium leaching in this lysimeter experiment compared with the previous experiment in Chapter 4. Several factors were different, however. Soil temperatures in June 2014 were warmer than in 2013, and with lower than average rainfall, and no simulated rainfall, meant the ammonium-N had a longer resident period in the topsoil and probably nitrified before it could be leached into the subsoil.

The breakthrough curves for each urine application show that most nitrate was leached from the lysimeter after the annual average drainage of ~260 mm (for an irrigated well-drained soil in Canterbury). Nitrogen leaching losses as a proportion of the N applied were high overall (33-77%) but were probably exacerbated by the artificial drainage occurring in late-spring when warm, wet conditions likely enhanced soil organic matter mineralisation. Although this doesn't diminish the ability of the catch crop to reduce nitrate leaching losses, our experimental conditions produced significant drainage in a relatively short period and probably exaggerated the potential N losses.

The oats sown soon after the early winter (June) urine application yielded about twice as much total DM at time of harvest as the later-sown treatments. Nitrogen uptake of around 60-80 kg N ha^{-1} covered all treatment combinations so despite a large difference in DM production, there was less effect on N uptake with significant N dilution occurring within the plants from the earlier sown oats. Low drainage over the period probably contributed to this reduced range as more rainfall, post-winter forage grazing, over the mid-to-late winter period, would have increased nitrate leaching and

reduced N uptake for later sowing dates. Francis (1995) found that an oats catch crop with a yield of about 3 tonnes ha^{-1} sown in autumn contained 80 kg N ha^{-1}, similar to the maximum in our study.

Normal practice, post-winter forage grazing, is to sow the land back into pasture or kale in the spring, but this may be up to three months after the final grazing, so the potential for large nitrate leaching losses from high soil mineral-N concentrations is considerable. Currently, there is little field data on soil mineral N concentrations post-winter forage grazing in Canterbury but Francis (1995) found that where an autumn-sown leguminous cover crop had been lightly grazed over the winter, soil mineral-N concentrations (to 600 mm depth) were much higher in the spring compared to when a catch crop had been incorporated (62-164 kg N ha^{-1} vs. 19-44 kg N ha^{-1}, respectively). The rapid rate of mineralisation of urinary-N over winter and early spring, in comparison with a green manure, means that winter forage grazing needs a strategy, such as a catch crop, to decrease soil mineral N concentrations to avoid large nitrate leaching losses.

5.4.2 Timing of catch crop sowing

The early planting of a catch crop like oats may mitigate N losses from winter forage grazing systems but it will depend on the prevailing climatic conditions, soil type, rainfall distribution and the speed of crop establishment. For instance, Francis *et al.* (1998) found that sowing a range of catch crops in Canterbury in March achieved DM production of 1440-3100 kg ha^{-1} by the start of winter but sowing in April, one month later, resulted in very little yield. This study enjoyed warmer than average air and soil temperatures for the oats sown in June so these established well initially, providing a good start for later growth when temperatures warmed again. There are differences, however, between using catch crops in arable mixed cropping sequences with their use in winter forage grazing systems. For example, incorporation of pasture in autumn means a build-up in soil mineral-N at deeper depths (>400 mm) by spring compared to urine application, from winter forage grazing, where there is initially a high soil mineral-N concentration close to the surface.

Currently, there is no published information available on the use of catch crops to capture N post-winter forage grazing; most New Zealand studies report the use of crops in more conventional mixed cropping-pasture sequences. Comparison with catch crop studies conducted in other countries is not possible since the catch crop is generally sown after the harvesting of a summer

crop to capture residual soil N for the following spring-sown crop (Thorup-Kristensen et al. 2003). Forage grazing over winter is also uncommon overseas since harsher climatic conditions mean most dairy cattle are housed and fed inside (Hopkins 2008).

Although N uptake from the soil is the primary benefit of sowing a catch crop, there is an indirect benefit on nitrate leaching of reduced drainage because there is increased evapotranspiration and water loss from the growing crop that exceeds surface soil evaporation alone. However, Francis (1995) and Francis *et al.* (1998) found that drainage in catch crop treatments was only lower than the fallow (ploughed pasture) treatment when the crops were sown in early autumn, giving them time to establish. This indicates a strong dependence on climatic variables, and urine-N deposited in early winter is likely to be at more risk from leaching than in late winter/spring onwards, when conditions are more optimal for crop growth (Teixeira et al. 2016).

5.4.3 Factors affecting nitrate leaching

Despite low soil temperatures, the bulk of the urinary-N deposited directly to the soil surface during winter forage grazing will undergo rapid hydrolysis to ammonium within hours (Sherlock & Goh 1984). Although some bypass or macropore flow of the urine can occur, the soil surface often becomes pugged by stock trampling during winter forage grazing so there is less likelihood of macropore flow occurring prior to nitrification (Houlbrooke et al. 2009a). A proportion of ammonium will be lost by ammonia volatilisation but in winter this is likely to be only a minor fraction (~12%) (Sherlock & Goh 1984) and indeed in the first field experiment (chapter 4.2.3) only 3-4% of the urinary-N applied was volatilised. Consequently, the bulk of urinary-N remains in soil solution or interacts with the soil cation exchange complex, awaiting nitrification (Haynes & Williams 1993); this process is slowed, if not halted, however, at low winter temperatures (<5°C) (Flowers & Arnold 1983; Cookson et al. 2002). Thus, it may take up to several months before peak soil nitrate concentrations are reached (Holland & During 1977). Depending on the prevailing winter conditions there may still be a sufficient window for a cool-season active crop like oats to assimilate significant quantities of N before declining temperatures reduce growth further. One effect of colder temperatures is to increase root N concentrations, especially in cereals and thus, early development of the catch crop root system is likely to be an important sink in storing some of the

deposited urinary-N and protecting it against leaching (Laine et al. 1994). Indeed, a winter wheat trial sown in Canterbury after pasture incorporation in May retained 55% of its total-N content (51 kg N ha^{-1}) in its roots by time of sampling in October (Francis et al. 1995).

The Balmoral soil used in the study is typical of land used for winter forage grazing in Canterbury, being mostly stony with rapid drainage (Cutler 1968). Nitrate leaching loss in this study declined significantly with each progressively later urine application and this is largely attributed to the lack of rainfall after the early and late July applications, allowing the urinary-N to remain longer in the topsoil compared with the June application that was followed by significant rainfall. In addition, the longer the urinary-N remains in the biologically-active topsoil the greater the opportunity for immobilisation and denitrification to occur. Denitrification losses in particular are enhanced under compacted soil conditions where the oxygen supply is reduced and a source of labile carbon and a high nitrate concentration co-exist (Bolan et al. 2004; Ball 2013; Saggar et al. 2013). Greater rainfall and drainage following the early-June urine application probably meant more urinary-N was leached below the biologically active topsoil, reducing the denitrification loss. In a field trial measuring nitrate leaching losses after winter forage grazing in the Waikato region of New Zealand, Carlson et al. (2013) also reported lower losses from a late-July compared with an early-June urine application (26 and 119 kg N ha^{-1}, respectively).

5.4.4 Paddock N losses

The relatively large nitrate leaching losses observed in this study relate to nitrate leaching directly under a urine patch (where the N loading is equivalent to 350 kg N ha^{-1}) and are not representative of losses at a paddock scale. Previous published (Malcolm et al. 2015) and unpublished work using this soil type in two similar lysimeter studies indicate N losses under a kale winter forage crop from non-urine affected areas are between ~30-40 kg N ha^{-1}. This will, of course, vary to some degree depending on the prior history of the paddock plus any contributions from residual fertiliser-N and mineralisation of the previous pasture or crop. The paddock scale loss will depend on the actual area occupied by urine patches, which is always likely to be a fraction of the total grazed area (Jenkinson et al. 2014). Currently, data from the P21-funded research programme at Ashley Dene research station at Lincoln University indicates urine area coverage of ~30% for a kale crop;

if the average fallow nitrate leaching loss across all urine applications is 223 kg N ha^{-1}, and background loss is 30 kg N ha^{-1}, then a weighted average for the total potential nitrate leaching loss can be calculated using the following:

$$(223 \times 0.3) + (30 \times 0.7) = 88\ kg\ N\ ha^{-1}$$

The average 21 or 42 days catch crop nitrate leaching values for all urine applications is ~138 kg N ha^{-1} so going through the same calculation:

$$(138 \times 0.3) + (30 \times 0.7) = 62\ kg\ N\ ha^{-1}$$

This gives a reduction in nitrate leaching loss of ~30% when a catch crop of oats is sown. However, these results are for one winter only and, given the high potential for large N leaching losses from winter forage grazing, and the range of farm, climatic and soil conditions, means there is clearly a need for further research on oats' effectiveness as a catch crop. One of the attractive advantages of the kale-oats catch crop system is the additional DM production from the oats which can be ensiled and used as low-N feed for cows in the following winter. Edwards *et al.* (2014b) found DM production and N use efficiency increased by 40% and 29%, respectively, over kale alone, by inclusion of oats as a catch crop in the winter forage cropping system. Given that results indicate earlier sowing of the catch crop offers greater potential for its effectiveness in reducing nitrate leaching, issues remain around the practicality of doing so and its effectiveness in a wet year.

5.5 Conclusions

The main conclusions drawn from this experiment are:

- Nitrate leaching losses from a series of winter urine applications were reduced by around a third (~34%; range 19-49%) after the sowing of an oats catch crop compared to the fallow treatments.
  - This result was partly due to an initially warmer, and overall drier, winter than normal.
  - The size of the decrease, however, suggests that there is significant potential to mitigate nitrate leaching in most conditions in these low-cost winter feed systems whilst improving N-use efficiency and DM production.
- Later sowings of oats leached up to 25% more nitrate than the earliest sowing.
  - Earlier sowing of the oats increased N uptake over later sowings by up to a third.
- Later winter urine applications had lower overall nitrate leaching losses (range 170-270 kg N ha^{-1}).
- Calculations of paddock N loss indicate a potential reduction in nitrate leaching overall of ~30%, from 88 to 62 kg N ha^{-1}.

From this study, the data could support the original three hypotheses.

Questions raised by this study:

Under the winter conditions experienced, there was a demonstrable ability of the oats catch crop to retain urinary-N against leaching. However, considerable uncertainty remains about its effectiveness over a wider range of climatic and soil conditions. Building on these results and that of the previous experiment requires greater understanding of how climatic conditions (leaving soil type aside) might affect N leaching. This poses a series of questions:

- How is the rate of urinary-N nitrification, and thus the potential for nitrate leaching, affected by differing soil temperatures and received light conditions?
- How does soil temperature interact with received light on the oat crop response and ability to take up urinary-N?
- What is the relative magnitude of the two main catch crop factors, N uptake and evapotranspiration, on N leaching?

Chapter 6

The Interactive Effects of Soil Temperature and Solar Radiation on Development of a Winter-Spring Sown Oats Catch-Crop

"A controlled climate growth chamber study to investigate catch crop development and N uptake under winter and early spring conditions and its effect on nitrogen leaching from a single urine application".

6.1 Introduction

The loss of urinary-N via nitrate leaching from winter forage grazing is a major issue in maintaining these low cost, dairy winter-feed systems. Field experiments have demonstrated a potential for cereal catch crops like oats to reduce nitrate leaching losses by up to 40% through winter establishment (Carey et al. 2016). However, there is considerable uncertainty around the size of this reduction under a wider range of climatic and soil conditions. Prolonged cool and wet conditions could hamper the preparation of a satisfactory seedbed for sowing the oats catch crop and/or reduce successful establishment. Findings from Chapter 5 indicated that the earlier the crop is sown, the greater the potential reduction in nitrate leaching; but these findings were made during a warmer and drier winter than average. A better understanding of the effect of growth factors is required to ensure that planting oats as a winter catch crop can make a significant reduction in nitrate leaching losses as well as improve N-use efficiency within the animal grazing system.

Given that soil type is a fixed variable in the context of farm management, and free-draining soils are preferred for growing and feeding of winter forage crops, then climate becomes the chief source of annual variability in determining the growth of the oats catch crop. The rate of growth is dictated by two main factors, soil temperature and intercepted solar radiation. If either is too low, then growth may not progress quickly enough for the oats to take up sufficient N to significantly reduce nitrate leaching. Findings from chapter 5 (experiment 2) suggested that eary sowing of oats after simulated winter forage grazing was advantageous in increasing N uptake over later sowings. However, part of this increase could have been due to the higher than average air and soil temperatures and greater sunlight hours experienced over the early winter period. Experiment 1 (Chapter 4) also suggested that the sowing and quick establishment of oats helped reduce drainage and, consequently, nitrate leaching. Determining the sensitivity of the development of the oats catch crop to winter light and soil temperatures is crucial to understanding how likely the crop is to reduce nitrate leaching from winter urine deposition i.e. how will nitrate leaching losses differ between a cooler and cloudier winter, as opposed to one that is warmer and sunnier?

The deposition of urine to the surface of a soil usually results in rapid hydrolysis of urea and related organic-N compounds to ammonium, whereupon the ammonium is typically nitrified under temperate conditions to nitrate over a 2-4 week period (Di & Cameron 2004c). However, the

ammonia oxidation to nitrite step is often rate limited and is enzymatically-mediated by ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA). In pasture and cropping soils most of this activity is undertaken by AOB rather than AOA (Di et al. 2009b; Ouyang et al. 2016). Animal deposition of urine during winter grazing occurs at a time when plant N uptake is usually minimal but at low soil temperatures AOB activity is reduced and, consequently, the nitrification rate is slowed.

This chapter reports a growth chamber experiment where nitrate leaching was measured after application of ^{15}N-labelled urine onto repacked tubes of a free-draining Balmoral soil where half the tubes were sown with oats (the remainder left fallow). Under controlled conditions of water, light and temperature, the experiment was designed to measure the effect of oats development and AOB activity on nitrate leaching losses.

The study tested the following hypotheses:

1. Nitrate leaching losses from a single urine application to fallow soil will be the same at 6ºC (winter temperature regime) as at 10ºC (spring temperature regime).
2. Nitrate leaching losses from a tube receiving a single urine application and sown with oats, will be greater at 6°C, that at 10°C, due to lower N uptake.
3. Nitrate leaching losses from a tube receiving a single urine application, and sown with oats, will be greater under low solar radiation (mid-winter regime) than under high solar radiation (mid-spring), due to lower N uptake.

The study therefore had the following objectives:

1. Measure nitrate leaching and N uptake from a single application of dairy cow urine to soil under simulated winter and spring soil temperature regimes for a period of three months, comparing treatments sown either with oats or left fallow.
2. Measure the effects and interactions of soil temperature and solar radiation on AOB activity and oats development.

6.2 Materials and Methods

6.2.1 Soil and tube preparation

The Balmoral stony silt loam soil (Acidic Orthic Brown soil; NZCS) used in the experiment was collected from the same location on Lincoln University's Ashley Dene research station, situated near Springston (NZGD2000: 43 38 42S 172 20 33E), as the soil monoliths used in the field studies. Soil texture ranged from silt loam to the first 15 cm depth but increasingly became stony, sandy and/or gravelly by 30 cm depth with up to ~50% of the soil volume occupied by stone. The soil was initially under established pasture consisting of a mixture of perennial ryegrass (*Lolium perenne* L., cultivar Grasslands Nui) and white clover (*Trifolium repens* L., cultivar Grasslands Huia). Key soil chemical and physical properties are presented in Table 6.2-1. The soil was collected from two depths, the A horizon, comprising the top 15 cm of the profile, and from the C horizon, the soil material at or below 50 cm. The soil was air-dried and sieved through a 5-mm sieve for the A horizon material, and through a 15-mm sieve for the C horizon material, to remove the largest stones.

Table 6.2-1. Key soil fertility (0-7.5 cm) and physical properties of the Balmoral soil.

Soil analysis	Value	Soil depth (cm)	Soil bulk density (g cm^{-3})	Stone volume (%)	Soil porosity (%)	Soil texture[1]
pH	6.1	0-10	1.14	11	58	stony ZL
Olsen-P	33 µg ml^{-1}	10-20	1.57	30	42	stony ZL
Exch-Ca	8.1 $cmol^{+}$ kg^{-1}	20-30	1.90	51	29	stony SL
Exch-Mg	0.5 $cmol^{+}$ kg^{-1}	30-40	2.05	54	24	stony S
Exch-K	0.4 $cmol^{+}$ kg^{-1}	40-60	1.96	51	27	gravelly S
Exch-Na	0.2 $cmol^{+}$ kg^{-1}					
CEC	16 cmol kg^{-1}					
Reserve-K	3.6 cmol kg^{-1}					
Sulphate-S	4 µg g^{-1}					
Organic-C	45 g kg^{-1}					
Total-N	4.0 g kg^{-1}					
Organic-S	5 µg g^{-1}					
Base saturation	65%					

[1]ZL -silt loam; SL -sandy loam; S –sand

A series of 50 cm long plastic tubes were prepared from 50 mm internal diameter Marley electrical conduit. Each was sealed at the bottom by a Marley end cap that was drilled and tapped to receive a 10-mm screw-in plastic nipple (Irrigation Express, Tauranga). The screw fitting, once in place, was milled to ensure that it was slightly below the level of the drilled hole in the base of the end cap. A 5-cm glass-fibre filter paper was placed in the base of each end cap before it was tapped securely on to the bottom of each tube. Wooden racks were prepared from 18 mm construction plywood to hold 32 tubes that sat on a lower shelf that was recessed and drilled to both hold the irrigation fitting and to allow the nipple to protrude into a 50-mm polythene collection bottle below (Figure 6.2-1).

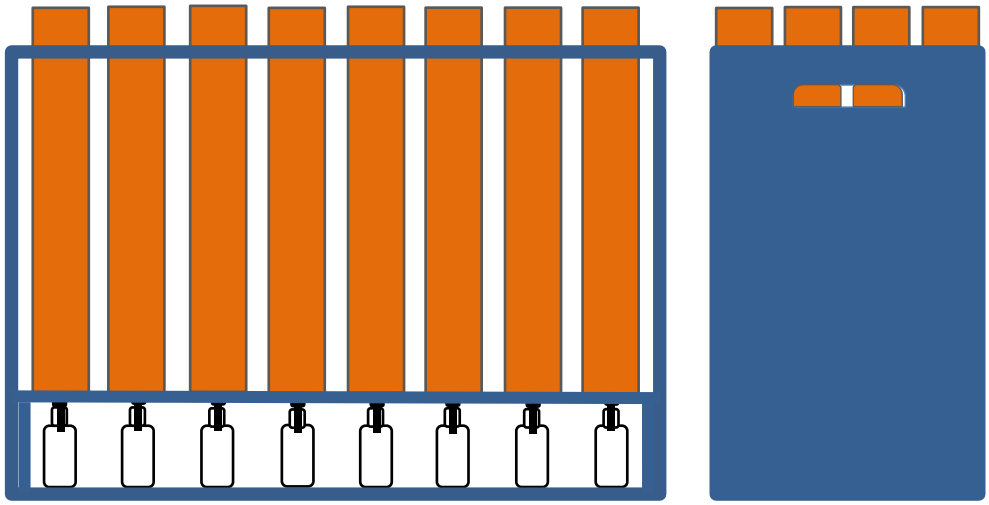


Figure 6.2-1. Profile and cross-section of racks showing tubes and bottles in position.

To approximate the Balmoral soil profile, each tube was repacked initially with 20 cm of material from the C horizon, comprising ~50% stone to a bulk density of 1.9 g cm^{-3}, whilst the upper 30 cm was repacked with the A horizon soil at a bulk density of 1.1 g cm^{-3}. The soil was tapped down progressively in a series of increments to ensure even packing. Once the tubes had all been repacked they were rewetted to field capacity and placed in an incubator at either 6°C or 10°C to equilibrate for three months prior to installation in the growth chambers. Approximately one pore volume of reverse osmosis-treated water (~500 ml) was flushed through each tube to remove any free nitrate prior to the study beginning.

6.2.2 Temperature and lighting treatments

The experiment was conducted in two Conviron Model BDW40 (Winnipeg, Canada) growth chambers, equipped with a CMP6050 controller with an Ethernet linked central management computer that can maintain air temperature from -10°C to 40°C and lighting levels from 500-1000 $\mu m\ m^{-2}\ s^{-1}$ (photosynthetic photon flux density). The chamber uses ceramic metal halide bulbs within a cooled light canopy with glass barrier to prevent heat interference from the light fittings. The chambers also have active CO_2, relative humidity (>10°C) and air management control.

Plate 6.2-1. Conviron growth chambers at Lincoln University where the experiment was conducted.

Temperature and lighting treatments were based on Lincoln mid-to-late winter (July-August) and early spring (September) average values of air and soil temperatures, solar radiation and relative humidity over the last 15 years recorded from the NIWA Broadfields meteorological station (5 km from Lincoln University).

Conversion of daily solar radiation to photosynthetic photon flux density (PPFD) was based on the following equation:

$$PPFD = MDSR \times Time \times CF$$

6.2-1

where: PPFD = Photosynthetic photon flux density or moles of photons per unit area per second ($\mu m\ m^{-2}\ s^{-1}$), MDSR = mean daily solar radiation in megajoules per unit area per day ($MJ\ m^{-2}\ day^{-1}$), Time = the number of seconds in a day to convert $MJ\ m^{-2}\ day^{-1}$ to watts per unit area ($W\ m^{-2}$), and CF = Conversion factor that converts megajoules (MJ) to joules ($x10^6$) and relates the proportion of the total radiation spectrum available to the plant for photosynthesis (plant available radiation or PAR; 400-700 nm, value ~2.1).

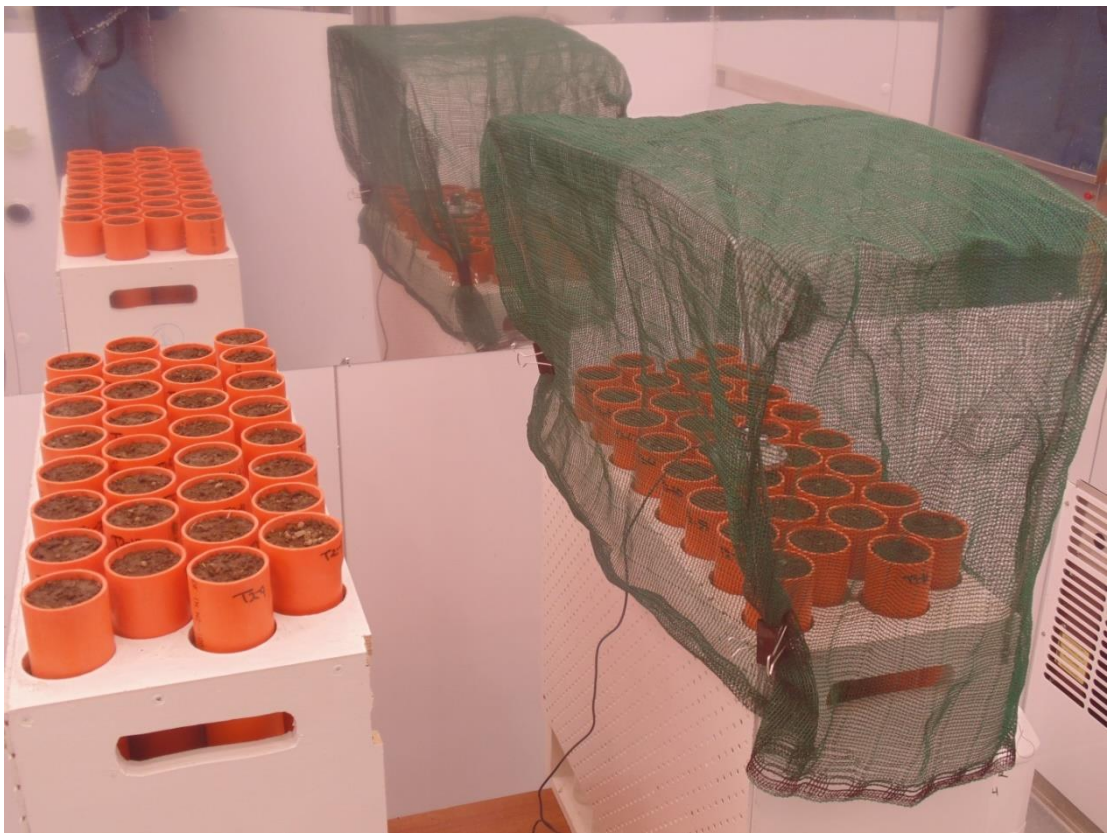


Plate 6.2-2. Racks of soil tubes, shaded and open, shown in place in a growth chamber prior to urine application.

Due to the inability to maintain the soil tubes at mean winter and spring soil temperatures and still allow for the diurnal fluctuation of daily air temperatures, the growth chambers were set approximately at the mean soil temperatures (10 cm) for the winter and early spring months; nominally 6 °C and 10 °C, respectively. Solar radiation levels were set at 5 and 10 $MJ\ m^{-2}\ day^{-1}$ to represent mid-winter and early spring light levels, respectively, and was achieved through chamber

lighting being set at the higher value with one half of the tubes shielded using 2 mm^2 shade-cloth netting over a wire frame that approximately halved the received light levels (Plate 6.2-2). Non-shaded racks were bordered by a clear plastic "fence" to minimise any airflow differences between racks. Light levels were controlled by the light meter reading within the chamber and confirmed with a handheld LiCor 250A light meter with a LI-190SA quantum (PAR) sensor (Nebraska, USA) to ensure consistent lighting over the tubes.

Table 6.2-2. Average Lincoln winter/spring monthly air and soil temperatures, daily radiation and relative humidity (NIWA 2016).

Month	Av. Daily air temperature (°C)	Av. Daily soil temperature (°C)	Av. Daily radiation (MJ m^{-2} day^{-1})	Relative humidity (%)
June	5.3	4.8	4.5	86
July	4.6	4.0	5.3	86
August	7.2	5.8	8.3	84
September	10.0	7.8	12.7	76
October	11.1	10.0	17.9	76
November	13.5	13.6	22.4	73

There were eight experimental treatments consisting of a 2x2x2 factorial of soil temperature (2 levels; 6 or 10°C) by solar radiation (2 levels; 5 or 10 MJ m-2 day-1) by crop (2 levels; oats or fallow) factors (Table 6.2-3).

Urine was collected from a mixed Friesian-Jersey herd one day prior to application and analysed overnight for total-N content as outlined in section 3.4.2. The urine was then adjusted for concentration to 3.5 g N L^{-1} using purified water and labelled with ^{15}N-urea and ^{15}N-glycine in a 10:1 ratio as per the method described in section 4.2.2. Final ^{15}N concentration was ~8.5% of the total-N present.

Table 6.2-3. Treatment ID and list of crop, soil temperature and solar radiation treatments.

No.	Treatment	Crop	Temperature (°C)	Daily radiation (MJ m^{-2} day^{-1})	PPFD[1] (μm m^{-2} s^{-1})
1	6°C-low light	Oats	6	5	286
2	6°C-high light	Oats	6	10	578
3	10°C-low light	Oats	10	5	286
4	10°C-high light	Oats	10	10	578
5	6°C-low light	Fallow	6	5	286
6	6°C-high light	Fallow	6	10	578
7	10°C-low light	Fallow	10	5	286
8	10°C-high light	Fallow	10	10	578

[1] Photosynthetic photon flux density

6.2.3 Experimental protocol

The ^{15}N-labelled urine was applied the day after collection at a rate of 69 mg N per tube (≅ 350 kg N ha^{-1}) in a volume equivalent to a 10-mm depth (20 ml). The tubes were watered 3-times weekly (Mon, Wed and Fri) to provide the average monthly rainfall rate (~59 mm per month) equating to a total weekly depth of 13.5 mm. Fourteen days after urine application, two oats seeds were sown per tube in every second row of eight tubes to a depth of 1 cm and covered lightly. The adjacent row was left fallow. A decision was made to increase the rate of watering, at the time the oats seeds were sown, to the 75th percentile for Lincoln for the winter months (86 mm), a 50% increase in the original depth applied (19.5 mm $week^{1}$). Each day's watering was split into four even increments over eight hours and applied by variable volume pipette. Leachate was collected weekly and frozen at -18°C until required for analysis.

Tubes were sequentially deconstructed starting from 30 days after sowing and thereafter at approximate 15-day intervals, out to 75 days. Vegetative material was removed from the tops of those tubes planted with oats, air-dried at 60°C overnight, and weighed. Tubes were then inverted and the bottom cap removed with the upper 30 cm extruded out of the tube using a large wooden dowel of the same internal diameter. The soil core was then gently pried apart to extract the bulk of the roots intact and set aside for later washing. The soil core, once the main root stem had been removed, was left largely intact and partitioned by ruler and knife into 3 segments, 0-10, 10-20 and 20-30 cm. Each segment was inspected and any remaining roots removed and added to those

removed earlier, before mixing and bagging. Fallow treatments were extruded in the same way. All soil segments were then weighed. Roots were washed using a high-pressure water spray head through a 2-mm sieve to wash the remaining soil out and allow removal of extraneous material (Plate 6.2-3). The roots were then placed in a 70-ml container, covered with water and refrigerated at 4°C for later analysis.

6.2.4 Leachate, soil and plant analysis

Leachate samples were analysed by flow injection analysis (FIA) to determine the NH_4^+-N and NO_3^--N concentration (Gal et al. 2004). Nitrogen leaching losses were calculated using the following equation (6.2-2):

$$N_{drn} = \sum_{i=1}^{n} (N_{conc} \times Vol) \times CF$$

6.2-2

where: N_{drn} = the sum of the cumulative nitrogen leaching loss (mg N) for *n* weeks, N_{conc} = leachate nitrogen concentration (µg N ml^{-1}), *Vol* = millilitres collected (ml), *CF* = conversion factor ($x10^{-3}$) to convert µg N to mg N

Measurements of the ^{15}N proportion of the ammonium and nitrate content of the leachates was undertaken by concentrating the $^{15}N/^{14}N$ on 7 mm glass fibre disks using the method described by Brooks et al. (1989) before combustion at 1000°C using a PDZ Europa 20-20 stable isotope mass spectrometer (Sercon Ltd., Cheshire, UK). Further details are outlined in Appendix C.

Mineral-N analysis was conducted on all soil samples following the method as outlined by Blakemore et al. (1987) but using 1 M KCL for the extraction instead of 2 M (exact method is described in Appendix C). Samples were centrifuged after shaking for 1 hour and filtered through a medium fast filter paper (Whatman 1 or equivalent) before freezing at -18°C until required for analysis by FIA. The gravimetric moisture content of each soil sample was calculated by drying 10 g of moist soil overnight at 105°C whilst another portion was dried at 40°C and pulverised to determine the N and ^{15}N contents using a Rocklabs mill (Dunedin, NZ).

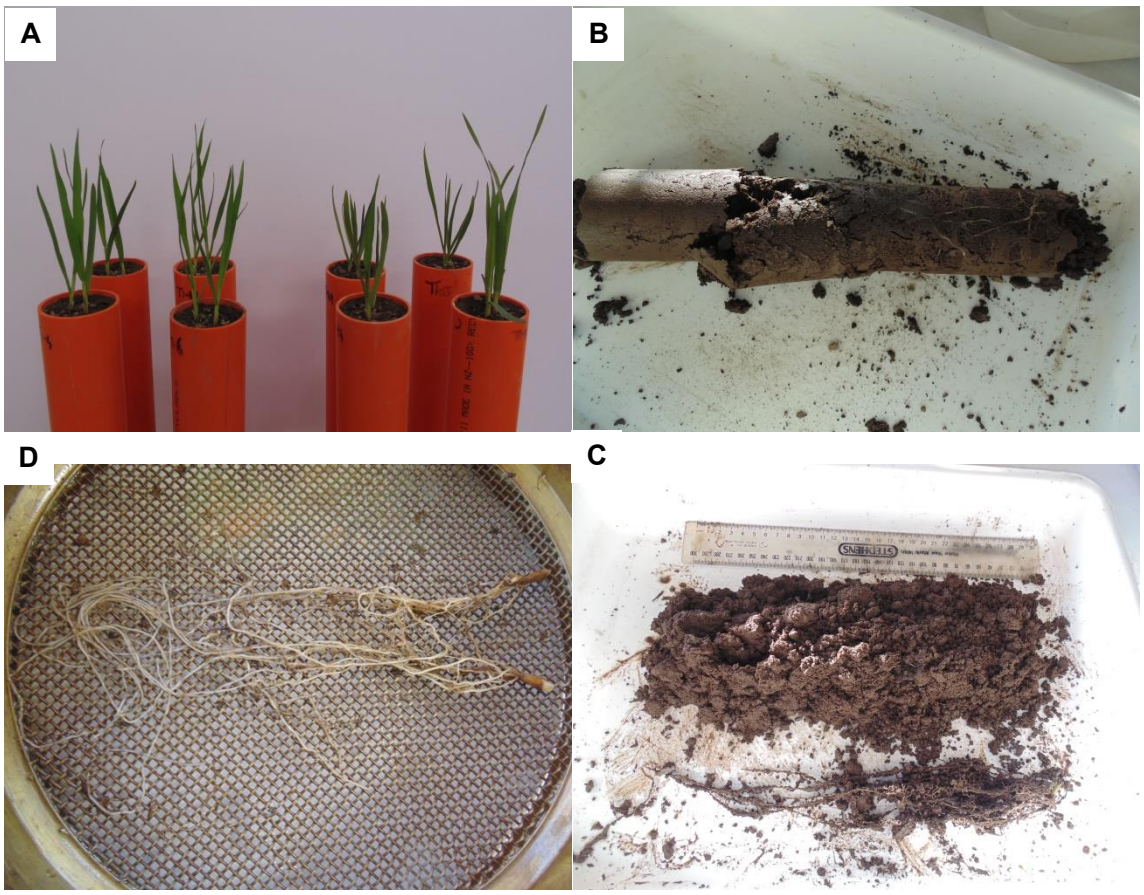


Plate 6.2-3. Stages in tube deconstruction; A) oats (6°C-60 days) prior to harvest, B) soil after initial extrusion from tube, C) separation of roots from soil prior to splitting into depth increments and D) after root washing (6°C-60 days).

Total root length, average root diameter, total surface area and total root volume were measured by computer scanning the root samples in water trays positioned in an Epson scanner (400 dpi, with a transmitted light unit, EPSON EXPRESSION 10000XL 3.49), creating grey scale images that were digitised and analysed by the computer software WinRHIZO (Reg V2009c; Regent Instruments Inc., Quebec City, Canada) (Himmelbauer et al. 2004). After digital analysis, the root samples were dried at 60°C, and those from the final harvest were weighed and ground for analysis. Soil and plant samples were submitted for total-N and ^{15}N analysis by combusting at 1000°C in an oxygen atmosphere in an automated Dumas-style elemental analyser which was linked to a 20-20 stable isotope ratio mass spectrometer (Sercon Ltd, Crewe, CWI6ZA, UK). Methods are more fully outlined in Appendix C.

6.2.5 Quantification of ammonia oxidising bacteria (AOB) abundance

AOB abundance was quantified using a real time quantitative polymerase chain reaction (qPCR) procedure to copy and amplify the *amoA* gene. Total soil DNA was extracted from 0.25 g of moist soil using MoBio Powersoil™ DNA isolation kits (MoBio Laboratories, Geneworks, South Australia) per the manufacturer's instructions. Concentration and quality of the extracted DNA were estimated using a Quant-iT™ dsDNA BR assay kit on a Qubit fluorometer (Life Technologies, Auckland, NZ) and NanoDrop ND-1000 spectrophotometer (Nanodrop Technologies, Montchanin, USA) using the primer pair *amoA*1F-mod and *amoA*RI-Mod to amplify particular regions of the bacterial *amoA* gene that were then measured for fluorescence intensity (Hornek et al. 2006). Results were expressed as million copies g^{-1} of dry soil. A fuller description of the method is presented in Appendix C.

6.2.6 Recovery of the urinary ^{15}N-labelled fraction

The recovery of the ^{15}N proportion in the applied urine was summed from that recovered in leachate, soil (mineral-N and total-N) and plant fractions. No gas measurements for N_2O or N_2 were made. The ^{15}N analysis of the leachates was confined to samples taken from the tube replicates that continued through the experiment to the last harvest (32 tubes; 4 replicates per treatment), and then only every second batch, meaning the ^{15}N fraction of the samples of the batches between was interpolated. The ^{15}N fraction of the soil from each core sampling (four in total) was determined for the top 30 cm only, except in the last deconstruction when the fine earth fraction (<2 mm) from the bottom 20 cm was also sieved, retained and dried for analysis. Top and root DM samples for each harvest were analysed for ^{15}N except harvest 1 (insufficient sample) for the 6°C treatments.

The recovery of the ^{15}N in the labelled urine was calculated using the formula provided by Cabrera and Kissel (1989) where the recovery is expressed as a percentage of the total ^{15}N in the initial application using the following equation (6.2-3):

$$ {}^{15}_{Rec}N\% = \frac{N_s \times ({}^{15}_{\%}N_a - {}^{15}_{\%}N_b) \times 100}{N_{urine} \times ({}^{15}_{\%}N_c - {}^{15}_{\%}N_b)} $$

6.2-3

where: ${}^{15}_{Rec}N\%$= the percentage of ^{15}N and consequently, the total-N recovered from that applied in the urine, N_s = milligrams of N in the leachate, plant or soil sample, N_{urine} = milligrams of N in the

applied urine, $^{15}_{\%}N_a$ is the atom % ^{15}N abundance in the plant or soil material, $^{15}_{\%}N_c$ = atom % ^{15}N abundance in the urine, and $^{15}_{\%}N_b$ = the natural abundance of atom % ^{15}N in untreated soil or plants grown in untreated soil.

6.2.7 Statistical analysis

The experimental treatments were an orthogonal design but the practical requirements of the experiment meant that the fallow and oats treatments were alternated between rows (Plate 6.2-4). Racks were rotated regularly within the chamber as a means of minimising the effect of any temperature or lighting variation with the selection of replicates for each deconstruction event done randomly within the rows so each row decreased by two with each sampling. Data was analysed as a completely randomised design using the General ANOVA function within the Genstat 9.0 statistical package (Lawes Agricultural Trust 2007). Modelling of data was done using the generalised mixed linear (GLM) model procedure within Genstat.

Plate 6.2-4. Rack showing alternating crop row treatments.

6.3 Results

6.3.1 Drainage

A maximum depth of 121 mm drainage was collected from the low-light fallow treatments of both chambers by the end of the experiment, with the oats treatments producing considerably less (crop effect; P<0.001, Table 6.3-1). By 30 days after sowing, drainage had started to decrease in the 10°C oats treatments compared with the fallow treatments (temp x crop 30 days; P<0.05) so that by 45 days, no further drainage was collected. By the experiment's end, drainage totals from the 10°C chamber oats treatments were only about a third of that for the fallow treatments (temp x crop 75 days; P<0.001, Table 6.3-1). Similarly, oats treatments in the 6°C chamber by the 60-day mark were also producing significantly less drainage although this continued through to the end of the experiment (Figure 6.3-1, Table 6.3-1). Drainage from the high-light treatments in both chambers was between 16-35% lower than from the low light treatments (P<0.001).

Table 6.3-1. Total inorganic-N drainage loss, drainage depth, nitrate-^{15}N and ^{15}N-fraction of total nitrate drainage loss for all treatments after 75 days.

Treatment			*Inorganic-N leached (mg N)*			*Drainage (mm)*	*Nitrate-^{15}N leached (mg N, %)*	
Temp	**Light**	**Crop**	**[a] NO_3^--N**	**NH_4^+-N**	**Total-N**	**Depth**	**NO_3^--^{15}N**	**[b] ^{15}N/N**
6°C	Low	Fallow	31.6 c	0.06 ab	31.7 c	117 e	8.7 b	27 cd
		Oats	22.2 b	0.06 ab	22.2 b	88 c	2.4 a	11 ab
	High	Fallow	17.0 b	0.31 c	17.3 b	86 c	3.0 a	18 bc
		Oats	9.3 a	0.10 b	9.4 a	56 b	0.8 a	8 ab
10°C	Low	Fallow	64.6 d	0.04 ab	64.7 d	121 e	23.0 c	36 d
		Oats	12.3 b	0.04 ab	12.4 ab	44 ab	0.1 a	1 a
	High	Fallow	38.1 c	0.03 a	38.2 c	102 d	8.3 b	20 bc
		Oats	11.0 a	0.03 a	11.1 ab	37 a	0.4 a	3 a
Temp			***	***	***	***	***	ns
Crop			***	***	***	***	***	***
Light			***	**	***	***	***	**
Temp x crop			***	ns	***	***	***	**
Temp x light			ns	***	ns	**	*	ns
Crop x light			***	***	***	ns	***	**
Temp x crop x light			ns	**	***	ns	***	ns
[c] LSD (5%)			6.5	0.06	6.6	11	3.0	9

[a] Means within a column followed by a letter in common are not significantly different according to Duncan's multiple range test (P < 0.05); [b] ^{15}N fraction of total nitrate loss; [c] Least significant difference at temp x crop x light level.

6.3.2 Nitrogen leaching

The overwhelming bulk (>99%) of the mineral-N recovered in drainage was as nitrate with only trace amounts of ammonium recovered (Table 6.3-1). Nitrate leaching losses from the fallow treatments of the 10°C chamber were about twice ($P<0.001$) those from the 6°C chamber (Figure 6.3-1), with an average of ~51 mg N collected after 75 days (Table 6.3-1). Sowing oats in the 10°C and 6°C chambers reduced nitrate leaching losses, on average, by around three-quarters and one-third, respectively ($P<0.001$; Table 6.3-1) so that final nitrate leaching losses from the oats treatments of the 6°C and 10°C chambers were similar, ranging from 9-22 and 11-12 mg NO_3^--N, respectively (Table 6.3-1). The higher lighting treatment in both chambers decreased nitrate leaching loss overall ($P<0.001$) for both cropping treatments although not significantly for the 10°C oats treatment, possibly due to drainage finishing so quickly by 45 days.

Much of the initial nitrate collected did not actually originate from the urinary-N as the ^{15}N-labelled fraction was only detected after ~45 days when the first traces were detected in the fallow treatments of both growth chambers. This increased, however, so ^{15}N-nitrate made up to 36% of the total nitrate loss from the fallow treatments by 75 days (Figure 6.3-1). However, for the oats treatments in both chambers, the ^{15}N fraction comprised a much smaller proportion, especially at 10°C, where it only made up between 1-11% of the total nitrate collected. This meant most of the nitrate collected from the 10°C oats treatments originated from soil nitrogen sources rather than from the ^{15}N-labelled urine (Table 6.3-1). Nitrate-^{15}N concentrations in drainage water remained low for all treatments till around 45-50 mm drainage whereupon concentrations for both 10°C and 6°C fallow low light treatments increased rapidly, reaching 175-285 mg NO_3^--N L^{-1} after 120 mm drainage (Figure 6.3-2 The lack of further drainage, however, meant no treatment breakthrough curves were completed.

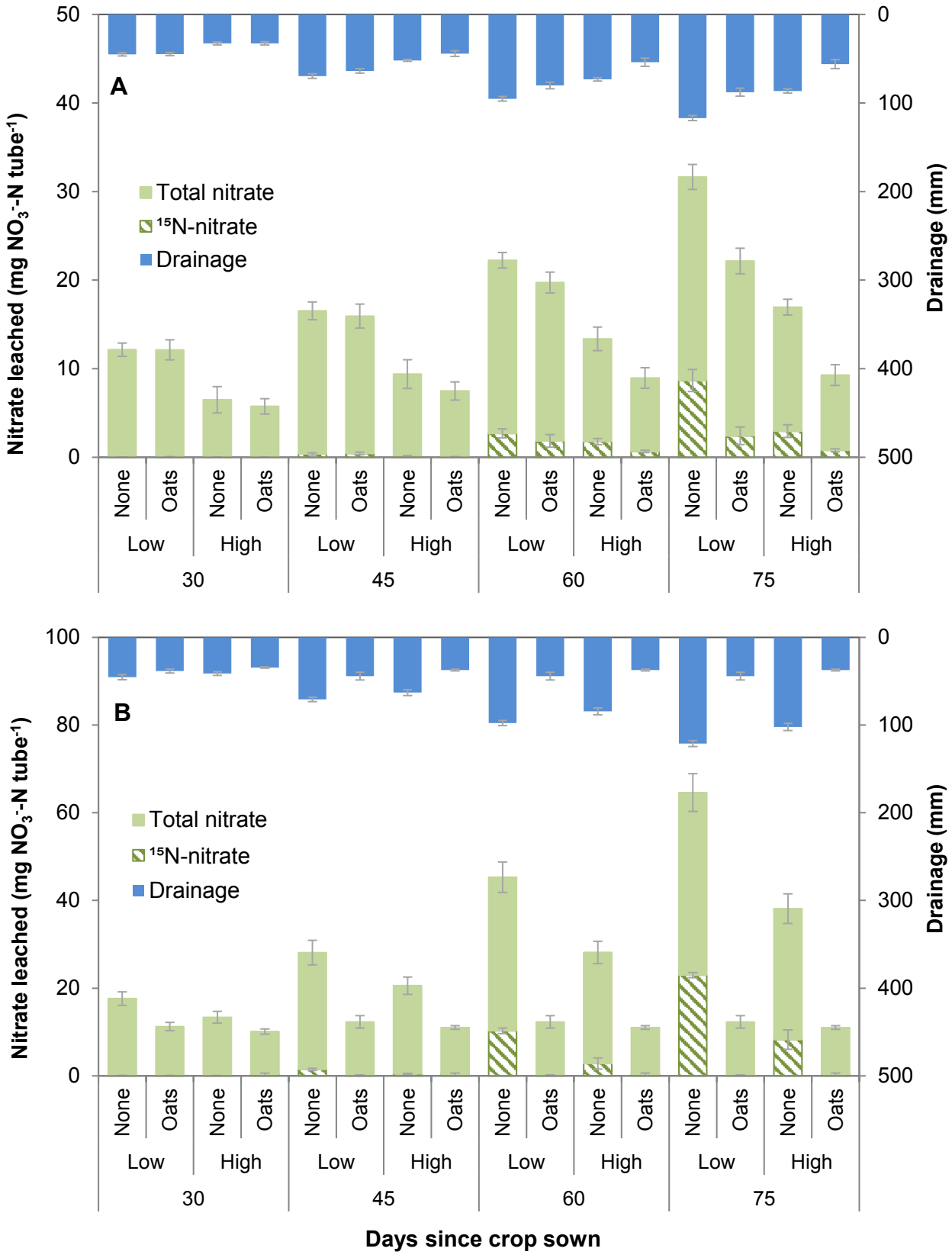


Figure 6.3-1. Total nitrate content in leachate (total and ^{15}N-nitrate fraction) and drainage depths for 30, 45, 60 and 75 days following sowing of oats at 6 °(A) and 10°C (B) and at mid-winter (low) or mid-spring (high) solar radiation levels. Standard error bars shown (±1 SE).

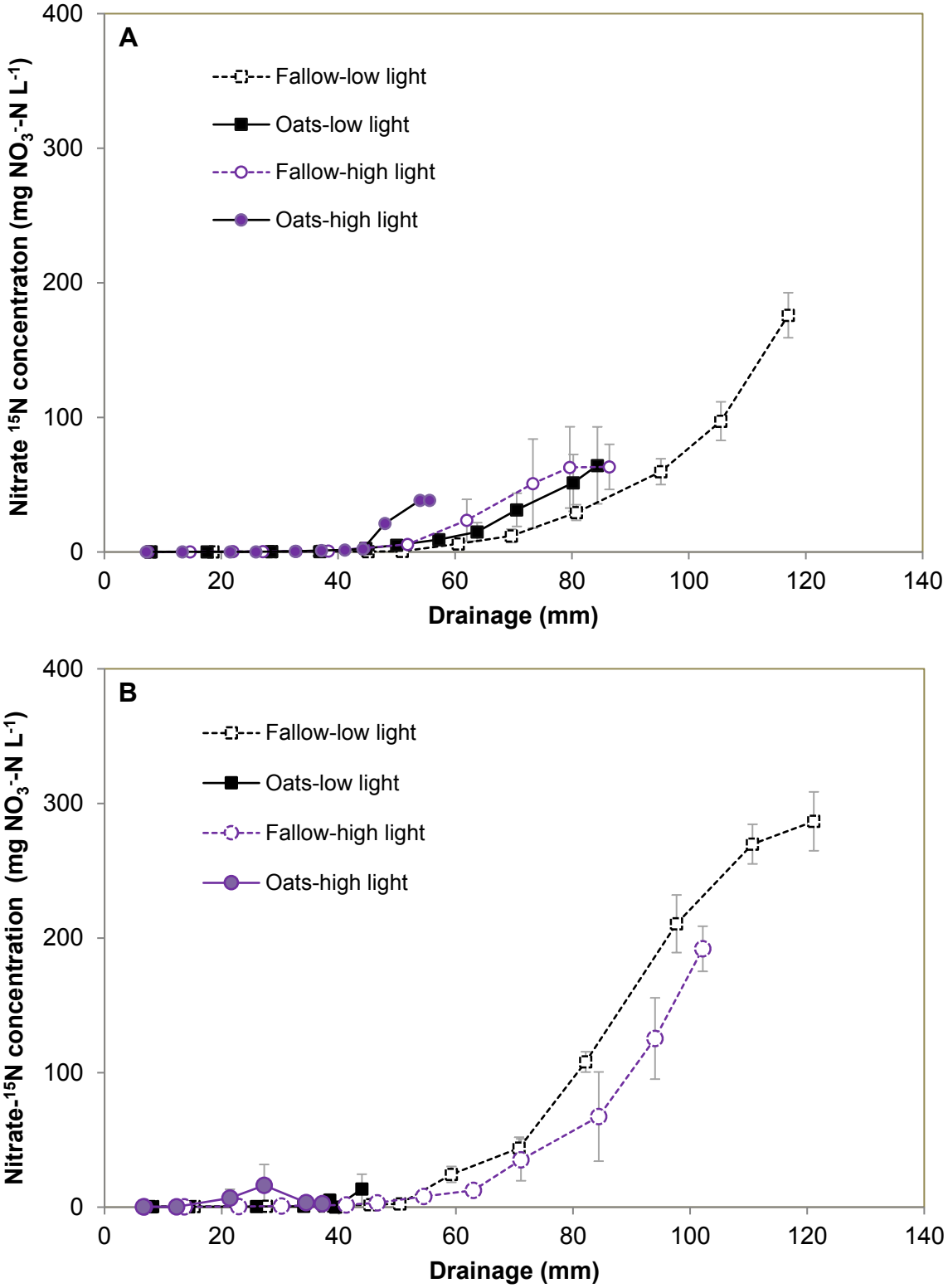


Figure 6.3-2. Nitrate-^{15}N concentration versus cumulative drainage over 90 days for fallow and oats treatments at 6° (A) and 10°C (B) and at mid-winter (low) or mid-spring (high) solar radiation levels. Standard error bars shown (±1 SE).

6.3.3 Soil mineral-N and AOB population abundance

By the time of the first core sampling (30 days after sowing of the oats), the overwhelming bulk of the soil mineral-N was present was as nitrate (>90%) with the amounts declining in all treatments by 75 days (Figure 6.3-3). The total amount of soil mineral-N present at 30 days as ammonium was small (~4 mg N per core), declining further over the following 45 days (< 2 mg N per core) (Figure 6.3-4). The total amount of nitrate present for both 6 °and 10°C treatments at 30 days was similar but its distribution was different, with more nitrate present in the lower depths for the 10°C chamber compared with the 6°C chamber (temp x depth; $P<0.001$) indicating a greater movement of nitrate after 45 days leaching (15+30 days after sowing). Peak nitrate content in the lowest depth (20-30 cm) of the fallow treatment in the 6°C chamber occurred at 60 days after sowing but it was 45 days for the equivalent 10°C treatment. Oats significantly reduced soil nitrate content in the 10°C chamber compared with fallow treatments by 45 days after sowing ($P<0.001$) and by 60 days in the 6°C chamber ($P<0.01$). By 60 days after sowing, nitrate all but disappeared in the 10°C crop treatments, and all together by 75 days. However, for the 10°C fallow treatments, 15-40% of the nitrate present in the top 30 cm at 30 days, remained at 75 days (Figure 6.3-3). Similar trends between the crop treatments occurred in the 6°C chamber although the rate of decrease in soil nitrate was not as rapid after 75 days.

There were many interactions in nitrate content and distribution through the profile (i.e. depth) between crop treatments, temperature and lighting levels. More intense lighting significantly changed the distribution of nitrate within the column (light x depth; $P<0.01$), with more present in the upper depths of the high-light treatments compared to the low-light treatments after 30 days for both 6°C and 10°C fallow treatments, and for the 6°C oats treatments. For the 10°C oats treatments, the crop-by-depth effect ($P<0.01$) was more important. By 60 days after sowing, light-by-depth ($P<0.001$), crop-by-depth ($P<0.01$) and crop-by-light ($P<0.05$) effects were all significant for soil nitrate in 6°C and 10°C fallow and crop 6°C treatments (Figure 6.3-3). In the 10°C chamber, both crop-by-depth ($P<0.001$) and light-by-depth ($P<0.05$) effects were evident after 45 days from sowing but the removal of nitrate from the crop treatments after 60 days by either uptake or leaching meant both these interactions diminished in significance (Figure 6.3-3).

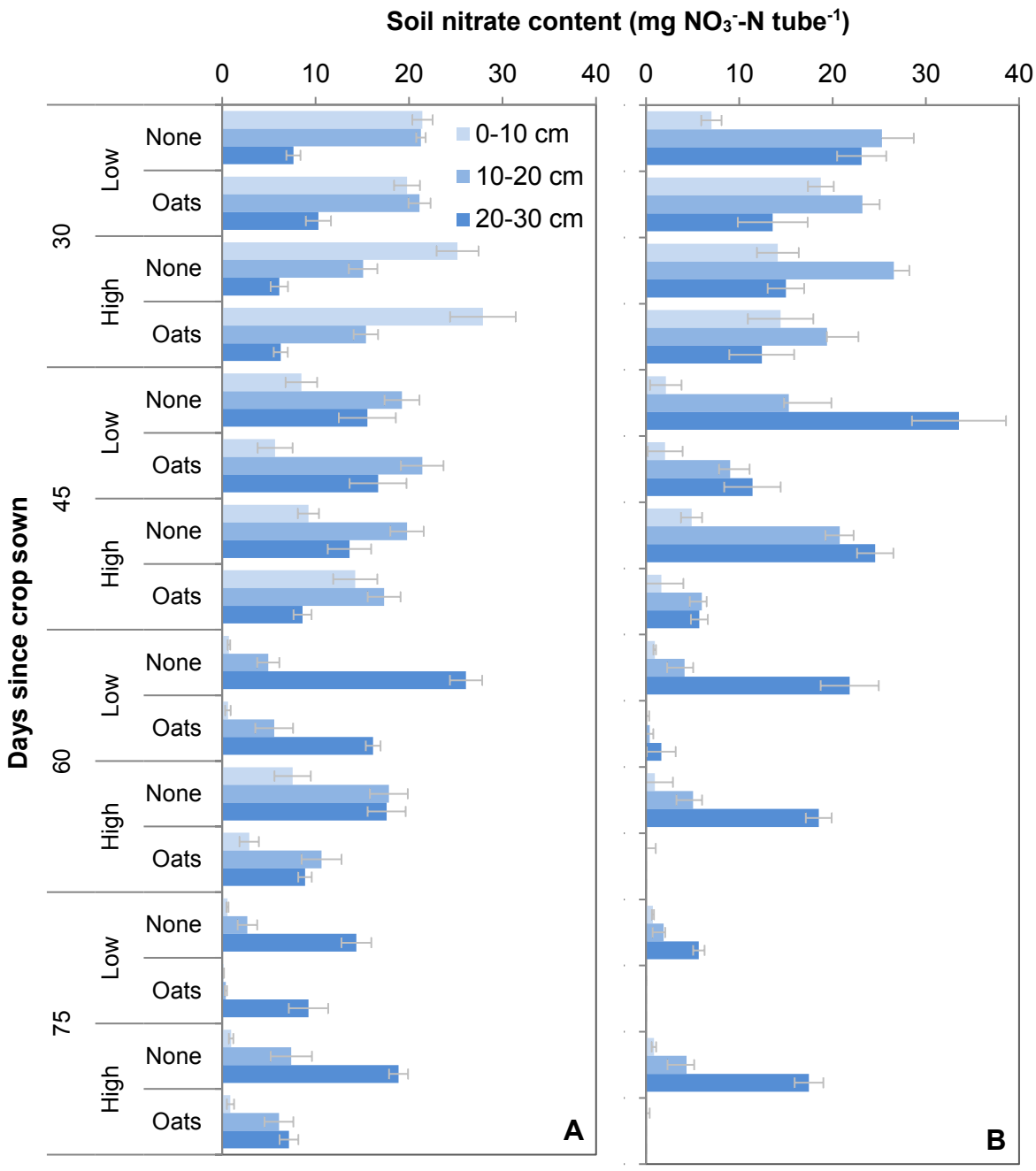


Figure 6.3-3. **Soil nitrate-N content per tube (to 30 cm) for deconstructed crop treatments (none, oats) held at 6° (A) and 10°C (B) and at mid-winter (low) or mid-spring (high) solar radiation levels after 30, 45, 60 and 75 days. Standard error bars shown (±1 SE).**

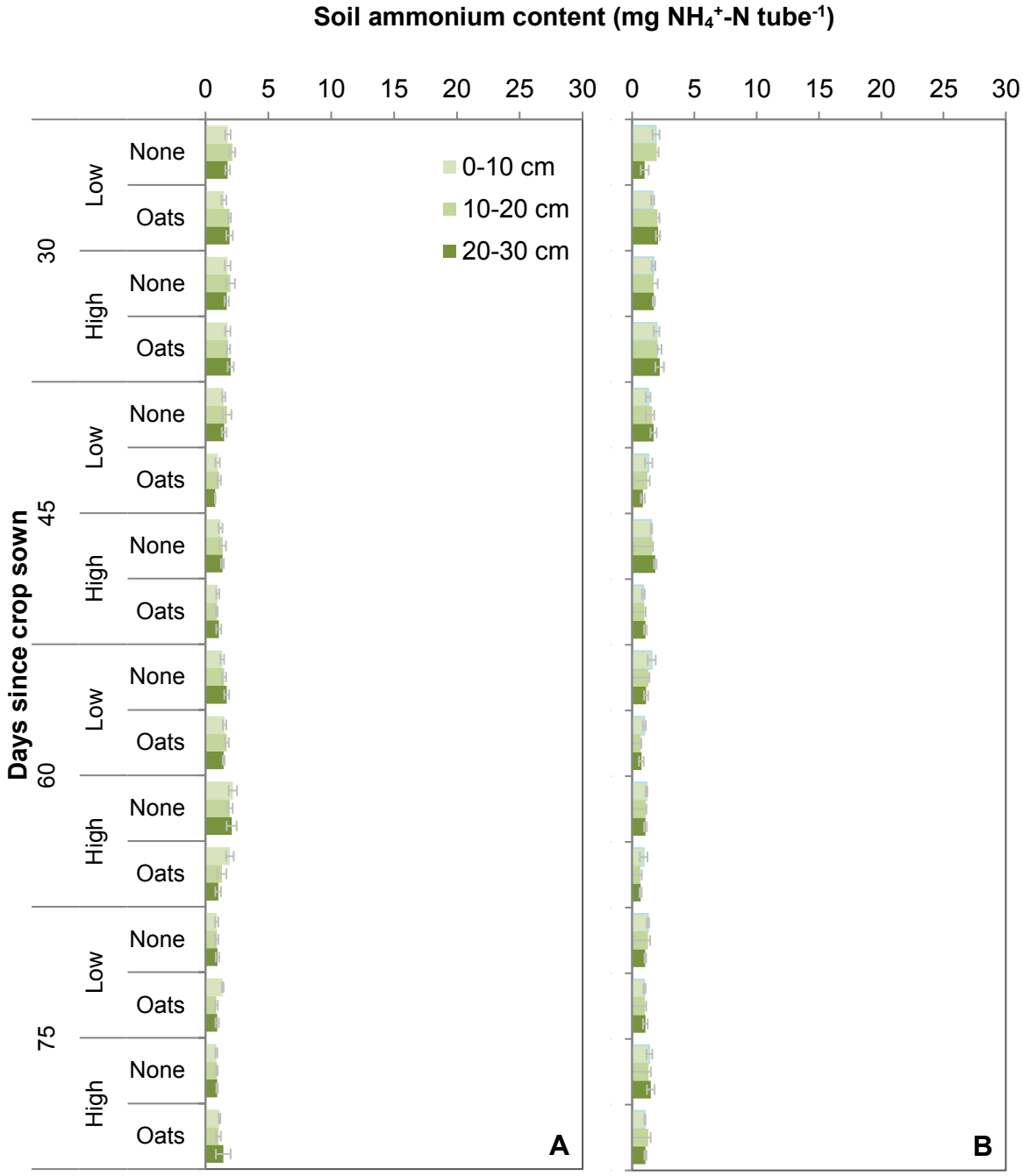


Figure 6.3-4. Soil ammonium content (to 30 cm) for deconstructed crop treatments (none, oats) held at 6° (A) and 10°C (B) and at mid-winter (low) or mid-spring (high) solar radiation levels after 30, 45, 60 and 75 days. Standard error bars shown (±1 SE).

The proportion of soil nitrate-N present initially as ^{15}N-nitrate at 30 days post-sowing for 6°C and 10°C treatments was, on average, about 60% (range 56-64%) with this proportion generally persisting in the 10°C treatments to 45 days, and in the 6°C treatments to 75 days, as nitrate content decreased or disappeared altogether (Table 6.3-1). The trends in both chambers for soil

^{15}N-nitrate loss were similar for soil nitrate generally, meaning statistical differences for main factors and interactions were also similar.

The effect of light intensity on soil ^{15}N-nitrate concentrations was similar to that for nitrate generally, particularly as an interaction with depth in the fallow treatments of both 6 ° and 10°C chambers. However, this effect manifested earlier (from 30 days; Figure 6.3-5) than for nitrate generally. More ^{15}N-nitrate was recovered after 30 days in the upper 10 cm of the 6°C high light treatments whereas for the 10°C treatments, more ^{15}N-nitrate was recovered lower down the tube profile (temp-by-depth; $P<0.001$), particularly in the fallow treatments as further leaching occurred (Figure 6.3-5). In the period from 30-75 days after sowing, differences in the depth distribution of ^{15}N-nitrate between 10°C fallow and oats treatments diminished as the effect of N uptake and/or leaching became more significant and lighting intensity less important; in the 6°C chamber, these effects persisted and the main and multiple factor interactions of crop, light and depth ($P<0.05$-0.001) continued to 75 days.

AOB population abundance values at each deconstruction event were highest in the 0-10 cm layer of each treatment where the urine was initially applied ($P<0.001$) (Figure 6.3-6). AOB abundance numbers for both fallow and oats treatments started to decline after 30 days in the crop treatments of the 10°C chamber but peaked in the 6°C chamber at ~45 days, particularly in the high light and/or crop treatments (temp-by-crop-by-light interaction $P<0.05$), before starting to decline after 60 days. By 75 days, AOB abundance had declined significantly in all treatments of both chambers although those in the 6°C chamber continued to remain higher overall ($P<0.01$).

There were significant interactions ($P<0.05$-0.01) between temperature, crop, light and depth, particularly for the 10°C treatments where differences were greatest and linked to N uptake and/or N leaching as values and differences declined over time. Crop treatments lowered AOB abundance significantly from fallow treatments after 30 days, particularly for the 10°C chamber but conversely AOB abundance was greatest for the 10°C fallow treatments. AOB numbers for fallow and oats 6°C treatments were generally intermediate (temp-by-crop; $P<0.001$) but followed similar trends over time (Figure 6.3-6).

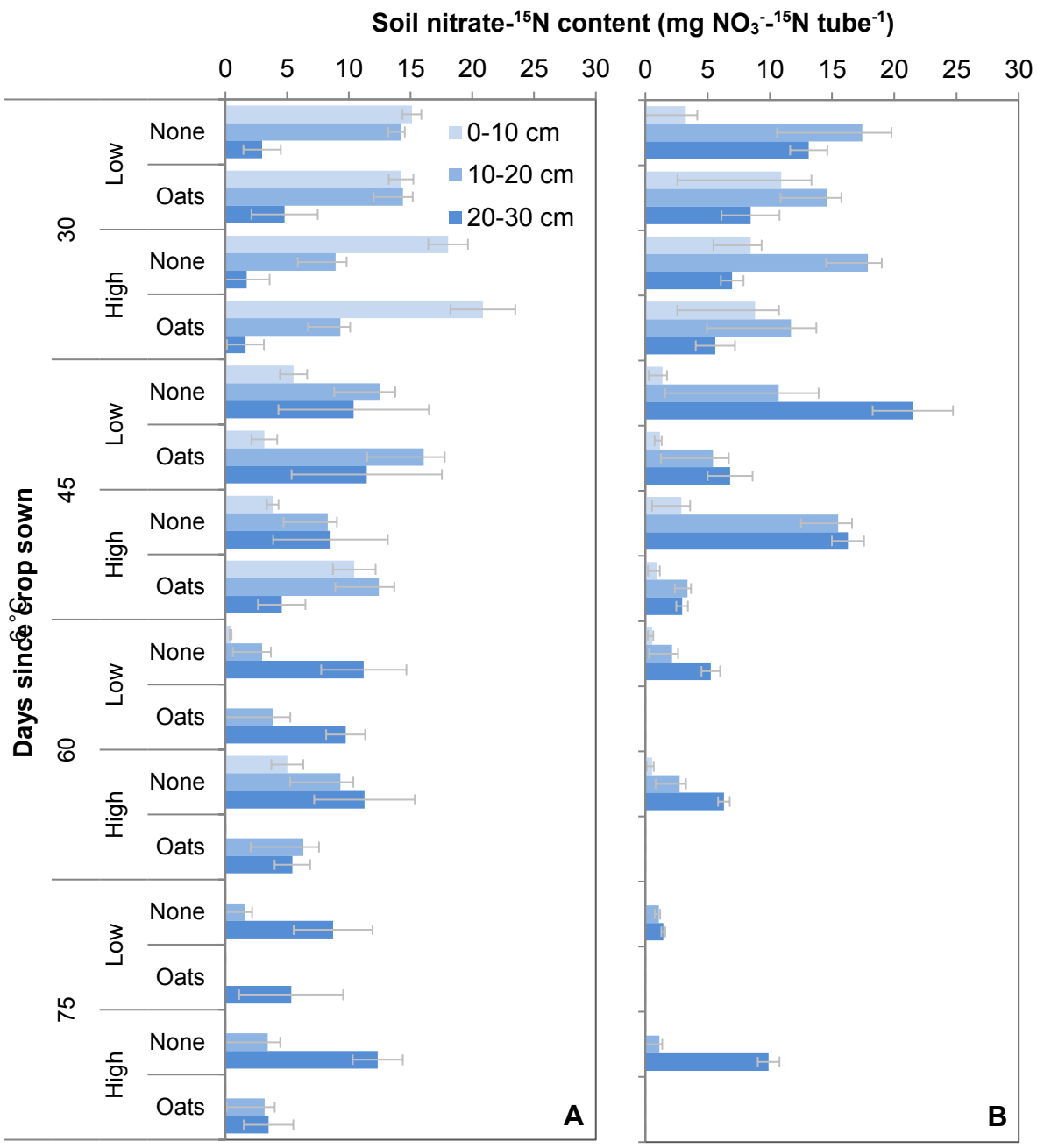


Figure 6.3-5. Soil ^{15}N-nitrate content (to 30 cm) for deconstructed crop treatments (none, oats) held at 6° (A) and 10°C (B) and at mid-winter (low) or mid-spring (high) solar radiation levels after 30, 45, 60 and 75 days. Standard error bars shown (±1 SE).

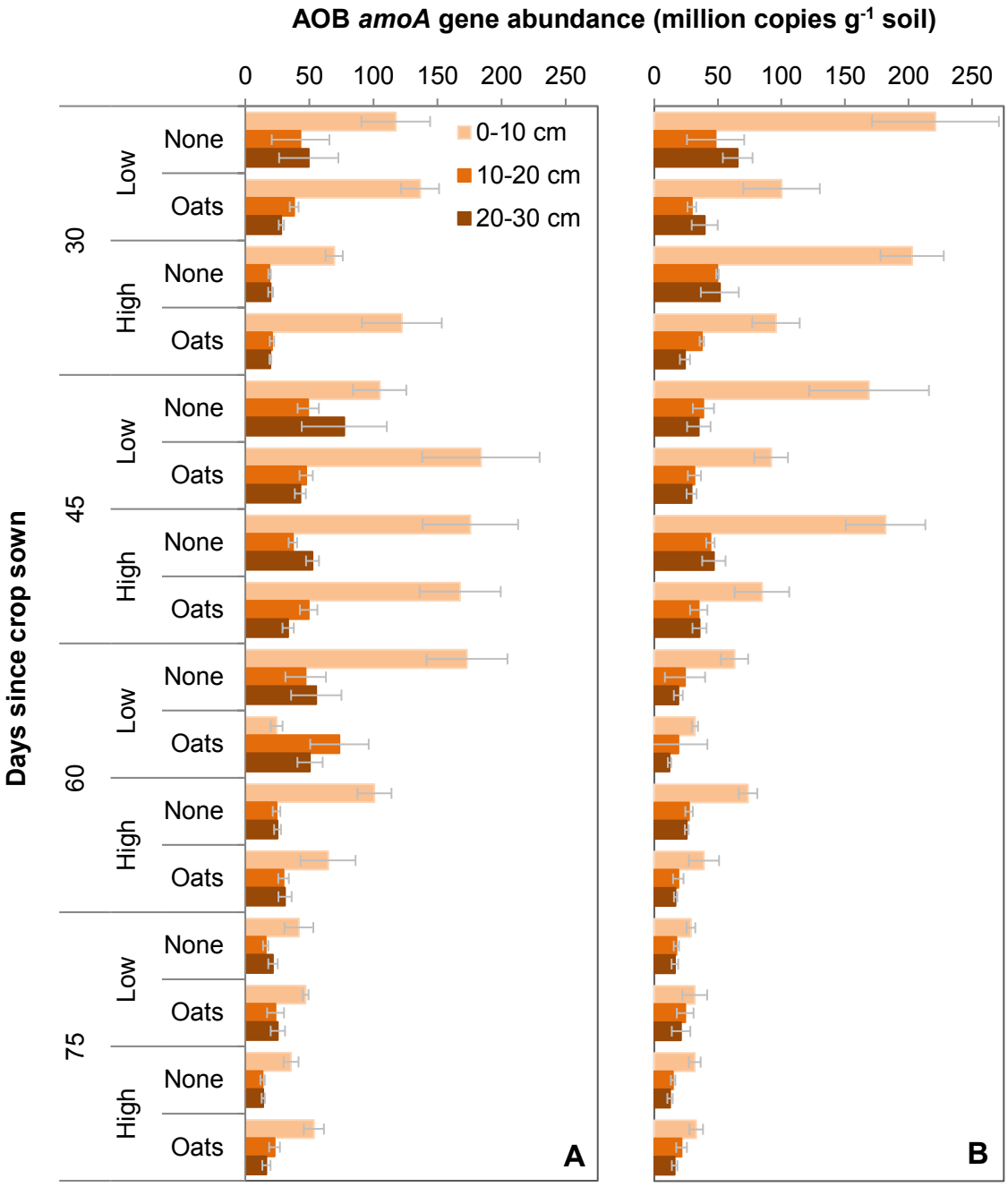


Figure 6.3-6. Soil AOB (*amoA* gene) abundance (to 30 cm) for deconstructed crop treatments (none, oats) held at 6° (A) and 10°C (B) and at mid-winter (low) or mid-spring (high) solar radiation levels after 30, 45, 60 and 75 days. Standard error bars shown (±1 SE).

6.3.4 Soil moisture content

Soil moisture content varied between treatments due to the evapotranspirative demand of the oats, despite watering at the 75th percentile of Lincoln winter rainfall inputs. By 45 days after sowing there were noticeable differences in soil moisture content between crop ($P<0.001$) and light

(P<0.01) treatments, especially for the 10°C chamber where there was also a significant crop-by-light interaction (P<0.05). By 75 days, these drying effects were large and soils taken from the deconstruction of the 10°C oats treatments had gravimetric moisture contents as low as 6%, compared to 26-28% in the fallow treatments (Figure 6.3-7). Similar effects were also noticeable between crop and light treatments, in the 6°C chamber by 75 days after sowing, albeit over a smaller range of soil moisture content.

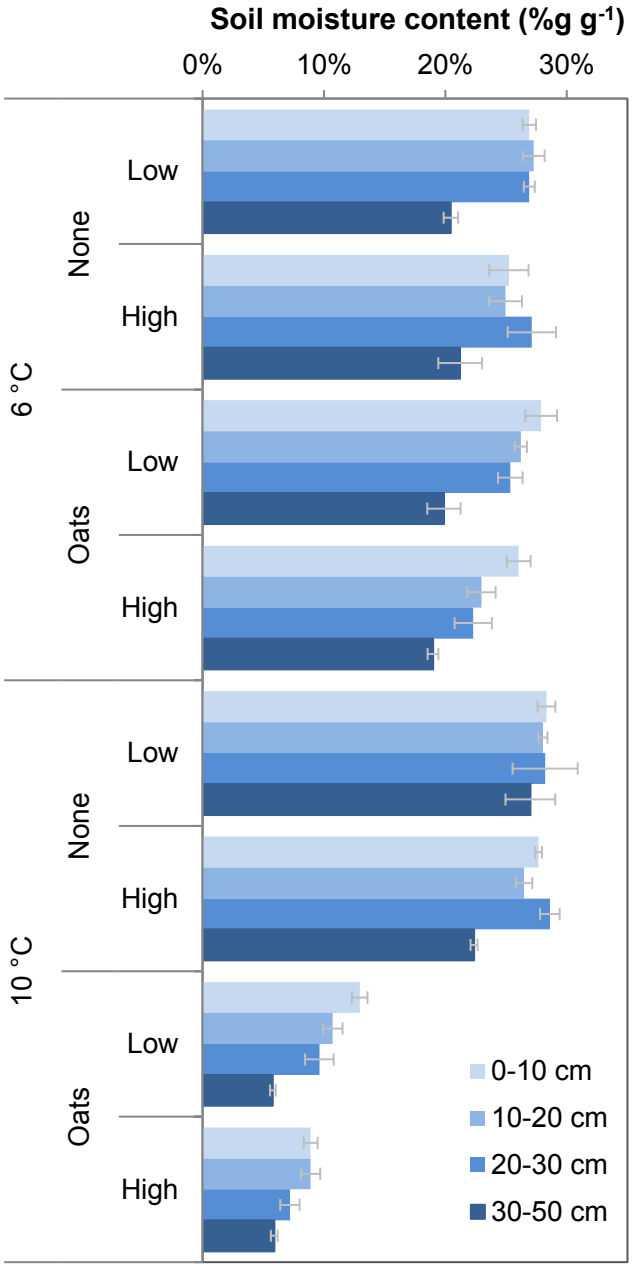


Figure 6.3-7. Soil moisture content for deconstructed crop treatments (none, oats) held at 6° and 10°C, and at mid-winter (low) or mid-spring (high) solar radiation levels, after 75 days. Standard error bars shown (±1 SE).

6.3.5 Dry-matter production, N and ^{15}N content

Oats germination occurred first in the 10°C chamber about 10 days after sowing, whilst germination in the 6 °C chamber began 13 days later. As expected, growth was more rapid in the 10°C chamber with production for both tops and roots an order of magnitude greater at 10°C than at 6°C to 60 days. Oats DM production of both tops and roots increased exponentially against degree days (sum of daily average chamber temperature exceeding 0°C) for both 6°C and 10°C chambers (Figure 6.3-8). Although those plants under low light grew taller than their full light counterparts (Plate 6.3-1), the total stem DM harvested was very similar (Figure 6.3-8a). However, root DM production in the 10°C chamber was consistently greater ($P>0.001$) for the high light over low-light treatment at each deconstruction day, by ~33% (Plate 6.3-2) but there was no difference between 6°C light treatments (Figure 6.3-8b). Overall, however, total DM production (roots and stems) was similar between light treatments for respective chambers (Table 6.3-2).

Plate 6.3-1. Oats top growth after 60 days for high and low light treatments at 10°C.

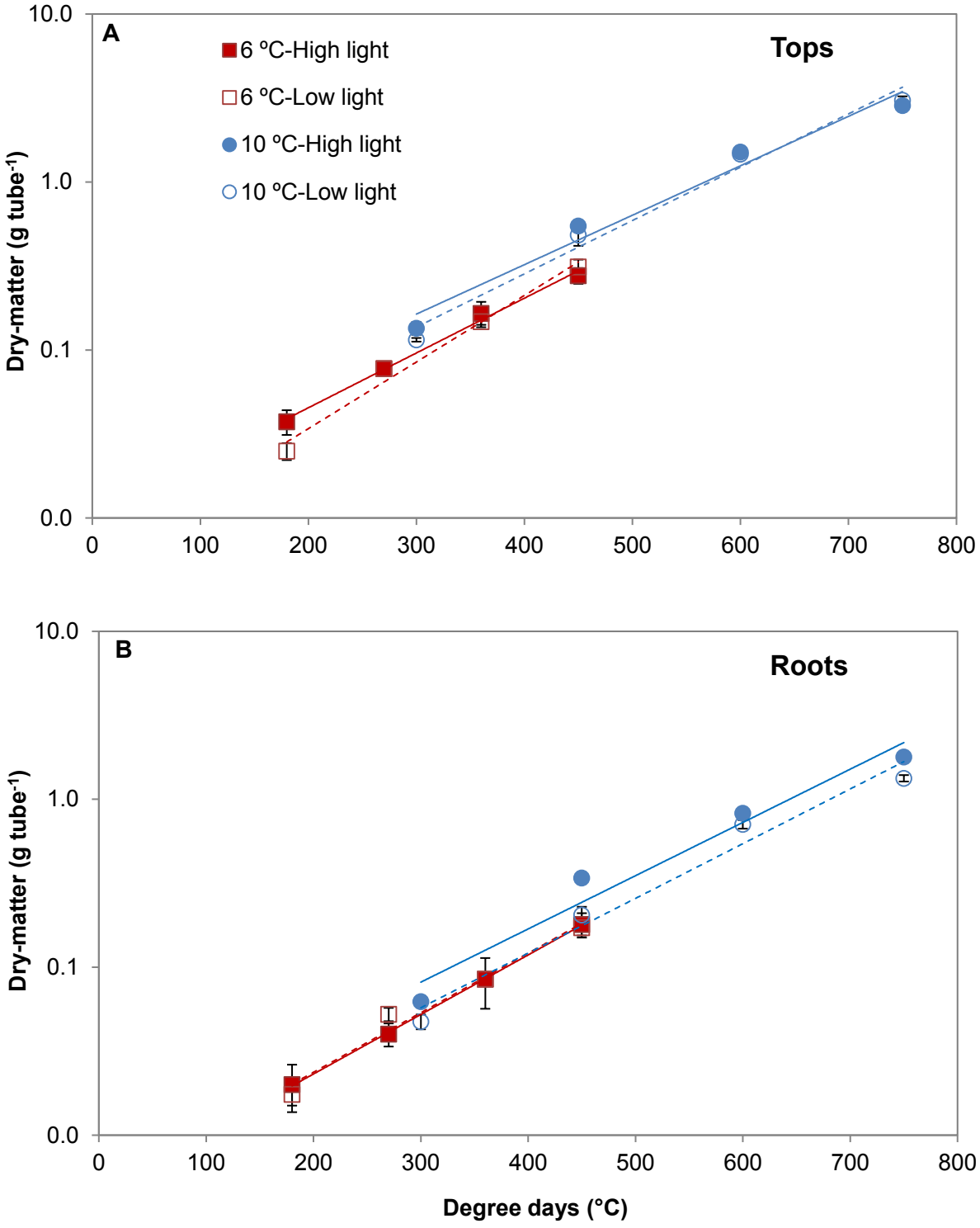


Figure 6.3-8. **Dry-matter production (log_{10} y scale) for oats tops (a) and roots (b) 75 days after sowing. Oats treatments were at two temperatures, 6° or 10°C, and two solar radiation levels, mid-winter (low) or mid-spring (high). Standard error bars shown (±1 SE).**

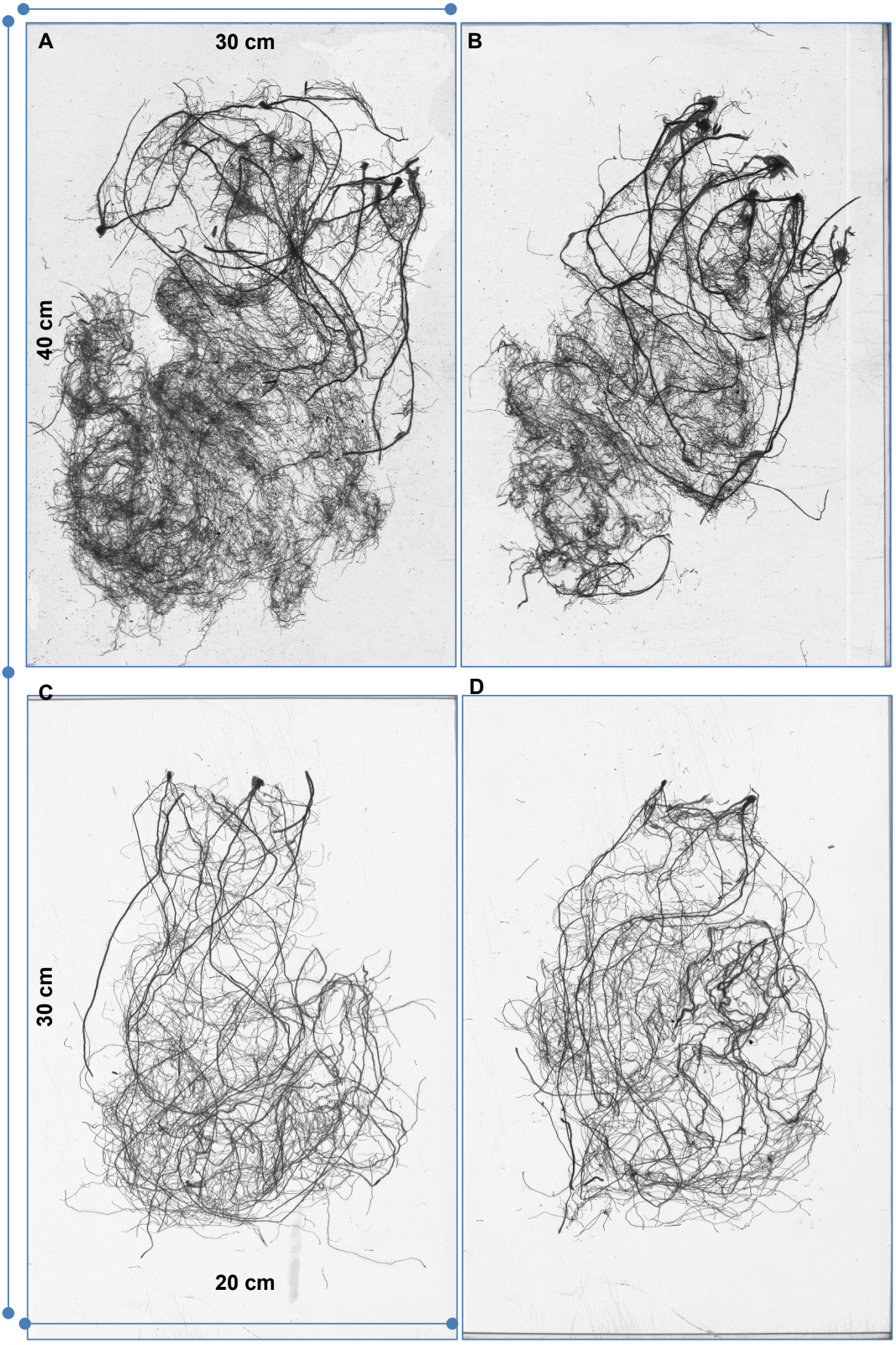


Plate 6.3-2. Digital scans showing typical root development after 75 days for 10°C mid-spring (A) and mid-winter (B), and 6°C mid-spring (C) and mid-winter (D) light treatments.

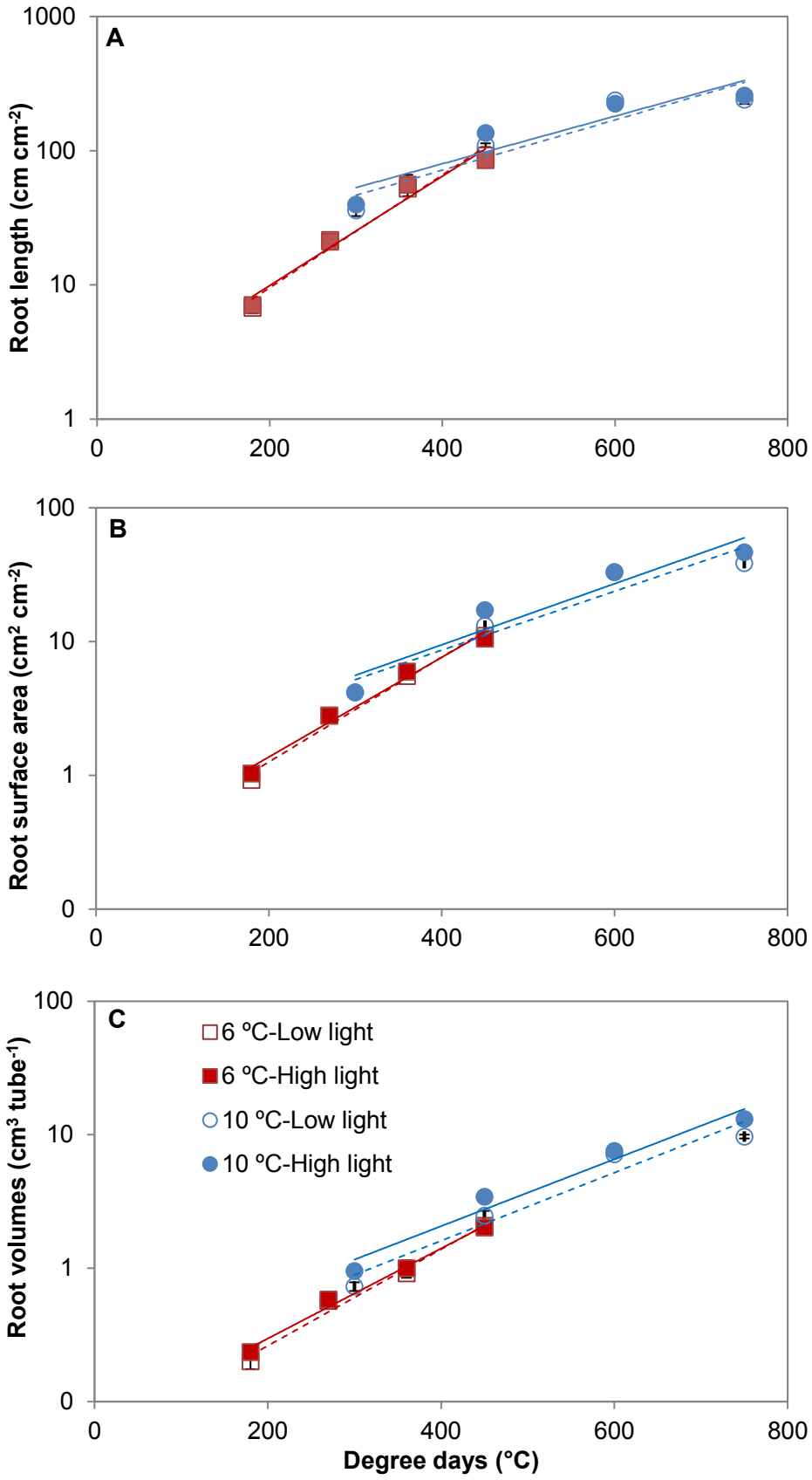


Figure 6.3-9. **Root lengths (a) surface areas (b) and volumes (c) for oats vs. degree growing days at two temperatures, 6° or 10°C, and two solar radiation levels, mid-winter (low) or mid-spring (high). Standard error bars shown (±1 SE). (note $\log_{10}$ y scale).**

Similar relationships to those for root DM were also shown for root length, surface area and volume (Figure 6.3-9a-c) although these tended to deviate from an exponential response by 75 days after sowing. Root length, surface area and volume were greater for the high light treatment for the 10°C chamber from 45 days ($P<0.05$-0.001, respectively) and although differences in root length appeared to vary, they remained significantly greater for both root volume ($P<0.01$) and surface area ($P<0.01$) for the high light treatments after 75 days (21% and 35% greater, respectively). There were no significant differences between the 6°C light treatments for root length, surface area or volume.

Despite an order of magnitude difference in DM production between chambers for both roots and stems throughout the experiment (Table 6.3-2), there was only a 2-4-fold difference in N content. This was due to the higher N content in both roots and stems of the 6°C chamber oats treatments and a "dilution" of the N content by the greater DM of the 10°C treatments. A similar pattern was repeated in ^{15}N content (Table 6.3-2) that made up about two-thirds of the total-N present although the proportion was a little less overall in the 10°C treatments (~60%) than for the 6°C treatments (~67%) (Figure 6.3-10 & Table 6.3-2).

As with DM yields, total-N and ^{15}N content (roots and stems) did not significantly differ between light treatments by the end of the experiment (75 days after sowing) but there were considerable differences between the stem and root components for the 10°C chamber from start to finish. More N and ^{15}N was stored in the roots of high light treatments from 30-75 days but this was compensated by less N present in stems of the same treatments by the experiment's end (Figure 6.3-10). Most of this additional N was due to the greater DM root weights rather than differences in N% (Table 6.3-2). Generally, the $^{15}N/N$ ratio was similar between treatments for roots, stems and totals at ~64% (range 54-70%).

A summing of ^{15}N recoveries in leachate, KCl-extractable soil nitrate (0-30 cm) and plant material showed that recovery of urine-N at 30 days was around ~38% and not significantly different between treatments. However, by 75-days, recoveries were greater overall for oats 10°C chamber treatments although generally the ^{15}N recovery did not exceed 50% (Table 6.3-3). Recoveries did not include any measurements of denitrification loss, soil organic-N or mineral-N resident in the gravel layer (30-50 cm).

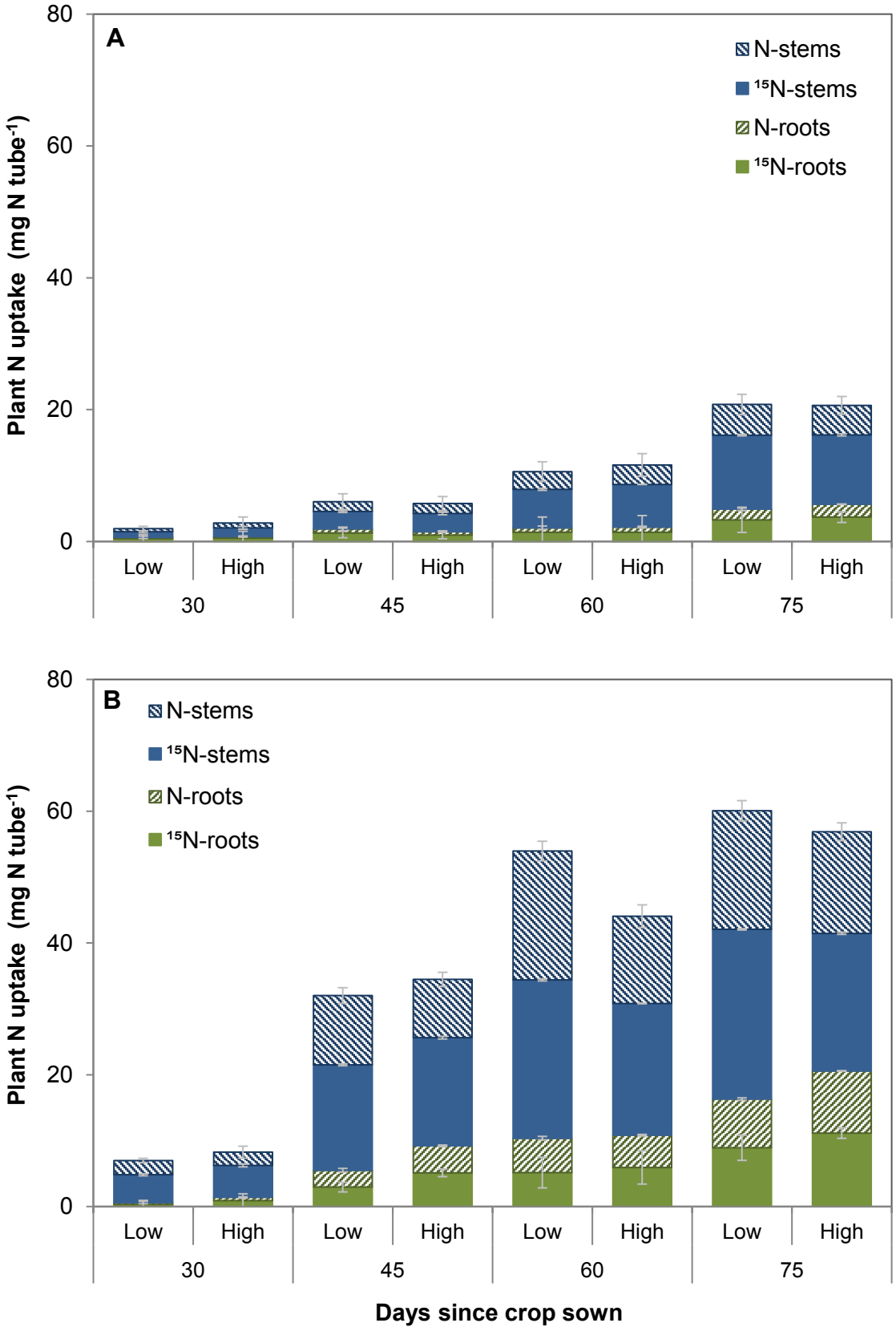


Figure 6.3-10. **Total N uptake (^{14}N and ^{15}N) in oats' stems and roots from 30, 45, 60 and 75 days after sowing, for two temperatures, 6° or 10°C, and two solar radiation levels, mid-winter (low) or mid-spring (high). Standard error bars shown (±1 SE).**

Table 6.3-2. Dry-matter yields, total-N and ^{15}N uptake and N concentration (%) per tube of oats stem and roots after 30, 45, 60 and 75 days from sowing.

	Treatment		*N content (mg N)*				*^{15}N uptake (mg N)*				*N%*				*Dry-matter (g)*			
Component	Temp	Light	30 d	45 d	60 d	75 d	30 d	45 d	60 d	75 d	30 d	45 d	60 d	75 d	30 d	45 d	60 d	75 d
Roots	6°C	Low	0.6	1.9	2.1	4.9	0.4	1.3	1.4	3.3	3.6	3.6	2.4	2.8	0.02	0.05	0.09	0.17
		High	0.8	1.5	2.2	5.6	0.5	1.0	1.4	3.7	3.8	3.8	2.6	3.1	0.02	0.04	0.09	0.18
	10°C	Low	0.6	5.5	10.4	16.3	0.3	2.9	5.2	8.9	1.3	2.7	1.5	1.2	0.05	0.21	0.71	1.34
		High	1.4	9.2	10.8	20.6	0.9	5.1	5.9	11.2	2.1	2.7	1.3	1.2	0.06	0.34	0.83	1.79
		Temp	ns	***	***	***	ns	***	***	***	**	***	***	***	***	***	***	***
		Light	ns	**	ns	*	ns	***	ns	*	ns	ns	ns	ns	*	**	ns	***
		Temp x light	ns	***	ns	ns	ns	***	ns	ns	ns	ns	ns	*	ns	***	ns	***
		[a] LSD (5%)	1.1	1.2	1.3	3.0	0.8	0.6	0.8	1.9	1.6	0.5	0.3	0.2	0.01	0.05	0.09	0.16
Stems	6°C	Low	1.3	4.1	8.5	15.9	0.9	2.6	5.8	11.2	5.4	5.4	5.8	5.1	0.03	0.08	0.15	0.31
		High	2.0	4.3	9.4	15.0	1.3	2.8	6.5	10.5	5.5	5.5	5.7	5.4	0.04	0.08	0.17	0.28
	10°C	Low	6.4	26.5	43.6	43.8	4.2	16.0	24.0	25.8	5.6	5.4	3.0	1.4	0.12	0.49	1.47	3.07
		High	6.9	25.3	33.2	36.3	4.8	16.4	20.0	20.9	5.1	4.6	2.2	1.3	0.14	0.55	1.51	2.85
		Temp	***	***	***	***	***	***	***	***	ns	**	***	***	***	***	***	***
		Light	ns	ns	***	*	*	ns	ns	*	ns	**	**	ns	ns	ns	ns	ns
		Temp x light	ns	ns	***	ns	ns	ns	*	ns	**	***	**	*	ns	ns	ns	ns
		[a] LSD (5%)	1.1	6.4	3.1	5.2	0.7	3.5	2.5	3.5	0.3	0.3	0.3	0.3	0.02	0.12	0.15	0.45
Total	6°C	Low	2.0	6.0	10.6	20.8	1.3	4.0	7.2	14.5	-	-	-	-	0.04	0.13	0.23	0.49
		High	2.8	5.8	11.6	20.7	1.8	3.7	7.9	14.2	-	-	-	-	0.06	0.12	0.25	0.46
	10°C	Low	7.0	32.0	54.0	60.1	4.5	19.3	29.2	34.7	-	-	-	-	0.16	0.69	2.18	4.41
		High	8.2	34.5	44.1	56.9	5.7	21.5	25.9	32.0	-	-	-	-	0.20	0.89	2.34	4.64
		Temp	***	***	***	***	***	***	***	***					***	***	***	***
		Light	ns	ns	**	ns	ns	***	ns	*					*	ns	ns	ns
		Temp x light	ns	ns	***	ns	ns	***	ns	ns					ns	ns	ns	ns
		[a] LSD (5%)	2.1	7.4	3.8	7.7	1.4	0.6	3.0	1.9					0.03	0.16	0.23	0.45

[a] Least significant differences at temp x light level (5% level); [b] insufficient sample present.

Table 6.3-3. ^{15}N contents in drainage, KCl-extractable nitrate-N and plant material, 30, 45, 60 and 75 days after sowing of oats (45, 60, 75 and 90 days after ^{15}N-labelled urine application).

Treatment			*Nitrate-^{15}N leached (mg N)*				*Soil ^{15}N-nitrate (mg N)*				*Plant-^{15}N (mg N)*				*Total-^{15}N (% of applied)*			
Temp	**Light**	**Crop**	**30 d**	**45 d**	**60 d**	**75 d**	**30 d**	**45 d**	**60 d**	**75 d**	**30 d**	**45 d**	**60 d**	**75 d**	**30 d**	**45 d**	**60 d**	**75 d**
6°C	Low	Fallow	0.0 a	0.4 a	2.7 b	8.7 b	25.9 b	18.9 cd	11.4 c	8.1 d	-	-	-	-	38 a	28 a	20 b	24 ab
		Oats	0.1 a	0.4 a	1.9 ab	2.4 a	27.0 b	24.4 de	10.8 c	4.2 c	1.3 a	4.0 a	7.2 a	14.5 a	41 a	42 b	29 cd	31 bc
	High	Fallow	0.0 a	0.2 a	1.8 ab	3.0 a	22.8 ab	13.7 bc	20.0 d	12.4 e	-	-	-	-	33 a	20 a	32 d	22 a
		Oats	0.0 a	0.1 a	0.7 a	0.8 a	25.2 b	21.9 de	9.3 bc	5.4 c	1.8 a	3.7 a	7.9 a	14.2 a	39 a	37 b	26 c	30 b
10°C	Low	Fallow	0.1 a	1.5 b	10.3 c	23.0 c	26.8 b	26.1 de	6.2 b	2.0 b	-	-	-	-	39 a	40 b	24 bc	36 c
		Oats	0.1 a	0.1 a	0.1 a	0.1 a	19.7 a	10.8 ab	0.0 a	0.0 a	4.5 b	19.0 b	29.2 c	34.7 b	35 a	43 b	42 d	50 d
	High	Fallow	0.1 a	0.5 a	2.8 b	8.3 b	26.8 b	27.3 e	7.6 b	8.6 d	-	-	-	-	39 a	40 b	15 a	24 ab
		Oats	0.3 a	0.4 a	0.4 a	0.4 a	20.9 ab	6.0 a	0.0 a	0.0 a	5.7 b	21.5 b	25.9 b	32.0 b	39 a	40 b	38 d	47 d
		Crop	ns	**	***	***	ns	***	***	***	-	-	-	-	ns	***	***	***
		Temp	ns	**	***	***	ns	ns	***	***	***	***	***	***	ns	***	**	***
		Light	ns	**	***	***	ns	ns	**	***	*	ns	ns	ns	ns	ns	ns	**
		Crop x temp	ns	**	***	***	**	ns	ns	ns	-	-	-	-	ns	**	***	*
		Temp x light	ns	ns	**	*	ns	***	*	ns	ns	ns	*	ns	ns	ns	***	***
		Crop x light	ns	*	***	***	ns	ns	***	***	-	-	-	-	ns	ns	*	ns
		Crop x temp x light	ns	**	***	***	ns	ns	**	ns	-	-	-	-	ns	ns	ns	ns
		[a] LSD (5%)	0.3	0.4	1.8	3.0	5.8	6.0	2.9	1.8	1.4	3.9	3.0	5.1	8	8	5	6

[a] LSD –least significant difference at 5% level of crop x temp x light; means within one urine application date followed by a letter in common are not significantly different according to Duncan's multiple range test ($P < 0.05$).

6.4 Modelling oats N uptake

Nitrogen uptake for both total-N and labelled ^{15}N content for the oats was modelled using a generalised linear mixed (GLM) model (Figure 6.4-1). The model used degree days and drainage as the fixed variables to produce the following equations:

$$^{15}N\ uptake = Degree\ days \times 0.062 - Drainage \times 0.064$$

6.4-1

$$N\ uptake = Degree\ days \times 0.11 - Drainage \times 0.173$$

6.4-2

where: ^{15}N uptake and N uptake are the total amount of N taken up from the ^{15}N-labelled urine and soil-N, respectively (mg); degree days is the cumulative number of days multiplied by the average soil temperature (>0°C) and drainage is the cumulative depth of drainage (mm) after 30,45, 60 and 75 days.

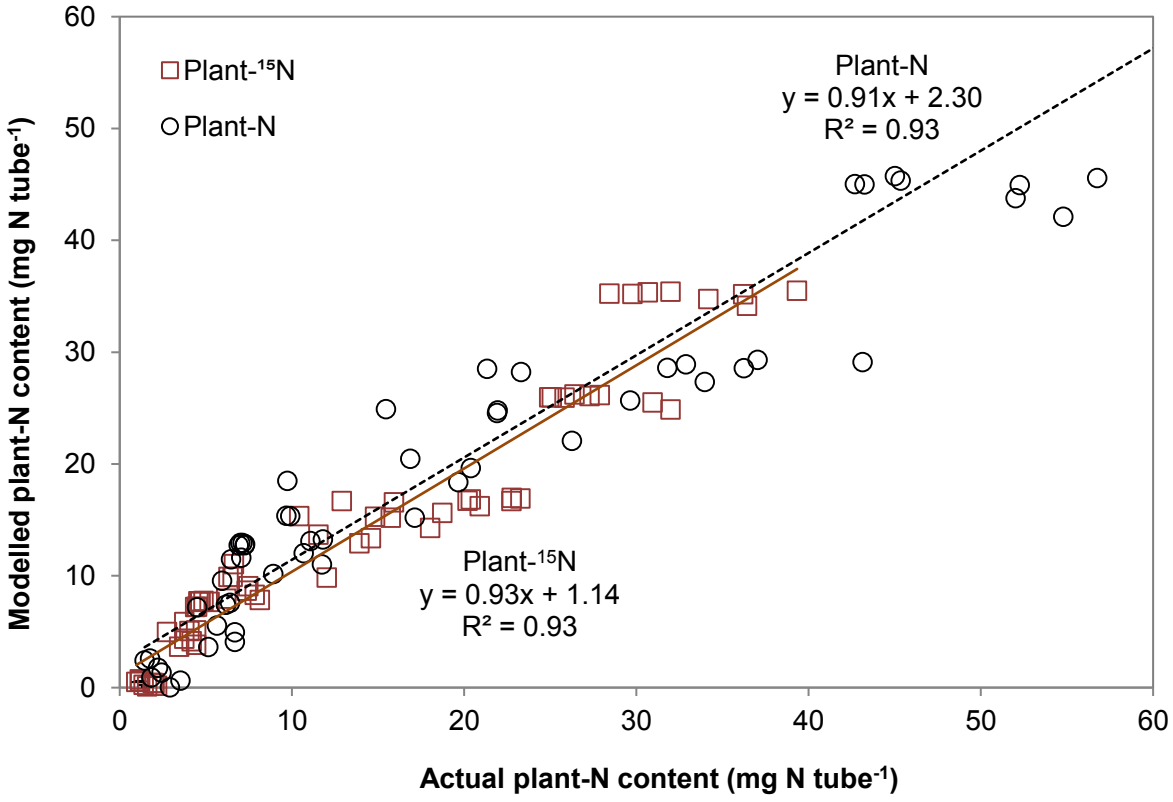


Figure 6.4-1. Actual and fitted GLM model data for total plant N and ^{15}N uptake from 30-75 days after sowing. Fixed variable degree days (days x average soil temp;°C) and drainage (mm).

6.5 Discussion

6.5.1 Drainage conditions

Drainage and N leaching losses in this experiment were invariably influenced using repacked soils rather than intact soil monoliths. Movement of nitrate down a soil profile occurs through a combination of solute transport processes, namely matric flow, preferential and bypass flow (see section 2.4.4). However, in a repacked soil it is reasonable to assume most drainage occurs through matric flow as large continuous pores and channels are largely absent. Although soil physical conditions for the experiment ensured free-drainage, the total drainage depth of ~120 mm for the fallow treatments was about 50% of water applied (~250 mm) whereas an average Lincoln winter would normally produce ~220 mm (~85%) drainage for a free-draining soil at field capacity (Lilburne et al. 2010). This difference is probably because air temperature conditions within the chambers were set to maintain soil temperatures, not air temperatures, and therefore more evaporation occurred from the soil surface than had been anticipated. Had the tubes been held under the conditions of a typical Lincoln winter diurnal air temperature environment, drainage would very likely have been greater. Nevertheless, sufficient consistent drainage occurred to enable study of the scale and size of the main factors involved in retention of nitrate with respect to the growing of the catch crop.

6.5.2 Effects of sowing oats on nitrate leaching

Sowing of the oats, particularly in the 10°C chamber, had such a strong evaporative demand on soil moisture that by 60-days, drainage ceased from the oats treatments altogether despite water application equivalent to the 75th rainfall percentile for Lincoln. The ability of oats to reduce drainage loss, even at a time when plant development appears slight, was also noted in experiments 1 and 2 (Chapter 4 and Chapter 5, respectively) and is undoubtedly due to the ability of the oat plants to remain active at low temperatures. Compared to pastures, oats can convert solar radiation to vegetative growth more efficiently over cool periods provided enough thermal time has elapsed for the leaf area of oats to increase sufficiently to intercept it (Noble 1972). With this achieved, oats can respond and grow quickly under Canterbury conditions as soil temperatures warm in response to the approaching spring (Martini et al. 2009).

The presence of a relatively large pool of nitrate within the repacked soil tubes, presumably derived from SOM mineralisation, meant that N leaching losses began immediately after the commencement of water application. Nitrate leaching from the ^{15}N-labelled urine was not detected until 60 days after application and then only in small amounts in the fallow treatments. Despite the unintended presence of a large resident nitrate concentration in the soil, oats in the 10°C chamber started to reduce nitrate leaching loss compared with fallow treatments after only 30 days from sowing (45 days after urine application) with the effect initially greater for the high-light treatments. This is attributable to a combination of reduced drainage and rapid N uptake by the oat plants. Indeed, in the period from 30-45 days after sowing, N uptake in the 10°C oats' roots and stems increased four-fold coinciding with an even more rapid growth in root length and mass, particularly in high-light treatments (Table 6.3-2). This rapid uptake effectively accounted for virtually all the ^{15}N-labelled urinary-N leaving very little urinary-N to leach. Consequently, oats sown early enough after urine application have the potential to capture N at the application rate used of 350 kg N ha^{-1}. In contrast, when the soil is fallow, the potential for a large nitrate leaching loss is equally high due to the high AOB activity.

A preliminary experiment conducted on 10 cm cores of the same soil incubated at 6°C and 10°C, respectively, found that complete nitrification of the applied urinary-N occurred by 42 days at 10°C but this did not occur till after 56 days at 6°C (Appendix F). Whilst these differences in nitrification rates might not appear large they are important windows in terms of nitrate leaching losses because they coincide with the 10°C 30-45-days rapid growth phase. For a catch crop well supplied with N, more than 1000 kg DM ha^{-1} can be grown in a two week period of active growth and thus, at 3-4% N content, 30-40 kg ha^{-1} can be removed from the soil (Vos & van der Putten 1997). In this experiment, growth rates in the 10°C chamber were up to 5-times that; consequently, N uptake in the period from 30-60 days after sowing was equivalent to ~220 kg N ha^{-1} of which ~120 kg N ha^{-1} was from the ^{15}N-labelled urine. Whilst the growth of oats was undoubtedly slower in the 6°C chamber, nitrate leaching losses after 75 days and total ^{15}N-nitrate losses were not that dissimilar to their 10°C counterparts, particularly at the higher light intensity. This was surprising as it might have been expected that slower crop growth would leave the built-up nitrate at a greater risk of leaching. Although more mineral-N did remain within the soil of the 6°C oats treatments by the end

of the experiment (90 days after urine application), this only amounted to ~10% of the ^{15}N-labelled urine, and although some residual nitrate may also have resided in the bottom layer gravels, the sum of both wouldn't account for such a large difference. N uptake in the same 30-60 days period after sowing as for the 10°C treatments shows a modest uptake of only ~50 kg N ha^{-1} (~30 kg ^{15}N ha^{-1}). The reduction in nitrate leaching in the 6°C oats treatments from 60 days after sowing is, therefore, probably attributable to several factors:

1) AOB abundance numbers built up more slowly, and persisted for longer, in 6°C treatments than at 10°C, indicating a slower nitrification rate and build-up in nitrate-N concentrations, leaving less to leach over the early drainage period,
2) Significantly higher N concentrations within the plant stems and roots by 60 days after sowing partially compensated for the reduced plant growth rates in the 6°C treatments, and
3) Less drainage, especially at the higher light intensity.

AOB abundance built and declined more slowly in the 6°C treatments indicating a slower rate of nitrification than for the 10°C treatments. This may offer some confidence that the window to retain urinary-N is longer than thought. Both Cookson et al. (2002) and Flowers and O'Callaghan (1983) found that nitrification rates for incubated clover material and ammonium sulphate-amended pig slurry at 4°C and 5°C, respectively, were approximately half those at 10°C whilst Di and Cameron (2004c) found that the half-life of NH_4^+-N in a urine incubation experiment was 44 days at 8°C but half that at 20°C. Although there is some evidence to suggest that the root systems of monocotyledons may be more sensitive to temperature in their N accumulation characteristics compared with, for example, crucifer species (Laine et al. 1994), the rapid development of cereal root systems means that they do have a capacity to absorb N. However, at 6°C root systems may not develop fast enough in the first 60 days after urine application (45 days after sowing) to make a significant impact on N uptake. The last factor, lower drainage, may have partly been an artefact of reduced humidity control in the 6°C chamber increasing evaporative loss. Although this would reduce nitrate leaching loss more than might have occurred naturally, actual leaching losses of ^{15}N-nitrate from the 6°C treatments, 90 days after urine application, were still

only 1-13% of the applied urinary-N so there is still a considerable margin to retain urinary-N against leaching.

In conclusion, sowing oats at 6°C, despite its modest growth, was still an effective method to reduce nitrate leaching losses. Indeed, it is critical if oats are to be effective as a catch crop to establish them early, even while soil temperatures are cool, to take advantage of the rapid growth period that follows soil warming.

6.5.3 Oats development – relationship with temperature and solar radiation

Research in New Zealand from the 1970s and 80s investigated the yields and development of forage oats in several North and South Island regions (Taylor & Hughes 1979; Hughes et al. 1984). Traditionally, these crops have been sown in autumn but the patterns of development are relevant to winter-sown crops where the crop yield models, especially in the cooler southern regions, have several distinct development phases (discussed previously in section 2.6.3). A yield model of oats' development can be represented by a three-part progression. The first, measured in degree days, covers the period when the crop emerges after sowing but essentially the yield is zero. In the second part, yield builds proportionally with degree days (i.e. aggregated daily average temperature) and is largely controlled by soil temperature until coverage is sufficient to intercept a large proportion of the available solar radiation. In the final phase the model switches from one where yield is proportional to temperature to one controlled by the received photosynthetically-active radiation component (Figure 6.5-1) (Hughes et al. 1984).

Results from this experiment can be broadly represented by the dashed box shown in Figure 6.5-1 where the growth of the crop is largely temperature controlled but is transitioning, at least for the 10°C chamber, to one controlled by intercepted light. For example, at 6°C for 75 days, a period that would cover most of a Canterbury winter, degree-days are only approaching 450 and oats yield is not much past emergence so at this point intercepted radiation is not a major factor in its development. At 10°C and 450 degree-days, however, canopy cover is sufficiently developed that the intercepted radiation is stimulating yield and is represented by the front part of the box. In this experiment, whilst higher radiation did increase root DM, there was less of an effect on stem DM. This could be because of the more rapid depletion of nutrients, N particularly, and of moisture under

the high-light treatments, where DM gains were limited in the latter part of the yield curve over low light treatments. This significantly affected stem N uptake although N uptake overall (roots+stems) was very similar between 10°C light treatments. This is encouraging because it shows that low light treatments, typical of the levels found in mid-to-late winter, did not significantly hamper oats N uptake. Given conditions less physically and nutritionally limiting, the N content in the stems of the 10°C high-light oats may well have been higher.

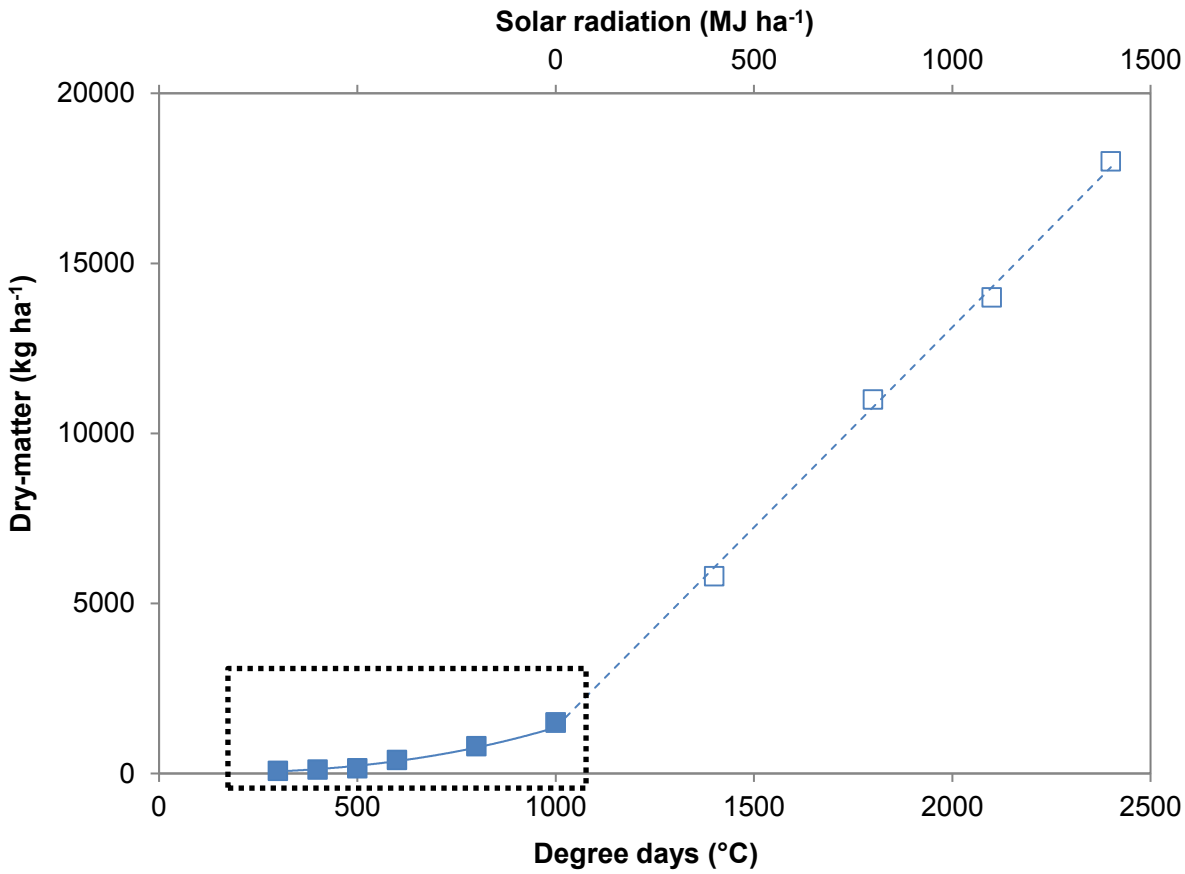


Figure 6.5-1. Representative cool season forage oats yield curve (after Hughes et al. (1984)). Box represents experimental period covering 6 ° and 10 ° C oats development.

6.5.4 Modelling N uptake

The GLM models used to predict N uptake for both total-N and ^{15}N uptake were simple and based on only two variables, degree-days and drainage. These were well correlated (R^2>0.9) with actual N uptake data but given the controlled environment this is not surprising. Field N uptake models based around catch crops are invariably more complicated as they are designed to deal with crop rotations and the residual soil mineral-N left over from the harvested crop and/or the mineralisation

of crop residues (Thorup-Kristensen et al. 2003). Water balances must also be considered if there is no direct measurement of drainage. In the growth chamber situation there is a defined pool size of N applied, and soil volume and drainage are easily measured. Nevertheless, there is some opportunity to predict N uptake in a field catch crop and thereby, infer what N has been removed from the soil mineral-N pool.

6.5.5 Relationship with field research reported in earlier chapters

This highly controlled growth chamber experiment provided further insight into the field experiments and where these succeeded or failed to mitigate nitrate leaching. N uptake in the oats and Italian ryegrass catch crops from the ^{15}N-labelled urine in field experiment 1 (Chapter 4) was low, amounting to only ~4% of that applied. The sowing of both in a soil with relatively low AWC, in the case of oats, seven weeks after urine application, and the Italian ryegrass, another four weeks later, failed to make an effective mitigation through N uptake. Nevertheless, nitrate leaching was lower in the field oats treatments than the Italian ryegrass treatments due to lower drainage. This was a fundamental, and possibly discounted part of mitigating nitrate leaching after winter forage grazing. Even at 6°C, this experiment has shown that oats emergence can reduce nitrate leaching loss, despite low yields, simply through increased evapotranspiration. Of course, soil temperatures go through minima around July and increase again into August, so maintaining soil temperatures at 6°C is somewhat artificial and it may be that the growth chamber conditions enhanced the reduction in drainage. Nevertheless, sowing the oats during this period appears critical to its success as a catch crop. Once the crop has emerged and soil temperatures warm, the crop is well placed to capture resident soil mineral-N. This was shown in the second field experiment (Chapter 5) where the planting of oats as soon as one day after urine application was effective in capturing N and reducing nitrate leaching, albeit over a warmer, drier winter.

6.6 Conclusions

Sowing oats in urine-treated tubes, in both 6° and 10°C chambers, proved highly effective in reducing nitrate leaching losses by around one-third and three-quarters, respectively. The factors, however, controlling N uptake and subsequently, nitrate leaching, differed between the 10°C and 6°C chambers. Urine applied to fallow treatments in the 10°C chamber increased AOB abundance rapidly, with nitrification complete by 42 days, whilst slower rates were observed in the 6°C fallow treatments, meaning less N was actually lost for the 6°C fallow treatments after 90 days. Oats treatments germinated and grew rapidly at 10°C, lowering nitrate leaching loss through N uptake and reduced drainage. At 6°C, plant growth and N uptake rates were less than at 10°C but a slower increase in AOB abundance meant nitrification rates were also slower, reducing the rate of soil nitrate accumulation. Decreases in AOB abundance were associated with falls in soil nitrate concentration due to leaching or N uptake and fell more slowly for the 6°C treatments. By 75 days, N concentrations in the roots and stems of the 6°C oats treatments were much higher that at 10°C and this appeared to assist in reducing nitrate leaching loss from the 6°C crop treatments over the experimental period, despite much lower DM growth rates. Although residual soil-N remained higher in the 6°C oats treatments by the end of the experiment (75 days after sowing), this point also coincides with a period of increasing soil temperature when oats undergoes a rapid development period, increasing N uptake.

The main effect of lighting intensity on treatments, in both 6 ° and 10°C chambers, was on evaporative water loss where higher lighting intensity produced a smaller amount of drainage and consequently, reduced nitrate leaching loss. This effect was especially evident in the oats treatments of both chambers where increased transpiration contributed to the decrease in drainage. The direct effect of higher lighting intensity on oats development was observed only in the 10°C chamber where it produced an increased rate of root development and root N uptake, resulting in soil mineral-N concentrations declining more rapidly in the 30-45 days period after sowing.

Chapter 7 General Discussion and Conclusions

"A summary of the main conclusions from this research programme and judgement of the potential effectiveness of a winter-sown catch crop to limit nitrate leaching under winter forage grazing".

7.1 General discussion

7.1.1 Field lysimeter experiment 1

Field lysimeter experiment 1 was designed to compare the relative performance of two potential catch crops, oats and Italian (It.) ryegrass, to capture ^{15}N-labelled urine applied after a simulated winter forage grazing event. The three hypotheses associated with this experiment were:

1. *That oats are a more effective catch crop than an It. ryegrass catch crop to reduce nitrate leaching losses in post-winter forage grazing systems,*
2. *That nitrogen leaching losses from single (350 kg N ha^{-1}) and double (2x 350 kg N ha^{-1}) urine applications post-winter forage grazing will be additive, and*
3. *That using a nitrification inhibitor (DCD) in conjunction with a catch crop will reduce nitrate leaching losses and increase N uptake from winter forage grazing compared with a non-DCD treated catch crop (control).*

Oats were sown in mid-August, 7 weeks after the first urine application and the It. ryegrass a further 4 weeks later as per recommendation. However, this was found to be too late and only 3-4% of the applied urinary-N was recovered in either crop. However, nitrate leaching losses were reduced under the oats crop by 25% over the winter-spring period compared with It. ryegrass. This difference was almost entirely due to the smaller amount of drainage from the oats treatments. **Hypothesis 1 -supported.**

A second application of urine a week later, at the same rate of 350 kg N ha^{-1}, increased nitrate- and inorganic-^{15}N leaching losses by 75% (range 68-81%) and 90% (range 81-108%), respectively. **Hypothesis 2 –supported.**

DCD applied a day after urine application reduced nitrate leaching losses on oats and It. ryegrass lysimeter treatments by 55% and 33%, respectively, and increased N uptake in the oats crop 3-fold (from ~4% to ~13%). **Hypothesis 3 -supported.**

7.1.2 Field lysimeter experiment 2

Experiment 2 specifically investigated the effect of the timing of the sowing of oats following urine application (1 day after, then at 21-day intervals) for three sequential winter applications (early June

to mid-July) on nitrate leaching and N uptake. This experiment tested the following three hypotheses:

1. *Sowing oats as a catch crop after simulated winter forage grazing will reduce nitrate leaching losses compared with a fallow treatment,*
2. *Nitrogen leaching loss will be greater from an early winter urine application compared to a later application date, and*
3. *Nitrogen uptake will increase, and nitrate leaching decrease, the sooner the oats catch crop is sown after winter forage grazing.*

The main outcomes from this experiment were:

- Nitrate leaching losses from a series of winter urine applications were reduced by around a third (~34%; range 19-49%) after the sowing of an oats catch crop compared to the fallow treatments. **Hypothesis 1 -supported.**
- The later the winter urine application date, the lower the nitrate leaching loss under the urine patch (range 170-270 kg N ha^{-1}). **Hypothesis 2 -supported**
- Earlier sowing of oats increased N uptake and decreased N leaching loss compared to later sowings. **Hypothesis 3 -supported**

7.1.3 Growth chamber experiment 3

Experiment 3 investigated more closely the interaction between soil temperature (6 ° and 10°C) and light intensity (winter vs spring light conditions) on the development of the oats plants and how this might affect N uptake and nitrate leaching using climate-controlled growth chambers. A further three hypotheses were tested:

1. *Nitrate leaching losses from a single urine application to soil will be lower under oats compared to soil left fallow.*
2. *Nitrate leaching losses from a single urine application to soil will be greater under a low temperature (6°C) regime compared with those under a high temperature (10°C) regime, due to lower N uptake by the oats crop.*

3. *Nitrate leaching losses from a single urine application to soil will be greater under a low solar radiation (mid-winter) regime compared with those receiving high solar radiation (mid-spring), due to lower N uptake by the oats crop.*

The main outcomes from experiment 3 were:

- After 75 days, the nitrate leaching losses from the fallow treatments, particularly those at 10°C, were large and up to 36% of the applied urinary-N. Sowing oats at 10°C substantially reduced both drainage values and nitrate concentrations, reducing the nitrate leaching loss to between 1-11% of the applied urinary-N. **Hypothesis 1 –supported.**
- Nitrate leaching loss at 6°C was lower than at 10°C for fallow treatments and was probably attributable to a slower nitrification rate and more N left resident within the soil. Sowing oats at 6°C, despite an order of magnitude lower DM production, reduced nitrate leaching losses to similar values observed for oats at 10°C, after 90 days. **Hypothesis 2 –not supported.**
- Higher light intensity increased evapotranspiration losses, reducing the amount of drainage. For the oats' treatments at 10°C, this also reduced the amount of drainage to less than a third of that in the fallow treatments. **Hypothesis 3 –supported.**

7.2 Final conclusions

This research programme has discovered that the use of catch crops, particularly oats, after winter forage grazing can significantly reduce nitrate leaching losses and increase N use efficiency within the South Island winter feed grazing system. Oats is a particularly useful catch crop because of its high cool season activity and vigorous growth habit that can maximise N uptake and reduce drainage under a Canterbury winter.

Sowing oats was shown to have the potential to reduce paddock N leaching loss by around 30% on a free-draining Balmoral soil.

7.3 Suggestions for future research

This study has highlighted several potential research areas for future investigation:

- There is a paucity of data around using catch crops to reduce N leaching losses from winter forage grazing. Therefore it is essential to conduct more experiments on different soils over a wider range of climatic conditions to assess whether the potential to reduce nitrate leaching, as shown in the lysimeter studies, can be realised more widely.
- There are several pragmatic issues around the sowing of the catch crop and how this might be done quickly and successfully given the large range of soil physical conditions that might be experienced in different regions on differing soil types. Some applied on-farm research is required here.
- DCD use has been voluntarily suspended in New Zealand; however, the research shows that a nitrification inhibitor can create an opportunity to supress nitrification whilst soil conditions improve sufficiently to enable sowing of a catch crop. Given there is no actual grazing or growing vegetation occurring when the chemical would be applied, there appears no impediment to its use. Initial investigative analysis suggests DCD applied in the post-grazing period is not taken up in the subsequent catch crop. A product that combines the oats' seed with DCD carried in an outer matrix might prove successful should DCD be approved again.
- One of the chief findings of this research programme was the reduction in drainage created by sowing the catch crop. In some regions irrigation is restricted over summer, therefore the water used by the oats crop might be essential for the subsequent crop. What are the strategies to capture urinary-N under those scenarios?
- Although oats are investigated here, other crops may also be viable and barley has shown some initial promise. Work around whether other cereals can offer similar or superior performance under the range of conditions experienced would be worth conducting.

Appendices

A. Site locations for Ashley Dene research station and Lincoln University

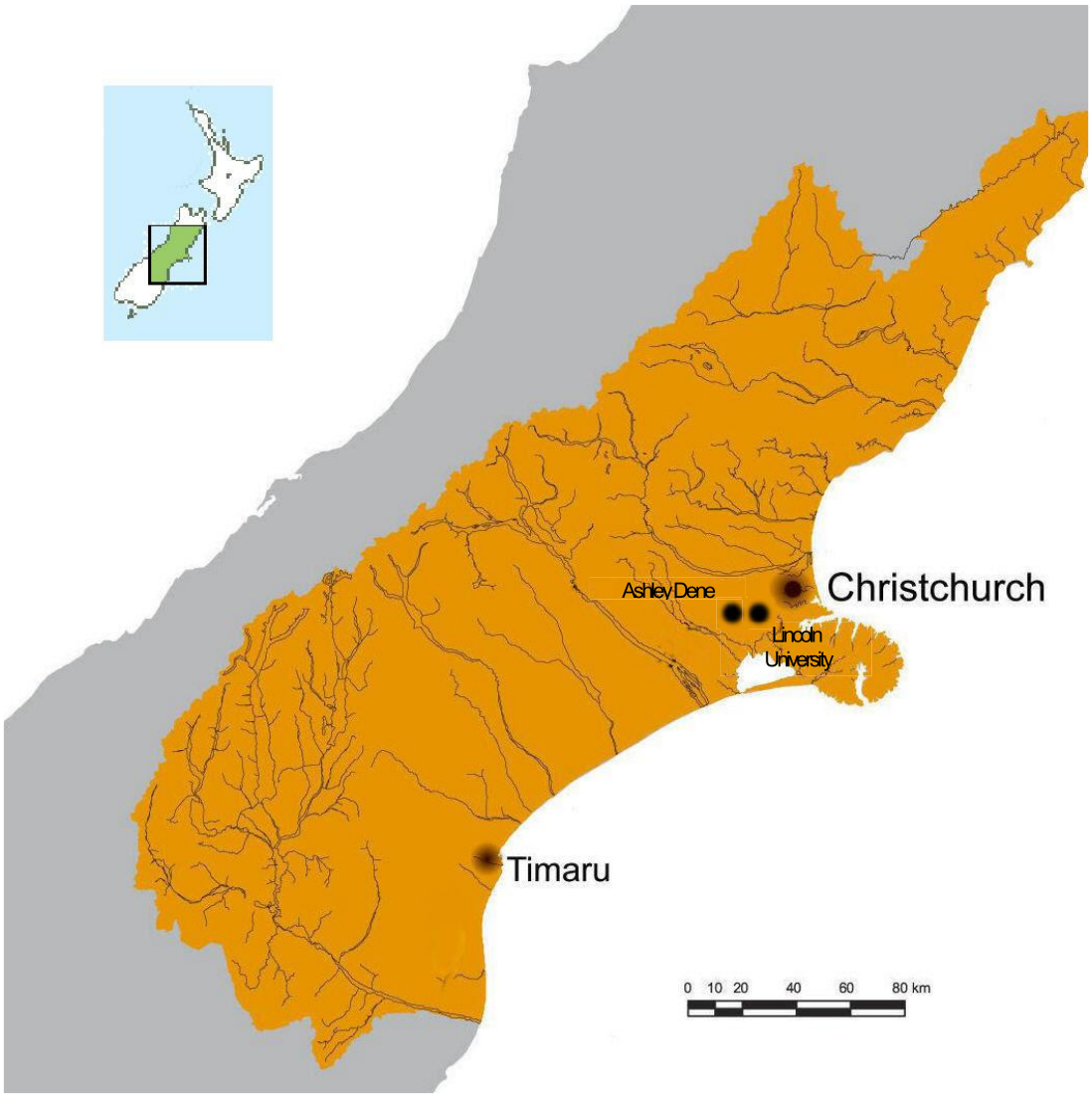


Figure A-1. Canterbury map showing approximate locations of Ashley Dene research station and Lincoln University field trench sites.

B. Soils and farm map of Ashley Dene research area

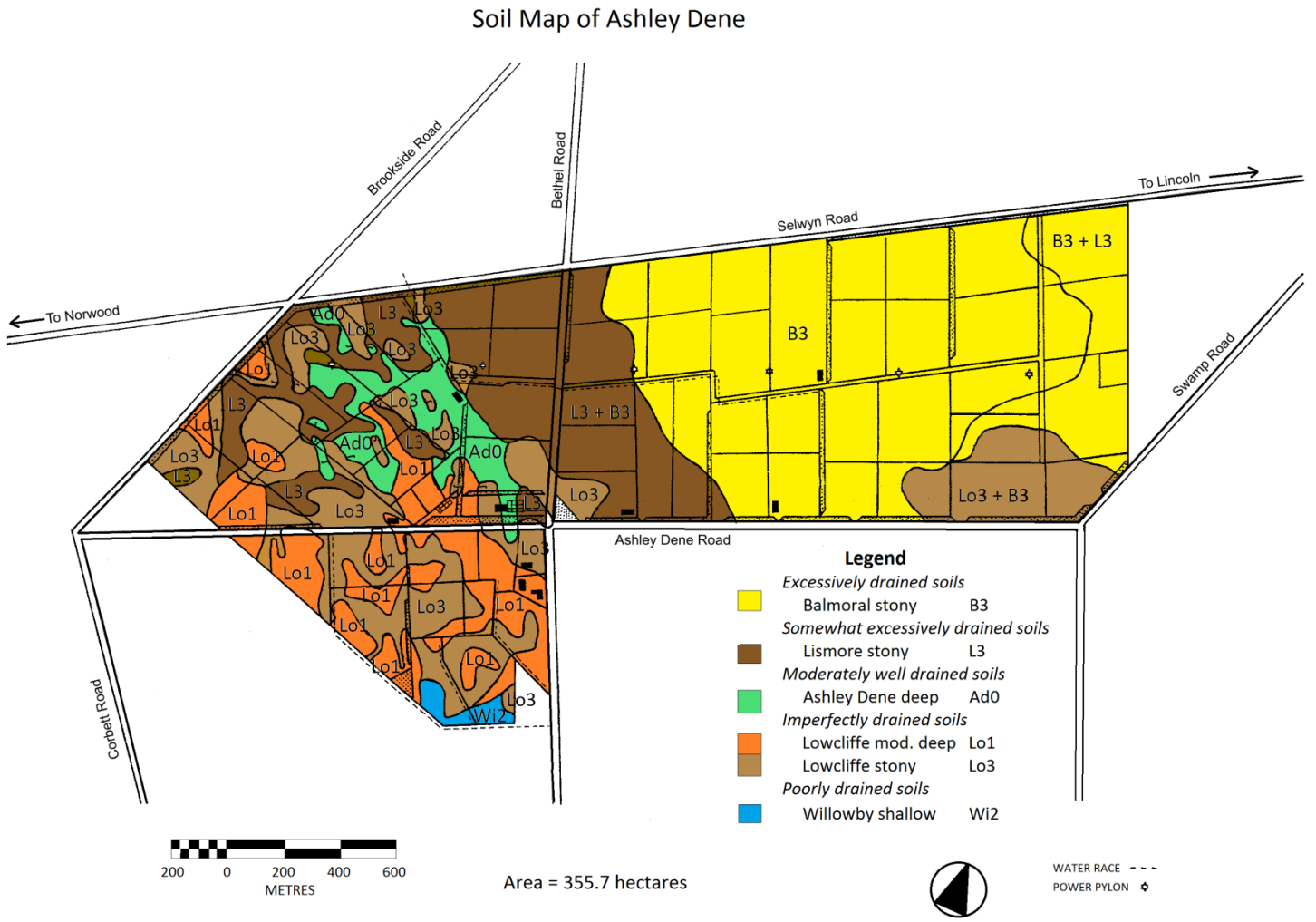


Figure B-1. Soil map of Ashley Dene research station and location of lysimeter collection site.

C. Field lysimeter treatments and placement (years 1 & 2)

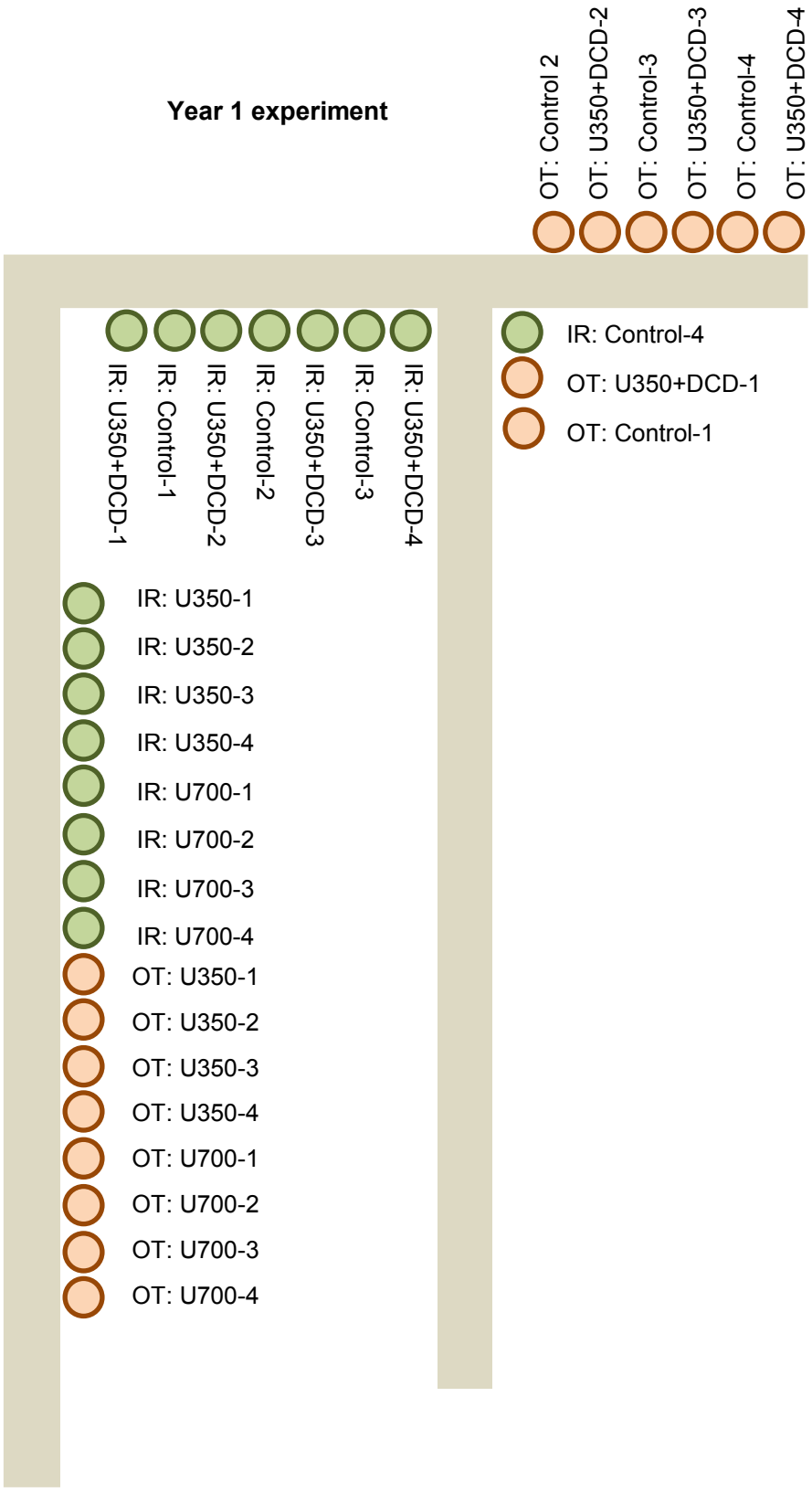


Figure E-1. Diagrammatic representation of lysimeters and treatments in the field trench in year 1

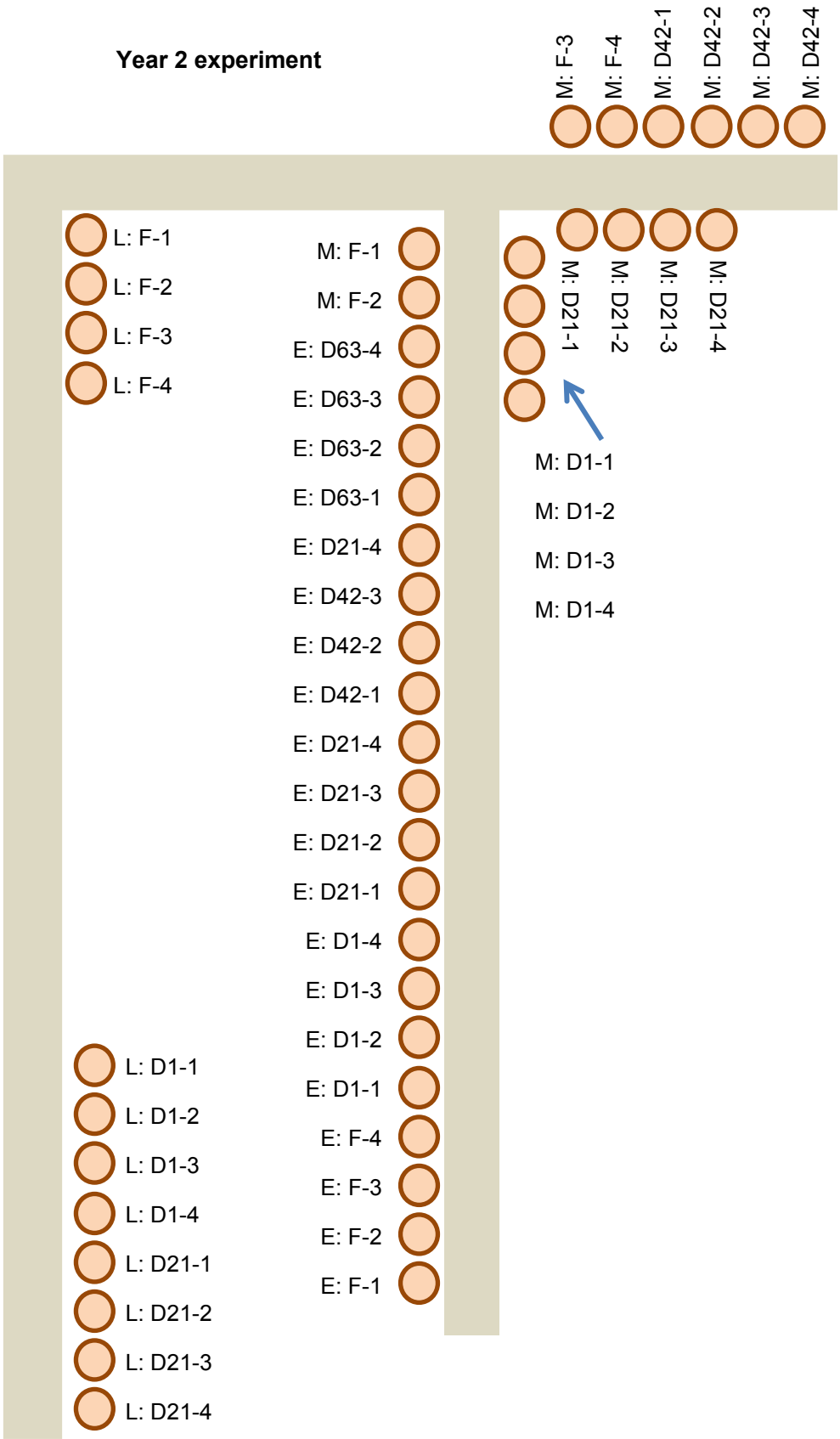


Figure E-2. Diagrammatic representation of lysimeters and treatments in the field trench in year 2.

D. Rainfall & irrigation simulation system

D.1 System design

The irrigation programme used in experiment 1 (Chapter 4) was set up to best simulate actual rainfall and irrigation events, particularly in terms of application frequency, intensity and rate. It also endeavours to generate sufficient drainage water during the winter/spring period to achieve a complete nitrate breakthrough curve. The system operates on predefined daily climate parameters derived from data collected by the NIWA Broadfield weather station, Canterbury, New Zealand. Daily average rainfall and evapotranspiration data to the 75th percentile between 1975 and 1999 are used as a basis for climate prediction, and around which the programme operates. The system was calibrated to apply water at a rate of 1000 ml per minute over the lysimeter area in 0.5 mm bursts (≅6 second burst).

Key definitions

Climate value: The daily seasonal requirement of water based upon previous climate data to the 75th percentile (rainfall and evapotranspiration) and leachate generation (Figure D.1; yellow line).

Climate accumulation line: The main reference line that is derived from accumulating daily climate values.

Target line: A line that randomly tracks within plus or minus 20 mm of the climate accumulation line to create variability and randomness around a constant reference. NB: This only occurs under rainfall simulation mode.

Tally: Accumulation of rainfall, simulated rain and irrigation.

Application: Amount of water to be applied by the system (mm).

D.2 Rainfall simulation mode (April to September)

At midnight (0:00) each day, the programme determines new climate accumulation and target values. The new climate accumulation value is calculated by the addition of the previous day's

climate accumulation value and the current climate value shown in Figure D-1. The new target is the addition of the climate value and previous target.

When the tally is less than or equal to the target, a new target is created by a random number generator, within the defined boundaries of the climate line. This new target may be below or above the tally. If the new target is less than the tally, no water is applied, and the system repeats the first operation each day until the condition of positive application is created. If the new target is greater than the tally, then the difference between the target and tally will be applied as simulated rain.

Figure D-2 illustrates the random nature of a) the target line around the climate accumulation line, and b) the event of simulated rain application. The concept of a randomly fluctuating target is to bring variability into the system which is a characteristic of an actual climate, and therefore no one season is replicated in the exact same way. The blue bars on the graph indicate randomly generated application amounts.

The application of simulated rain is done so by a randomly generated 'pulse pattern,' otherwise known as 'random intensity.' The rate of intensity (mm hr^{-1}) is weighted towards lower values, and the overall range of these possible values is weighted by the actual amount to be applied. Lower application amounts equal lower intensity range rates; higher application amounts equal higher possible range rates.

D.3 Irrigation mode (October to March)

The procedure for irrigation application is like that of the rainfall simulation methodology, however, the amount, frequency and intensity is defined by user-set variables. It uses the climate accumulation line as a reference point instead of the fluctuating target line and therefore application trends are more linear. This is set to simulate irrigation through a centre pivot.

In this trial, the irrigation regime was comprised of applications every three days, with single applications of 15 mm at an intensity of 20 mm per hour. In the event of rain, the time interval between applications was extended to remain on track with the climate accumulation line.

Figure D-3 illustrates actual data from the 2011/2012 trial during a proportion of time when the system was in 'irrigation mode.' Unlike Figure D.2 where there is variability around the target

amount and frequency, Figure D-3 illustrates the consistent pattern of applications when in 'irrigation mode,' which are typical of irrigation practice, and more specifically that of a centre pivot. Also, note that the target line (dotted red line) tracks the exact same path of the climate accumulation line.

D.4 Exceptions

All applications halt for real rainfall events and defined environmental conditions (e.g. wind speed greater than 3 m sec^{-1}). For rainfall simulation, the amount of real rainfall is deducted from the quantified application amount and added to the tally.

Further, the daily climate values (Figure D-1; yellow line) are subject to real climate conditions, and can be adjusted for either wet or dry years to achieve complete breakthrough curves.

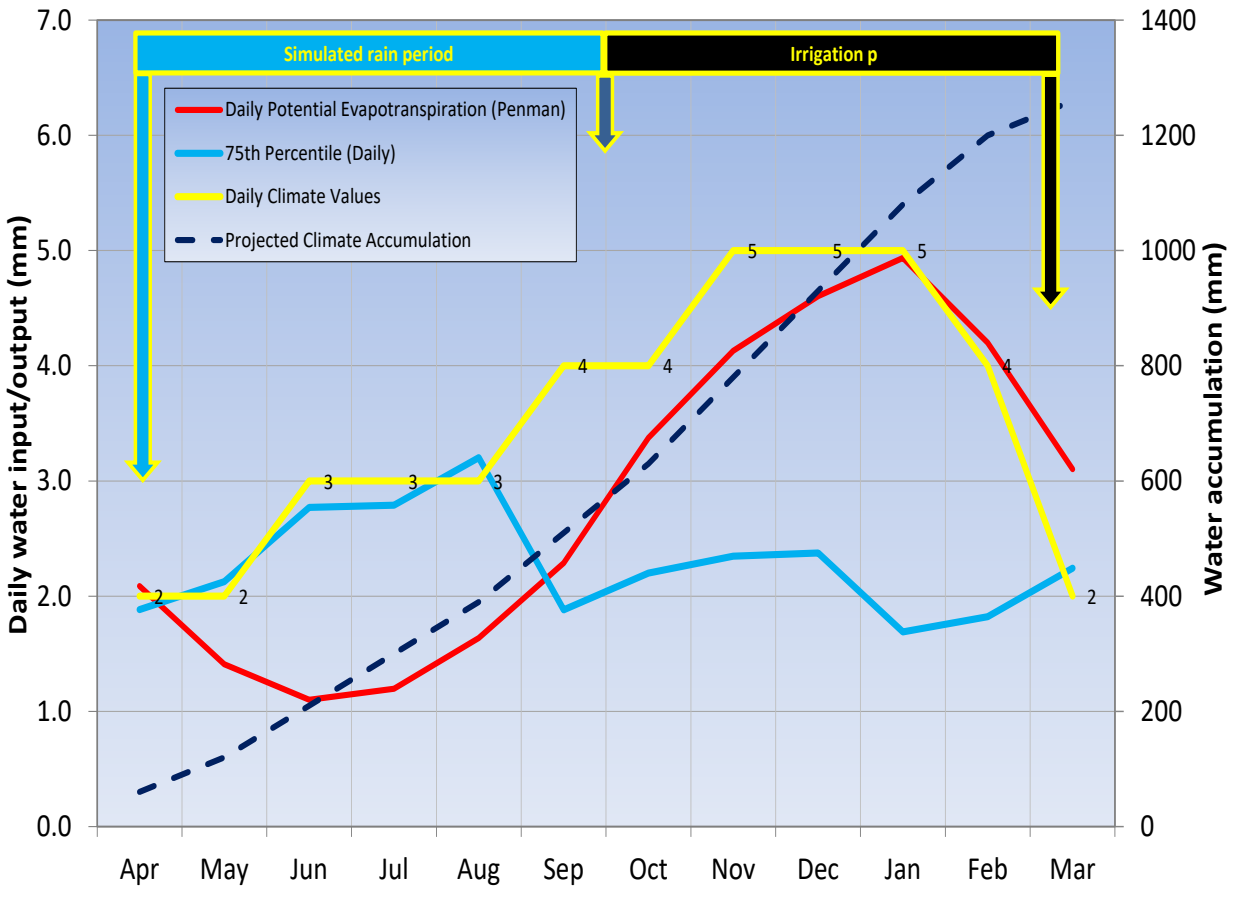


Figure D-1. Pictorial description of model controlling the automatic simulated rainfall and irrigation programme.

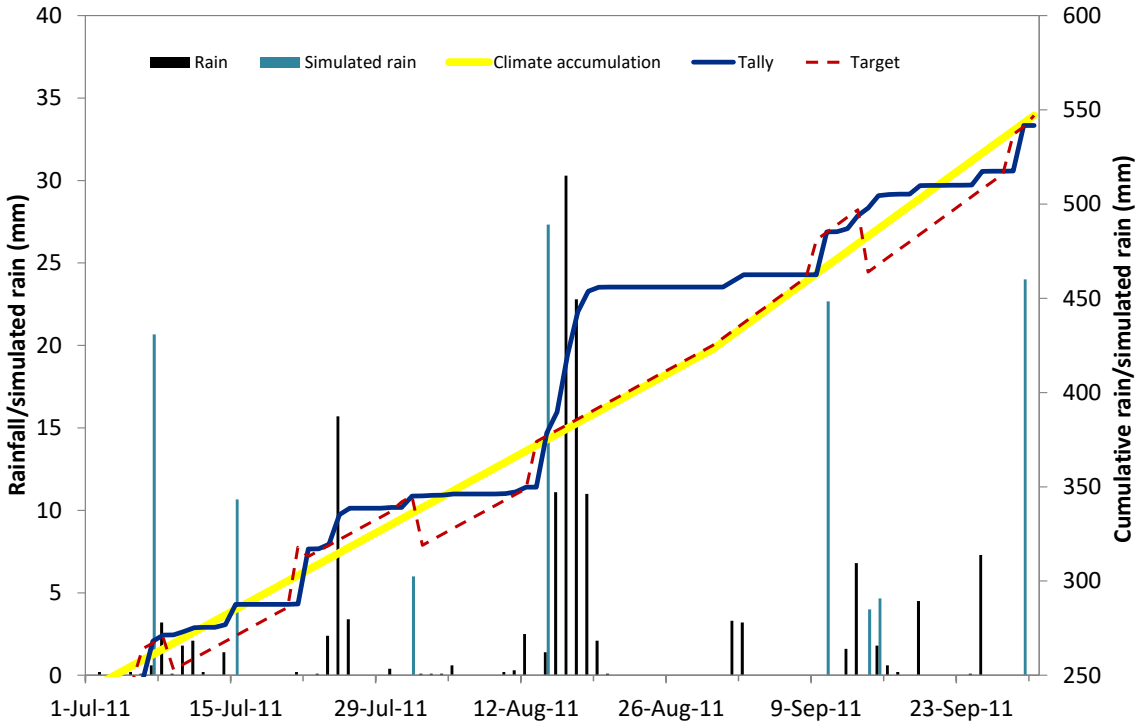


Figure D-2 Rainfall, simulated rain, tally, climate accumulation and target of the 2011/2012 lysimeter trial under 'rain simulation mode' between July and September.

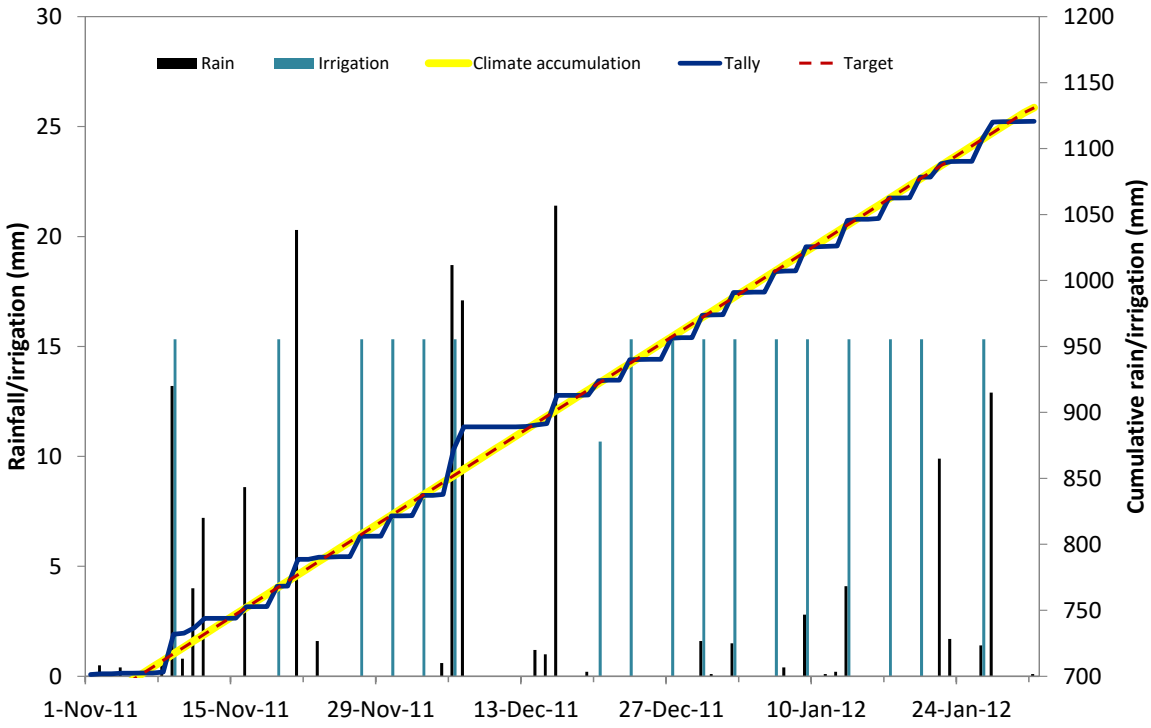


Figure D-3 Rainfall, irrigation, tally, climate accumulation and target of the 2011/2012 lysimeter trial under 'irrigation mode' between November 2011 and January 2012.

E. Methods of analysis

E.1 Leachate N content

Ammonium and Nitrate

Ammonium and nitrate-N concentrations in leachate samples were analysed by Flow Injection Analysis (FIA) (Gal et al. 2004). The analyser was a FOSS FIAstar 5000 triple channel analyser with SoFIA software version 1.30. Ammonium-N was analysed using a gas-diffusion membrane and sodium hydroxide to diffuse ammonia gas into an indicator stream that monitors a proportional change from red to blue at 590 nm due to the concentration of ammonium ions.

Nitrate-N was analysed by initial reduction of nitrate-N to nitrite-N using a cadmium reduction coil (OTCR – open tubular cadmium reactor). The nitrite-N was then reacted with sulphanilamide/NED to form an azo dye compound. The intensity of this compound is determined spectrophotomically at 540 nm. Adapted from FOSS Application Note AN 5206; FOSS Tecator AB, Hoganas, Sweden.

^{15}N-nitrogen

Leachate samples containing ^{15}N-labelled ammonium or nitrate (determined by FIA) were diffused following the procedures outlined in Brooks et al. (1989) in preparation for ^{15}N analysis by a mass spectrometer. The volume of leachate sample diffused varied depending on ammonium or nitrate-N concentration so that the total amount of N contained in 50 ml of solution was no more than 120 μg N (usually containing 5-50 μg ^{15}N-nitrogen).

The method uses 120-140 mL disposable specimen containers to hold the sample, and 7-mm diameter disks of acidified GF/D glass fibre filter paper on stainless steel wire as the acid trap. Where the leachate contained significant amounts of ammonium the solution first received magnesium oxide (MgO) to create basic pH conditions and convert the NH_4^+ ions present to NH_3 gas, allowing diffusion of the gas onto the acid disk. The first step required sealing the containers and sitting for 6 days at room temperature without shaking. The retrieval of nitrate required the addition of Devarda's alloy as the reductant to the MgO-treated solution to convert the NO_3^- to NH_4^+ and repeat the ammonia diffusion process. Once the initial ammonium diffusion step was completed the wires were removed and new wires and disks installed, or if no ammonium was

present the first step was skipped and the MgO and Devarda's alloy added together. The containers sat for a further 6 days.

^{15}Nitrogen enrichment in diffused leachate samples was analysed by a mass spectrometer. The analyser was a continuous flow isotope mass spectrometer, enabling measurement of stable isotopes at both enriched and natural abundance levels. The instrument was manufactured by Sercon Ltd, Crewe, CWI6ZA, UK. Solid samples were initially combusted at 1000°C in an oxygen atmosphere in an automated Dumas-style elemental analyser which was linked to a 20-20 stable isotope ratio mass spectrometer.

E.2 Herbage

Total nitrogen

Total nitrogen contents of dry pasture samples and urine were analysed using an Elementar Vario-Max CN Elemental Analyser (Elementar GmbH, Hanau, Germany).

The sample was combusted at 900°C in an oxygen atmosphere. The combustion process converts any elemental nitrogen into N_2 and NO_x. NO_x is subsequently reduced to N_2 so that all N present in the sample is in N_2 form. These gases are then passed through a TC (thermal conductivity) cell to determine the total amount of N_2.

^{15}Nitrogen

Herbage samples were analysed for ^{15}N content by mass spectrometer using the same solid sample combustion process outlined above.

E.3 Volatilization

Measuring N loss from volatilization was done by automating the monitoring of the airflow using a Campbell Scientific CR10x data logger with multiplexer and measuring the sampled air using Omega FLT 1000 (1.0-5.0 L/min) airflow sensors. The non-sampled air is measured using Omega FLR1203 (10-50 L/Min) sensors. Ambient air was continuously drawn through each enclosure at 0.41 L s-1 (approx. 17 air changes min-'). The flow from each gas enclosure was partitioned such that ~10% passed through an acid trap containing 50 ml of 0.05 M H_2S0_4 whilst the remaining flow

was vented. Solution from each trap was changed daily with any evaporative loss replaced prior to collection (Black et al. 1985a). The solutions were stored in 50 ml plastic bottles and frozen until the ammonium content of the solutions could be determined by FIA by Analytical Services at Lincoln University as described in section E.1

E.4 Lysimeter soil sampling and bulk density determination

Measurement of soil bulk density and sampling of the lysimeters for experiment 1 was done by deconstructing the lysimeters as outlined in chapter 4. Once the first increment was exposed after winching down the lysimeter casing, the vaseline-coated soil on the outside was cut away into a tared bin, then weighed and discarded. The remaining soil was levelled down to the top of the lysimeter and removed into another tared bin. Any pasture or kale root mass present was removed after being shaken to remove loose soil, bagged and stored in a cool store at 4°C. Soil in the bin was sorted and major stones removed and then soil and stone weighed separately. The remaining soil was mixed and a 3-kg sample removed to a drying tray. A further moist sample of soil of about 1 kg was removed and stored at 4°C for soil mineral-N analysis. The soil on drying trays was placed in a forced air-dryer at 30°C and once air dry, the soils were weighed again and sieved to remove any stone greater than 5 mm. The stone fraction was then weighed and discarded whilst the remaining soil was stored until required for ^{15}N analysis.

Root mass was washed by hand with a forced jet wash on a 0.5 mm sieve and the recovered root material dried in an oven at 60°C until ground for analysis using the Retsch mill (<0.5 mm). Dried soil was finely ground for ^{15}N soil analysis using a Rocklabs ring mill pulveriser (Dunedin, NZ). From the weighed soil and stone fractions, the calculation of dry soil bulk density for each soil lysimeter layer was determined.

E.5 Soil mineral-N analysis

Soils from the deconstruction of the soil monoliths were extracted for soil mineral-N after approximately 2 weeks in storage using a 10 g moist sample and 50 ml 2M KCl as per the method outlined by Blakemore et al. (1987). The filtrate was frozen until required for nitrate and ammonium analysis by FIA using the procedure in E.1.

E.6 Ammonia oxidising bacteria (AOB) abundance

Bacterial and archaea amoA gene abundances were quantified using real-time quantitative PCR (qPCR). Total soil genomic DNA was extracted from 0.4 g soil (fresh weight) using MoBio Power-soil™ DNA isolation kits (MoBio Laboratories, GeneWorks, South Australia, Australia) as per the manufacturer's instructions. Concentration and quality of the extracted DNA were estimated using Quant-iT ™ dsDNA BR assay kit on a Qubit fluorometer (Life Technologies, Auckland, New Zealand) and NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Montchanin, USA). All DNA extractions were diluted 1:10 with nuclease-free DI water to reduce potential PCR inhibition. The PCR primer pair amoA1F and amoA2R (Rotthauwe et al. 1997), and the primer pair Arch-amoAF and Arch-amoAR (Francis et al. 2005) were used to amplify regions of the bacterial and archaea amoA genes, respectively (Table C.5-1). A typical 16 µL reaction contained 8.0 µL of SYBR®Premix Ex Taq™ (TaKaRa, Norrie Biotech, Auckland, New Zealand), 0.4 µL of each primer (250 nM final concentration), 1.5 µL of template DNA and nuclease-free DI water to the final volume. All reactions were set up using the CAS-1200 Robotic liquid handling system (Corbett Life Science, BioStrategy, Auckland, New Zealand) and real-time qPCR analysis was performed on a Rotor-Gene™ 6000 real-time rotary analyser (Corbett Life Science, BioStrategy, Auckland, New Zealand). Fluorescence intensity was measured at the end of each extension step at 72°C. To confirm specificity of the reaction, a melting curve analysis was performed at the end of each run and melting peaks from the analysed samples were compared with the melting peaks of standards. The standards used in the qPCR analysis were prepared by cloning respective bacteria and archaea amoA gene amplicons into the pGEM-T Vector (Promega, In Vitro Technologies, Auckland, New Zealand) and subsequently transforming into JM109 High Efficiency Competent Cells (Promega, In Vitro Technologies, Auckland, New Zealand) per the manufacturer 's recommendations.

Plasmids were subsequently extracted from over-night cultures using PureLink™ Quick Plasmid Miniprep Kit (Life Technologies, Auckland, New Zealand) and concentration and quality of the extracted plasmid DNA were estimated using Quant-iT ™ dsDNA BR assay kit on a Qubit fluorometer (Life Technologies, Auckland, New Zealand) and NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Montchanin, USA). Standard curves used for each

gene quantification were generated using a series of 1:10 dilutions of plasmid DNA, giving a concentration range from 10^2 to 10^7 copies per ml. Data analysis was carried out using Rotor-Gene™ 6000 series software 1.7 (Corbett Life Science, BioStrategy, Auckland, New Zealand).

Table C.5-1. PCR primers used in *q*PCR analysis

Target group	***Primer name***	***Sequence (5'-3')***	***Length of amplicon (bp)***	***Primer final concn. (nM)***	***Thermal profile***	***Amplification efficiency (%)***
Bacterial *amoA*	*amoA*1F	5'-GGGGTTTCTAC TGGTGGT-3'	491	250	94°C for 2 min x1 cycle 94°C for 20 s, 57°C for 30 s, 72°C for 30 s x40 cycles	95-102
	*amoA*2F	5'-CCCCTCKGSA AAGCCTTCTTC-3'				

F. Preliminary nitrification rate experiment

F.1 Introduction

The purpose of this experiment was to measure the nitrification rates of urinary-N applied to the Balmoral soil at the same temperatures (6°C and 10°C) as those used in the growth chamber experiment in Chapter 6. This information would be used to assess when the maximum pool size of available nitrate had likely been reached in each chamber and therefore, the period when there was the greatest potential for nitrate leaching. A number of authors have shown that soil nitrification rates from a range of applied readily-mineralisable N-containing substrates increase with increasing soil temperature but these tend to be soil and site specific (Flowers & O'Callaghan 1983; Cookson et al. 2002; Di & Cameron 2004c). Consequently, the objective of this experiment was to measure nitrification rates under conditions like those in each growth chamber.

F.2 Materials and methods

The Balmoral soil used in the experiment was the same as the A horizon collected for the experiment used in Chapter 6, comprising the top 15 cm of the profile. The soil was air-dried and sieved through a 5-mm sieve and repacked in 11 cm tubes (5.0 cm diameter) to a height of 10 cm as outlined in section 6.2.1. Each tube was sealed with fine netting and maintained near field capacity. Urine was applied at a rate of 350 kg N ha^{-1} (69 mg N $tube^{-1}$) as per chapter 6.2.2 and the tubes held in two controlled temperature incubators (6 ° and 10°C) for 1, 7, 14, 28, 42 and 56 days before sampling. At each sampling date the soil was removed from the tube and mixed, with two duplicate samples taken for mineral-N (NH_4^+-N and NO_3^--N), AOB abundance and moisture content analysis. Mineral-N and ammonia oxidising bacteria (AOB) numbers analysis was as outlined in sections 6.2.4 and 0.

F.3 Results and discussion

Results indicated that for the 10°C soils, mineralisation of the urine occurred within 24-hours, with soil ammonium content at a maximum one day later but decreasing by a third over the following seven days (Figure F-1A). Ammonium derived from the ^{15}N-labelled urine reached a similar content after 24 hours but the rate of decrease thereafter was considerably slower and was maintained in

the first 14 days before decreasing in the following period. The bulk of the ammonium-N measured in both chambers was derived from the ^{15}N-labelled urine but a greater proportion of ammonium-N in the 10°C soils was apparently derived from mineralisation of SOM (i.e. non-labelled ^{14}N) than at 6°C (Figure F-1A).

Nitrification of the ammonium was slower at 6°C than at 10°C such that by 56 days after urine application there was no indication that nitrate content had peaked although with ammonium content having decreased considerably it was probably close to this point. Conversely, for the 10°C soils, this point had already been met by 42 days. This represented a 25% longer period of incubation at 6°C than at 10°C to reach and maintain similar maximums for nitrate content.

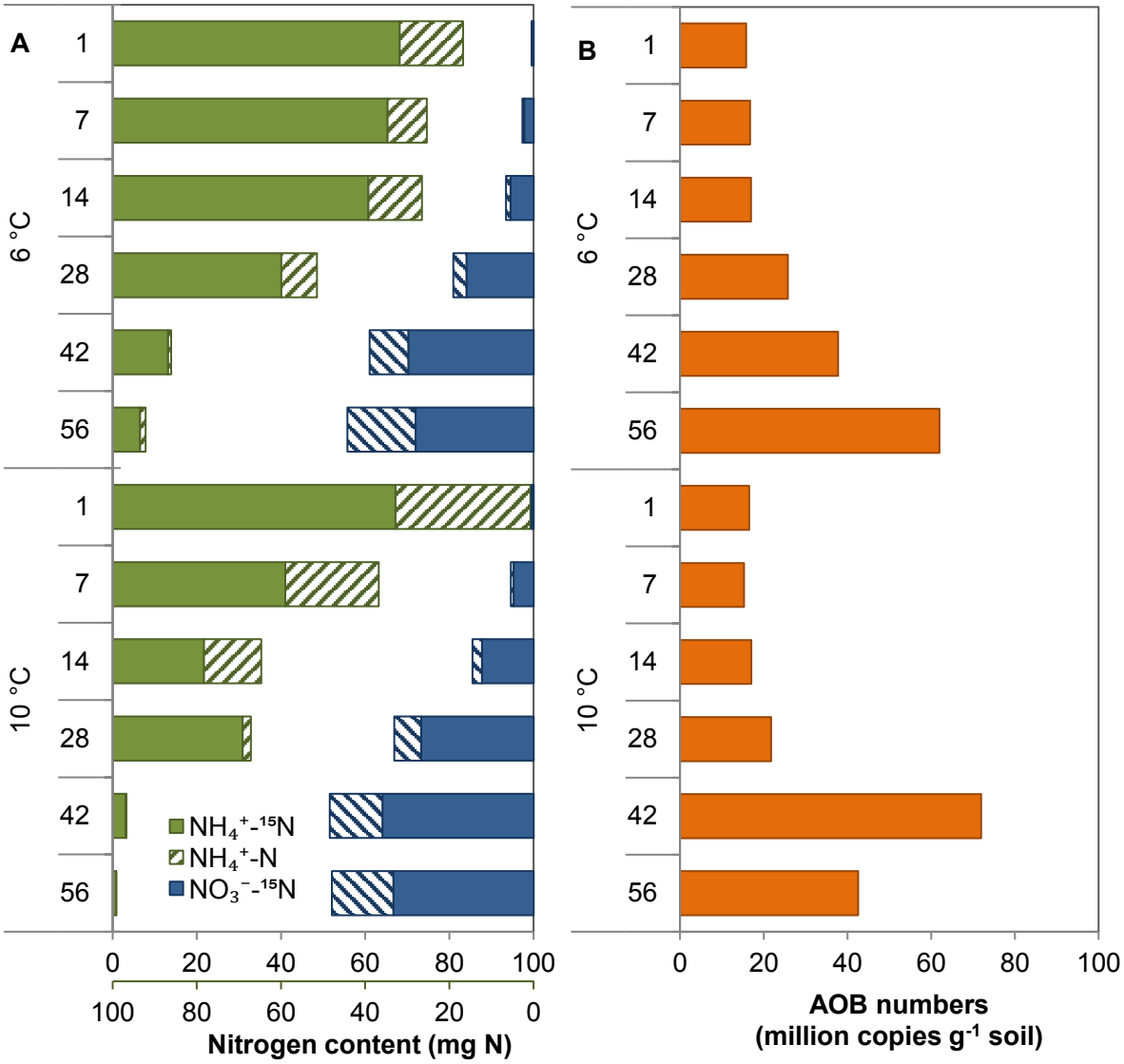


Figure F-1. Soil mineral-N (A: NH_4^+-N and NO_3^--N) concentrations and AOB abundance (B) for soils incubated at 6°C and 10°C, 1-56 days after urine application (350 kg N ha^{-1}).

AOB abundance increased and decreased in accordance with the difference in nitrification rates with numbers still showing an increasing trend for the 6°C soils at 56 days but showing a decline after 42 days for the 10°C soils (Figure F-1B). This is in line with expectations that AOB abundance reflects the rate of nitrification activity (Di et al. 2014).

F.4 Conclusion

The rate of nitrification of the applied urinary-N was shown to be slower at 6°C than at 10°C, taking ~25% longer to reach similar maximums in soil nitrate content at the two temperatures. This represented a difference of 14 days.

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