The impacts of urease inhibitor and method of application on the bioavailability of urea fertiliser in ryegrass (*Lolium perenne* L.)

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by Khadim M. Dawar

UNIVERSITY OF CANTERBURY
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This thesis is dedicated

to my late mother who has been

so supportive all this time

The work presented in this thesis is, to the best of my knowledge and belief, original. The material has not been submitted, either in whole or in part, for a degree at this or any other University.

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ABSTRACT

The use of urea fertiliser has been associated with relatively poor nitrogen (N) use efficiency (NUE) due to heavy N losses such as gaseous emissions of ammonia (NH_3) and nitrous oxide (N_2O) and nitrate (NO_3^-) leaching into surface and ground waters. Improving N use-efficiency of applied urea is therefore critical to maximise its uptake and to minimise its footprint on the environment. The study was conducted under laboratory-glasshouse conditions (Chapter 2-4) and lysimiter-field plot studies (Chapter 5). In chapter 2, Two glasshouse-based experiments were conducted to investigate the potential of incorporating urea fertiliser with urease inhibitor, (N-(n-butyl) thiophosphoric triamide (nBTPT) or 'Agrotain') to enhance fertiliser N uptake efficiency. Urea, with or without Agrotain, was applied to Ryegrass (*Lolium perenne* L.) grown in standard plant trays maintained at soil moisture contents of 75–80% field capacity, at rates equivalent to 25 or 50 kg Nha^{-1}. These treatments were compared with other common forms of N fertilisers (ammonium nitrate, ammonium sulphate and sodium nitrate). In a separate pot experiment, granular ^{15}N urea (10 atom %) with or without Agrotain, was applied at 25 kg N h^{-1} to track N use-efficiency and the fate of ^{15}N-labelled fertiliser. In both experiments, Agrotain-treated urea improved bioavailability (defined as the fraction of total soil N that can interact with a biological target in the plant or that can be taken up by plant) of added N and resulted in significantly higher herbage DM yield and N uptake than urea alone or other forms of N fertilisers. Results from the ^{15}N experiment support the suggestion that a delay in urea hydrolysis by Agrotain provided an opportunity for direct plant uptake of an increased proportion of the applied urea-N than in the case of urea alone.

In chapter 3, two more glasshouse-based experiments were conducted to investigate if urea applied in fine particle application (FPA), with or without Agrotain, had any effect on

fertiliser-N uptake efficiency (defined as the difference in N uptake between the fertiliser treatment and the control as a percentage of the amount of N applied) under optimum soil moisture (75-80% field capacity) and temperature (25 ^{0}C) conditions, in comparison with other common forms of N fertilisers applied, either in FPA or in granular form. In a separate pot experiment, ^{15}N urea (10atom %), with or without Agrotain, was applied to either shoots or leaves only or to the soil surface (avoiding the shoots and leaves) to determine urea hydrolysis, herbage DM and ^{15}N uptake. In both experiments, herbage DM yield and N uptake were significantly greater in the FPA treatments than in those receiving granular application. Agrotain-treated urea FPA resulted in significantly higher N response efficiency (difference between the dry matter produced by the various fertiliser treatments and the control, divided by the amount of N applied) than urea FPA alone or other forms of N fertilisers. Results from the ^{15}N experiment support the idea that Agrotain treatment improves the N response of urea applied in FPA form due to a delay in hydrolysis of urea, thus providing herbage an extended opportunity to absorb added urea directly through leaves, cuticles and roots.

A further glasshouse-based study was conducted to investigate the effect of Agrotain and irrigation on urea hydrolysis and its movement in a Typic Haplustepts silt loam soil (Chapter 4). A total of 72 repacked soil cores (140 mm inner diameter and 100 mm deep) were used - half (36) of these cores were adjusted to soil moisture contents of 80% field capacity (FC) and the remaining 36 cores to 50% FC. Granular urea, with or without Agrotain, was applied at a rate equivalent to 100 kg N ha^{-1}. Twelve pots were destructively sampled at each day after 1, 2, 3, 4, 7, and 10 days of treatment application to determine urea hydrolysis and its lateral and vertical movement in different soil layers. Agrotain-treated urea delayed urea hydrolysis compared with urea alone during the first 7 days of its application. This delay in

urea hydrolysis by Agrotain enabled added urea to disperse and move away from the surface soil layer to the sub-surface soil layer both vertically and laterally. In contrast, most urea in the absence of Agrotain hydrolysed within 2 days of its application. Irrigation after 1 day resulted in further urea movement from the surface soil layer (0-10 mm) to the sub-soil layer (30-50 mm) in Agrotain-treated urea. These results suggest that Agrotain delayed urea hydrolysis and allowed more time for rainfall or irrigation to move the added urea from the surface layer to sub-soil layers where it is likely to make good contact with plant roots. This distribution of urea in the rooting zone (0-200 mm) has the potential to enhance N use efficiency and minimise N losses via ammonia (NH_3) volatilisation from surface-applied urea.

Finally, a field study using lysimeters (300 mm inner diameter and 400 mm deep), and small field plots (1 m^2 in area) was established using a silt loam Typic Haplustepts soil (Soil Survey Staff 1998) to investigate the effect of FPA and granular applications of urea, with or without Agrotain, on N losses and N use efficiency (Chapter 5). The five treatments were: control (no N) and ^{15}N-labelled urea (10 atom %), with or without Agrotain, applied to lysimeters or mini plots (un-labelled urea), either in granular form to the soil surface or in FPA form (through a spray) at a rate equivalent to 100 kg N ha^{-1}. Gaseous emissions of NH_3 and N_2O, NO_3^- leaching, herbage production, N response efficiency, total N uptake and total recovery of applied ^{15}N in the plant and soil were determined up to 63 days. Urea-alone and urea with Agrotain, applied in FPA form, was more effective than its granular form and reduced N_2O emissions by 5-12% and NO_3^- leaching losses by 31-55%. Urea-alone applied in FPA form had no significant effect in reducing NH_3 losses compared with granular form. However, urea with Agrotain applied in FPA form reduced NH_3 emissions by 69% compared with the equivalent granular treatment. Urea-alone and with Agrotain applied in FPA form increased

herbage dry matter production by 27% and 38%, and N response efficiency compared with the equivalent granular urea application, respectively. Urea applied in FPA form resulted in significantly higher ^{15}N recovery in the shoots compared with granular treatments – this was improved further when urea in FPA form was applied with Agrotain. Thus, treating urea with Agrotain in FPA under field conditions has the potential to delay its hydrolysis, minimise N losses and improve N use efficiency and herbage production. The lower dry matter production and N-response efficiency to urea applied in FPA form in Chapter 3 are probably because of additional factors such as lower application rates (25 kg N ha^{-1}) or lack of interception of urea by the leaves. Applying urea in FPA form is a good management strategy and I conclude that combining FPA urea with Agrotain has the potential to increase N use efficiency and herbage production further.

Chapter 1

Introduction, review of literature and rationale

1.1 **Introduction**

Nitrogen (N) is an essential nutrient for growing plants, animals and microbes. It is an important component of proteins and chlorophyll that builds cell materials and plant tissues (Vickery 1981). It is also an important determinant of the rate of key physiological processes in plants, such as photosynthesis and respiration (Lewis et al. 2004; Takashima et al. 2004). Nitrogen is often limited in most agricultural ecosystems, therefore N fertilisers (i.e. chemical or organic) are frequently applied to meet the N demand of growing plants and to improve soil fertility. Current global N fertiliser consumption is 100.1 Mt and according to the International Fertiliser Industry Association (IFIA 2007) predicted global N demand is likely to increase to 107.5 Mt by 2011-12. Urea [(CO (NH$_2$)$_2$] constitutes the major type of chemical fertiliser as 50% of the world N demand is met through urea application. This large urea use is due to a number of factors including its high N content (46% N by weight), high solubility in water, ease of transportation, handling and application.

In New Zealand, the major form of agriculture (52% of the total New Zealand area) is legume-based pasture (approx. 8m hectares). The permanent-pasture vegetation, over sown after burning of the original scrub at least 75 years ago, consists predominantly of ryegrass (*Lolium perenne* L.) and some white clover (*Trifolium repens* L.). These pastures are sown every 5 to 7 years and are regularly grazed by approximately 5.2 m dairy cows, 4.4 m beef cattle and 40 m sheep throughout the year except the winter months of June and July (Southern Hemisphere) when excessive soil water contents force farmers to keep especially

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dairy cows in winter feed pads to avoid soil pugging and animal injury in slippery conditions.

It is estimated that New Zealand agricultural systems receive an annual input of about 3

million tones of N, with 1.58 million tones from animal excreta (urine + faeces), 0.9-1.1

million tones from biological N fixation (BNF) of atmospheric dinitrogen (N_2) and about

0.01-0.015 million tones from atmospheric deposition (Saggar 2004). In addition, 0.33

million tones of chemical fertiliser is added to New Zealand pastures each year.

Urea is the predominant form (80%) of chemical fertiliser in New Zealand, and is commonly

applied to legume-based pastures (typically 25 to 50 kg N ha^{-1} application) after 1-2

rotational grazing cycles to meet animal feed demand and to sustain productivity (Ledgard et

al. 1990; Saggar 2004; Quin et al. 2005; Zaman et al. 2008). Urea is generally applied at

relatively higher rates in spring because of low BNF due to low temperature and slow organic

N mineralization in winter. However, with recent intensification in pastoral farming in New

Zealand, there has been a shift from reliance on BNF towards a markedly increased use of

chemical fretilisers, mainly urea for grazed pastures. During the past 2 decades, there has

been a six-fold increase in N fertiliser use, from 0.05 to 0.33 Mt N yr^{-1} according to the

Ministry for the Environment (MfE 2007). Factors such as recent rapid conversion of sheep

to dairy farming, high stocking rate (>3.5 cows ha^{-1}), early calving, and high payout for milk

solid are driving farmers to apply greater quantities of urea fertiliser onto their pastures

(Bolan et al. 2004; Saggar et al. 2004b, 2005).

Whatever the N inputs to grazed pastures are, it is well understood that significant amounts of

N added to pastures are not utilized efficiently and are lost to the atmosphere as NH_3 and N_2O

and into surface and ground waters as NO_3^- (Martikainen 1985; Raun and Johnson 1999;

Baligar et al. 2001; Follett et al. 2001; IPCC 2007; Zaman et al. 2009). These losses have

both economic and environmental impacts. New Zealand is mindful of the climatic and

environmental implications of land management practices, and consequently research has been focused in this area and various mitigation options have been proposed and tested to improve N efficiency of applied fertilizers and to minimise losses. One such approach is the use of N inhibitors. Recently there has been increased interest in the use of urease and nitrification inhibitors (Sing et al. 2008). A number of studies have reported increased pasture dry matter and N uptake after application of urea coated with urease inhibitor (Watson et al. 1998; Xu et al. 2000; Zaman et al. 2008; Zaman et al. 2009; Zaman and Blennerhassett. 2010).   However none of these studies have assessed the relationship between increased herbage dry matter and N uptake, and particularly have not investigated the mechanisms underpinning improved plant-bioavailability of urea by urease inhibitor. The present study was undertaken to explore the relationship between increased N uptake and herbage dry matter to improve our understanding of the mechanism involved in such processes.

1.2 Review of literature

The main purpose of this chapter is to review the current state of knowledge and understanding regarding N transformations in legume based pasture systems. A simplified version of the transformation of N in a legume-based pasture is presented in Fig. 1.1. Nitrogen transformations in soils processes - urea hydrolysis, NH_3 volatilisation, nitrification, N_2O emission, NO_3^- leaching and plant N uptake - will be discussed.

Chapter 1
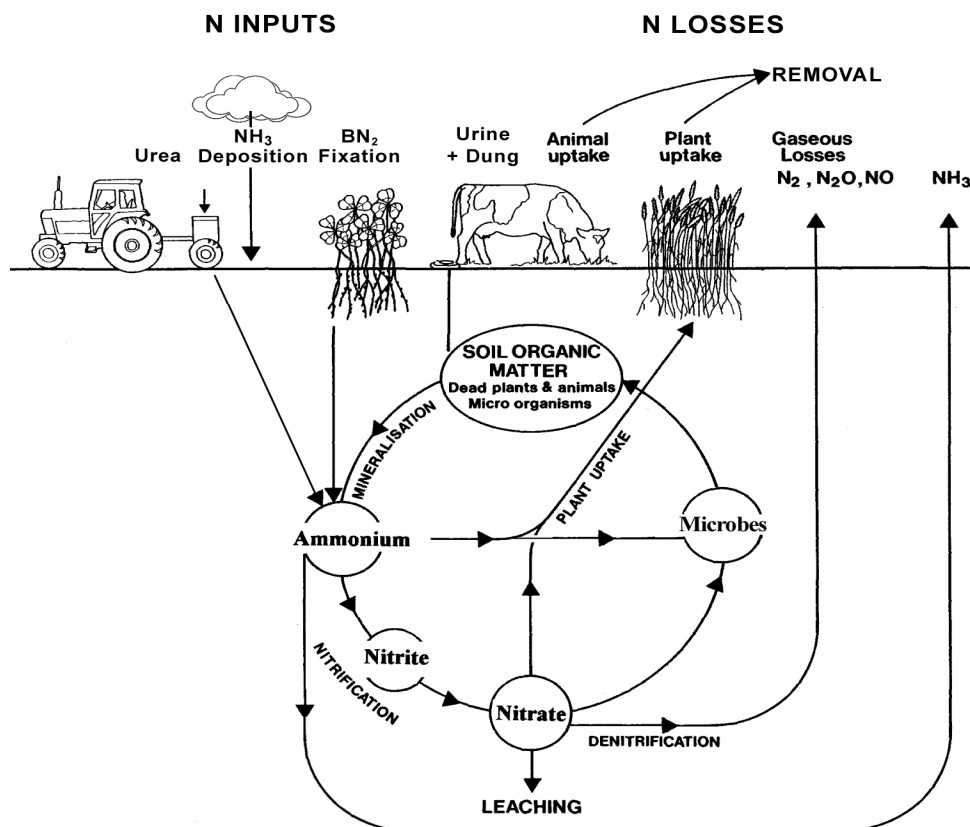
Fig.1.1. Schematic diagram of N cycle in grazed pasture systems

1.2.1 Urea hydrolysis

Urea hydrolysis starts soon after application of urea fertiliser or deposition of cow urine in grazed pastures and is completed with 1 to 2 days (Zaman et al. 2008; 2009). Urea hydrolysis is a chemical reaction and is carried out by urease enzyme (urea amiodrolase). When it is applied to soil, urea undergoes hydrolysis. This is catalysed by the urease enzyme to form ammonium carbonate ($(NH_4)_2CO_3$) (eq. 1.1), which in turn, being unstable, dissociates into ammonium (NH_4^+) and carbonate (CO_3^{2-}) ions. The (CO_3^{2-}) ions release hydroxyl (OH^-) ions, thereby resulting in a high pH close to the site of hydrolysis. Each molecule of urea produces 2 molecules of NH_4^+-N ions (eq. 1.1).

$$H_2N-C(=O)-NH_2 \quad + \quad 2H_2O \xrightarrow{\textit{Soil Urease}} \quad (NH_4)_2CO_3 \qquad \text{(eq. 1.1)}$$

$$2H^+ \longrightarrow 2NH_4^+ \; + \; CO_2 \; + \; H_2O$$
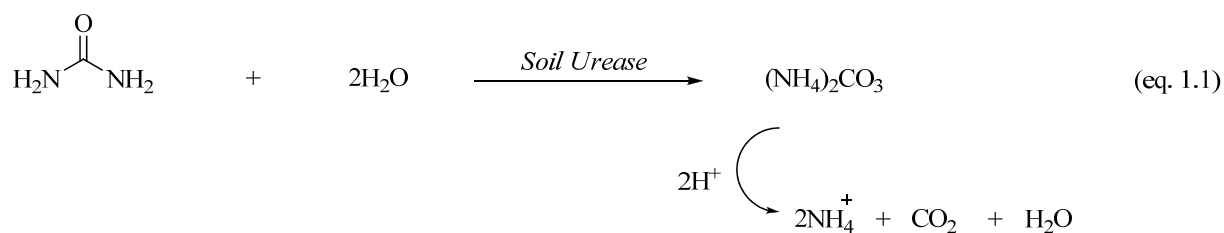
Soil urease are generally thought to be of microbial origin (most species of bacteria, yeast and fungi), however there is evidence that some soil urease may be derived from plants (Frankenberger and Tabatabai 1982; Freney and Black 1988). Urease enzymes are ubiquitous and reported to be found under both aerobic and anaerobic soil conditions (McCarty and Bremner 1991; Zaman et al. 1999). Factors known to increase urease activity include soil temperature (above 5^0C), soil pH (above 6.5), soluble organic C, total N and cation exchange capacity (CEC) (Bremner and Mulvaney 1978 Kissel and Cabrera 1988; Zaman et al. 1999). Urease activity is greater in grassland than in cultivated soils (O' Toole et al. 1985; Reynolds et al. 1985; Whitehead & Raistrick 1993), probably because of the high organic C in the former than in the later. The major factors influencing the hydrolysis of urea (urea concentration; soil pH, soil water content and temperature) will be discussed below.

1.2.1.1 Urea concentration

Urea hydrolysis follows simple Michaelis-Menten kinetics (Tabatabai and Bremner 1972; Dalal 1975). When urea fertilisers are applied to soil, the concentration of the urea solution may range from very low at some distance from the granule or site of application to very high at the surface of the prill or centered at the application site. Cabrera et al. (1991) measured the hydrolysis of urea at urea concentrations ranging from 0.01 to 10 M. They found two possible reactions, one with high affinity and another one with low affinity for urea. The high affinity reaction was responsible for most of the hydrolysis at urea concentrations lower than 0.1 M, although its contribution at greater than 8 M is smaller. In general, the urea-N

concentration at which low and high affinity enzyme reactions contribute equally is 0.5 M. In addition, Cabrera et al. (1991) found that at higher urea concentrations (i.e. > 6 M), the rate of urea hydrolysis decreases, possibly due to enzyme denaturation or substrate inhibition (Kistiakowsky and Rosenberg 1952).

1.2.1.2 Soil water content

Soil water is the pre-requisite for urea hydrolysis to occur (eq 1.1). Urea is hygroscopic and can absorb water from surface soil as well as water vapor in the air (Wahl et al. 2006). Urea dissolution starts immediately after its application to the soil because of its high solubility in water (i.e. about 1080 g L^{-1} can be dissolved at 20 $^{\circ}$C (Wahl et al. 2006) - this increases with temperature (Wahl et al. 2006)). Black et al. (1987) reported that urea hydrolysis was very slow when applied to air-dry soil and about 73± 14% of the applied urea remained un-hydrolyzed after 30 days of application. However, Watson and Miller (1996) found that when urea was applied to a soil at field capacity, hydrolysis was rapid with only 1.3 % urea-N remaining in the soil after 1.75 days. Vlek and Carter (1983) reported that urease activity is generally greater near field capacity and declines as soil moisture decreases. Yadav et al. (1987) found that the rate of urea hydrolysis was not a linear function of moisture content. When expressed as a percentage of field capacity (FC) the rate of hydrolysis in the soil followed the order: 20% FC < 40% FC < 80% FC = 100% FC, although Yadav et al. (1987) reported that the added urea was completely hydrolysed in 3 days at all moisture regimes except 20% FC.

1.2.1.3 Soil pH

Soil pH is another major factor that can affect the rate of urea hydrolysis. The products of urea hydrolysis are NH_4^+ and one or more inorganic carbon (C) species depending on the soil

pH. In a soil of near neutral pH, the predominant C species will be HCO_3^- (eq.1.2), whereas in a soil of pH < 6.3, the predominant C species will be H_2CO_3 (eq.1.2) (Ferguson et al. 1984). The urea hydrolysis reactions can be represented as follows:

$$H_2N-\overset{\overset{\displaystyle O}{\|}}{C}-NH_2 \; + \; 2H_2O \; + \; H^+ \; \longrightarrow \; 2NH_4^+ \; + \; HCO_3^- \quad (pH \; 7.0\text{-}9.0) \qquad (eq. \; 1.2)$$

$$H_2N-\overset{\overset{\displaystyle O}{\|}}{C}-NH_2 \; + \; 2H_2O \; + \; 2H^+ \; \longrightarrow \; 2NH_4^+ \; + \; H_2CO_3 \quad (pH < 6.3) \qquad (eq. \; 1.3)$$
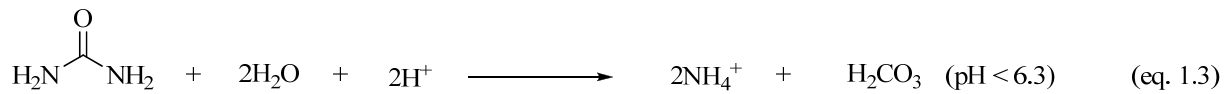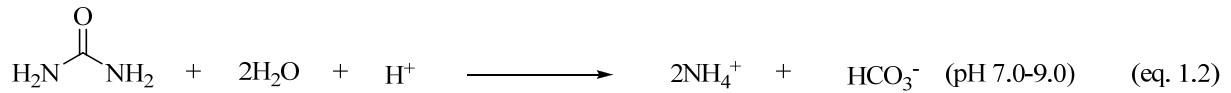
The HCO_3^- produced in (eq.1.2) can react with another H^+ to maintain a chemical equilibrium in the system (eq. 1.4) (Ferguson et al. 1984).

$$H_2CO_3 \; + \; H^+ \; \longrightarrow \; CO_2 \uparrow \; + \; H_2O \qquad (eq. \; 1.4)$$

The majority of pasture soils in New Zealand have a pH of less than 6 (unless recently limed). Urea hydrolysis consumes two protons (H^+) for each mole of urea hydrolyzed. This reaction tends to increase the pH around urea-granules, and thus increases the rate of urea hydrolysis (Ferguson et al. 1984). Longo and Melo (2005) measured the rate of urea hydrolysis under laboratory conditions using a range of soil pH from 2.2 to 8.0. They found that as the soil pH increases the rate of urea hydrolysis increases almost exponentially. In addition, they found that the highest rate of urea hydrolysis was at pH 8.0. Similar results were found by Cabrera et al. (1991) where the rate of urea hydrolysis increased up to a pH of 9.5.

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1.2.1.4 Temperature

Temperature is an important factor affecting the process of urea hydrolysis. Temperature can affect the rate of dissolution of a urea granule in water (Wahl et al. 2006). Sadeghi et al. (1989) showed that elevated temperature increases urea hydrolysis probably because of increased urea diffusion increases the rate of movement of urea toward the urease enzyme. An increase in soil temperature is also reported to enhance microbial growth (Zaman and Change 2004), which is likely to increase urease production. Moyo et al. (1989) found that increasing temperature from 5 to 45 ^{o}C greatly increased urease activity. They also found that the mean energy of activation (Ea) for soil urease was about 51.5 kJ mol^{-1}. This value corresponds to a Q_{10} of approximately 2, which indicates that reaction rate of urea hydrolysis doubles for every 10 ^{o}C rise in temperature. Yadav et al. (1987) reported that the amount of urea remaining after 12 h of incubation was 64% at 10 ^{o}C and 27% at 35 ^{o}C. Lai and Tabatabai (1992) found that urease activity increases with temperature and reaches a maximum between 60 and 70 ^{o}C.

1.2.2 Ammonia (NH$_3$) volatilisation

Ammonium produced after urea hydrolysis in the soil can follow different pathways including NH$_3$ volatilisation, plant uptake, immobilisation, nitrification and clay fixation (Fig. 1.1; eq. 1.5).

$$2NH_4^+ \quad + \quad OH^- \quad \rightleftharpoons \quad NH_3\uparrow \quad + \quad H_2O \qquad \text{(eq. 1.5)}$$
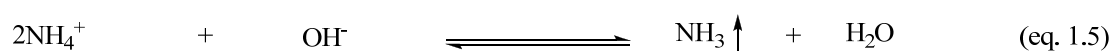
Among these pathways, NH$_3$ volatilisation is regarded as critical because of the negative effects of NH$_3$ losses on crop productivity as well as on the environment. Ammonia itself is not a greenhouse gas; however after deposition on land, NH$_3$ produces N$_2$O through the microbial processes of nitrification and denitrification and thus contributes to global warming

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and ozone (O_3) depletion (Martikainen, 1985). Ammonia emissions also represent a major agronomic loss (Rochette et al. 2009) and result in degradation of air and water quality (Galloway et al. 2003).

Urea applied to the soil surface, in the form of fertilisers or in urine, is quickly hydrolysed within one to two days depending on soil moisture content and temperature. In New Zealand NH_3 losses from grazed and fertilised pastures from 1.7 to 36% of applied N have been reported (Sherlock and Goh 1984; Zaman et al. 2008; 2009) if urea present in sufficient amount near the soil surface (Black et al. 1985; Koelliker and Kissel 1988; Sommer and Jensen 1994; Bussink and Oenema 1998). Ammonia emission from urea fertiliser or urine-N is reported to be affected by a number of soil and environmental factors such as, soil water content, pH, temperature, wind-speed, cation exchange capacity and buffering capacity. These factors are discussed below.

1.2.2.1 Soil water content

Nitrogen loss via NH_3 volatilisation after surface application of urea is strongly influenced by soil water content. Ammonia losses occur when there is free NH_4^+ present at the soil surface. Soil water not only influences urea dissolution and hydrolysis but also movement of urea product (NH_4^+) in soil surface layers (Ferguson and Kissel 1986). Vlek and Carter (1983) suggest that low urea hydrolysis after application of granular urea at low water contents may be due to poor urea diffusion which is likely to limit the contact between urea and urease. The rate of NH_3 volatilisation varies with the amount and timing of irrigation or rainfall events. For example Craig and Wollum (1982) found that a light rainfall (< 15 mm) after applying granular urea to dry soil stimulated urea hydrolysis, but did not result in washing applied urea from surface soil to sub-surface layers due to a dry soil conditions, and thereby increasing NH_3 volatilisation. Van Der Weerden and Jarvis (1997) reported that 20% of the

applied N was lost via NH_3 emission despite 14 mm of rainfall which occurred 3 days after fertiliser application. This was probably due to most of the urea being hydrolysed in the first 3 days following application. In contrast, Kissel et al. (2004) found that simulated rainfall applied immediately after urea application reduced NH_3 volatilisation losses to <1% of the applied urea. Similarly in another study, Bussink and Oenema (1996) reported reductions of NH_3 losses with 9 mm of rain following applications. Zaman et al. (2008) also reported lower NH_3-N losses after application of urea with or without Agrotain which they attributed to wet soil conditions at the time of fertiliser application and a rainfall of 17 mm, which occurred 1 day after fertiliser application. These results highlight the fact that both the timing as well as the amount of irrigation or rainfall event is critical to minimise such losses.

1.2.2.2 Soil pH

Losses of N due to volatilisation are often greater in soils with a higher pH (Bouwmeester et al. 1985; He et al. 1999) because the concentrations of NH_4^+ and NH_3 are determined by the pH of the soil solution. Urea applied to acidic or neutral soils results in an increase in soil pH around the urea granule (eq. 1.2) which drives the equilibrium to the right, thereby resulting in higher NH_3 emission (eq. 1.5). Fan and Mackenzie (1993) measured the effect of soil pH on NH_3 volatilisation losses from two soils with pH of 5.2 and 6.0. They found that NH_3 volatilisation was lower at pH 5.2 than that from the soil with a pH of 6.0.

1.2.2.3 Buffering capacity

Hydrogen ion (H^+) buffering capacity is another soil property that affects NH_3 volatilisation losses. Buffering capacity is defined as the ability of the soil to resist changes in pH. The H^+ buffering capacity of a soil is determined by its soil minerals and organic matter content, among other soil properties (Meisinger and Jokela 2000). The ability of the soil to resist an increase in pH during urea hydrolysis affects the amount of NH_3 loss due to its effect on the

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ratio of NH_3 to NH_4^+ (Avnimelech and Laher 1977). A soil with more H^+ supplying ability than another will have less potential for NH_3 volatilisation provided all the other factors remain the same (Ferguson et al. 1984). Ferguson et al. (1984) measured the effect of H^+ buffering capacity on NH_3 volatilisation losses in two different soils, and they found that when soils were amended with a resin that increased the H^+ buffering capacity, the amount of N lost through volatilisation was smaller compared to unamended soil.

1.2.2.4 Temperature

An increase in temperature is reported to accelerate the rate of NH_3 volatilisation (Olesen and Sommer 1993). Temperature has a triple effect on the process of NH_3 volatilisation. High temperature not only can increase urease activity and thus, NH_4^+ and OH^- in the soil solution (Lai and Tabatabai 1992), but also can increase the conversion of NH_4^+ to NH_3 and the diffusion of NH_3 from the aqueous phase to the gaseous phase (Sander 1999). Staudinger and Robersts (2001) found that Henry's constant (K_H) is temperature dependent. Therefore, as temperature increases by 10 $^\circ$C the diffusion of NH_3 from the aqueous phase to the gaseous phase increases by a factor of 1.88 (an 88% increase). McGarry et al. (1987) measured the effect of three soil temperatures (8, 13 and 18 ^{0}C) on NH_3 volatilisation when a solution of urea was surface applied on pastures and found that NH_3 losses increased with an increase in temperature. Even though high temperatures have been shown to increase NH_3 volatilisation losses, Steenhuis et al. (1979) found that NH_3 losses do not stop at near-freezing temperatures. Losses near freezing can occur because a lower, but still substantial, rate of volatilisation occurs for a longer period of time (Sommer and Olesen 1991). Zaman et al. (2009) found that cow urine applied to pasture lost 8.2% of the applied N in summer as opposed to 3.6% in spring because of the higher temperature in former than the latter. In another field experiment, Zaman and Blennerrhassett (2010) observed lower NH_3 losses from urine applied to pasture

soils in late autumn than those applied in early spring because of the lower temperature in autumn.

1.2.2.5 Wind speed

Wind speed accelerates NH_3 losses by increasing mass transfer and air exchange between the NH_3 on the soil surface and in the atmosphere. The effect of wind speed on NH_3 volatilisation was clearly demonstrated by Fillery et al. (1984), who found that the rate of NH_3 loss from a flooded rice (*Oryza sativa* L.) field increased linearly with wind speed over the range of 0 to 8 m s^{-1}. Thompson et al. (1990) found that wind speed had a positive effect on NH_3 volatilisation, although the effect was small in relation to the total loss; increasing the wind speed from 0.5 to 3.0 m s^{-1} increased the total 5 days loss by a factor of 0.29 (29%). In this experiment, the effect of wind speed was also more pronounced in the first 24 h when much of the NH_3 loss took place. Sommer and Ersbøll (1996) measured NH_3 volatilisation from surface-applied urea, diammonium phosphate (DAP), and calcium phosphate (DP), ammonium sulphate (AS), and calcium ammonium nitrate (CAN) using chambers, through which air was passed continuously. They found that NH_3 losses were related to the air flow rate and a transfer coefficient (K_a) and that K_a increased exponentially with the flow rate. At a flow rate above 3.9 liters min^{-1} (20 volume exchanges min^{-1}) no further increase in NH_3 volatilisation was observed.

1.2.2.6 Cation exchange capacity (CEC)

The CEC of a soil is the amount of positively charged ions that a soil can hold. Generally, texture is an important indicator of CEC and the greater the clay content and organic matter content, the greater the CEC of the soil (Havlin et al. 1998). A high CEC can reduce NH_3 loss principally in two ways: by restricting pH changes or increasing the buffering capacity and by increasing the adsorption of NH_4^+ produced after the process of urea-hydrolysis is completed.

Ahmed et al. (2006) conducted a laboratory study showing the differences on NH_3 volatilisation losses when urea-fertiliser was mixed with triple superphosphate (TSP), humic acid and zeolite materials having the property to enhance soil CEC. The results indicate that applying urea with humic acid and zeolite significantly reduces NH_3 volatilisation losses from 48% to 18% of the total applied N when compared to urea without additives. In summary, the decreased loss of NH_3 from surface applied urea in soils with high CEC is possibly due to a lower formation of NH_3 over NH_4^+, a greater buffering capacity, and a greater retention of NH_4^+ ion within the soil (Ahmed et al. 2006).

1.2.3 Nitrification

Nitrification refers to a two-step process (eq. 1.6) of biological oxidation of NH_4^+ in the presence of oxygen (O_2) by gram-negative bacteria of the genera *Nitrosomonas* and *Nitrobacter* to produce nitrite (NO_2^-) and nitrate (NO_3^-), respectively (Bremner and Blackmer, 1981):

$$2NH_4^+ \quad + \quad 3O_2 \quad \xrightarrow{\textit{Nitrosomonas}} \quad 2NO_2^- \quad + \quad 2H_2O \quad + \quad 4H^+ \qquad \text{(eq. 1.6)}$$

$$\textit{Nitrobacter} \longrightarrow 2\,NO_3^-$$
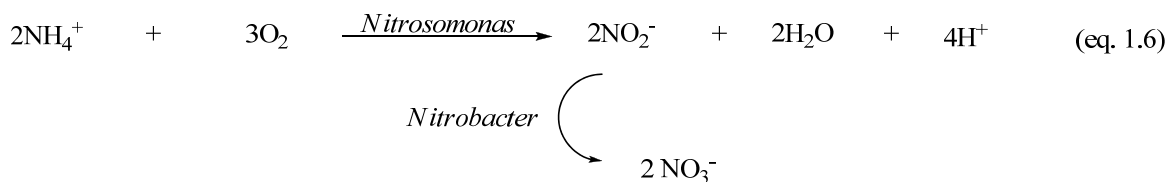
Nitrification can be either autotrophic or heterotrophic, but the former is known to be predominant in most soils. Autotrophic nitrification also produces nitrous oxide (N_2O), a greenhouse and ozone (O_3)-depleting gas, as a by-product (Mulvaney and Bremner 1981). The presence of O_2, available NH_4^+ as a substrate, soil water content to range from 75 to 100% FC (Zaman et al. 1999; Sierra and Marban 2000), favorable temperature above 5 ºC (optimum 25 to 35 ºC) (Paul and Clark 1996; Zaman et al. 1999; Sierra and Marban 2000)

and soil pH above 5 (optimum 7 to 9) (Kyveryga et al. 2004; Zaman and Chang 2004; Sahrawat 2008) are known to accelerate autotrophic nitrification.

1.2.3.1 N_2O emission

Nitrous oxide emissions from soils do not pose an economic loss as they account for less than 2.5 % of the applied N (Bouman 1996), however mitigating N_2O emission is of particular interest because it is one of the key greenhouse gases, constituting about 7 % of the anthropogenic greenhouse effect (Houghton et al. 2001; IPCC 2007). The global atmospheric concentration of N_2O has increased from 270±7 in pre-industrial-period to 319±12 ppbv in 2005 (IPCC 2007). Over the last two decades a nearly linear increase of 0.26% (per year) in the concentration of N_2O has been measured. On a molecular basis, N_2O has approximately 310- and 16-times greater global warming potential than carbon dioxide (CO_2) and methane (CH_4), respectively over a 100 year period (IPCC 2007). Moreover, due to its relative stability, after emission from the soil surface, N_2O acts as a source of nitric oxide (NO) in the stratosphere. It thus indirectly accelerates depletion of ozone (O_3), and so can increase the risk to the biosphere from harmful ultraviolet (UV) radiation (Crutzen 1981). Since 1750, the concentration of N_2O present in the atmosphere has increased by 17% and continues to increase by 0.3% yr^{-1} (Houghton et al. 2001; IPCC 2001). Modern agriculture and human activities, such as the increased use of N fertilisers 100.1 Mt N (IFA 2007), irrigation, increased pasture areas and introduction of management practices to enhance soil organic N mineralisation, are reported to be the major contributing factors for increased N_2O emissions (Duxbury at al. 1993; Jenkinson, 2001; Simek and Cooper 2001; Rochester 2003). In New Zealand, the major source of N_2O emissions is the urine deposited by grazing ruminant animals (e.g. Di and Cameron 2002; Saggar et al. 2005), which accounts for 80% of New Zealand's total agricultural N_2O emissions (De Klein and Ledgard 2005). The second largest source of N_2O is N fertiliser, contributing approximately 14% of agricultural N_2O emissions

and about 4% originates from other sources such as, N-fixing crops, crop residues, and storage of animal manure.

In addition to NO_3^-, N_2O and NO are formed as byproducts during autotrophic nitrification as shown in eq. 1.7 (Firestone and Davidson, 1989).

$$NH_4^+ \longrightarrow NH_2OH \longrightarrow HNO \begin{matrix} NO \\ \nearrow \end{matrix} \quad NO \quad NO \longrightarrow NO_2^- \longrightarrow NO_3^- \qquad (eq.\ 1.7)$$
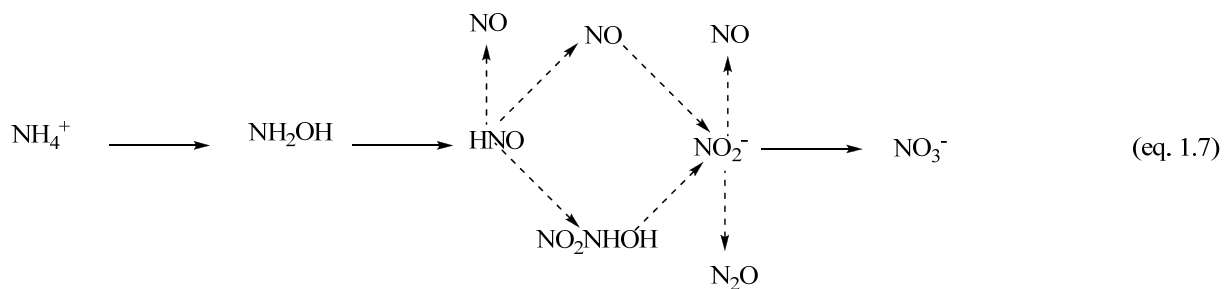
Broken lines show the unconfirmed pathways of the biological reaction.

Several intermediate and unstable compounds such as hydroxylamine (NH_2OH) and nitroxyl (NOH) are also formed during the oxidation of NH_4^+ to NO_2^-. Under low O_2 conditions, chemical decomposition of NH_2OH and NO_2^- produce substantial amounts of N_2O (Firestone 1982; Schmidt 1982; Firestone and Davidson 1989). The second proposed mechanism for N_2O and NO production is related to nitrification (or more precisely to nitrifiers) and is connected to the activity of ammonia oxidisers. In the first step of nitrification, ammonia oxidisers consume relatively large amounts of molecular O_2 causing anaerobic conditions in the microsites. Such anaerobicity then leads to reduction of NO_2^- to N_2O and N_2. (Poth and Focht 1985; Zart and Bock 1998; Colliver and Stephenson 2000). Heterotrophic nitrification, the oxidation of reduced N compounds or NH_4^+ to NO_3^- in the presence of O_2 and organic C, can also produce N_2O from NO_2^- and typically occur in acidic soils (Wood 1990). However, high rates of heterotrophic nitrification relative to autotrophic nitrification have been measured in a riparian wetland soil of pH close to 7 that was exposed to O_2 (Matheson et al.

Chapter 1

2003). Production of N_2O via heterotrophic nitrification is poorly understood because autotrophic and heterotrophic nitrification can occur simultaneously in a given soil and it is difficult to separate the end products of these two processes without the use of a ^{15}N tracer (Robertson and Kuenen, 1991).

Denitrification is a process by which oxidised N compounds, principally NO_3^- and NO_2^-, are reduced to N_2O and N_2 in respiratory metabolism (eq. 1.8). During respiratory denitrification, denitrifiers couple reduction of N-oxides to oxidation of organic C under anaerobic conditions and produce ATP by phosphorylation (Firestone 1982; Linn and Doran 1984; Tiedje 1988; Cavigelli and Robertson 2001). Four different enzymes (reductases) are involved in a complete denitrification reaction, usually distributed in different microorganisms:

$$NO_3^- \xrightarrow{\text{nitrate reductase}} NO_2^- \xrightarrow{\text{nitrite reductase}} NO \xrightarrow[\text{reductase}]{\text{nitric oxide}} N_2O \xrightarrow[\text{reductase}]{\text{nitrous oxide}} N_2 \qquad \text{(eq. 1.8)}$$
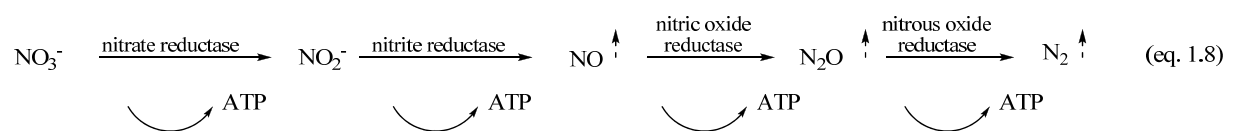
Denitrifiers are normally aerobic bacteria; however they are capable of using N-oxides at low O_2 level (Tiedje 1988). Biological denitrification thus requires the absence of O_2, which is related to high soil moisture content of >60% water filled pore space (WFPS), NO_3^- (as an electron acceptor), available organic C (as an electron donor), suitable soil pH (generally ranging from 5 to 8, optimum at 7) and temperatures ranging from 5 to 30 ^{o}C, optimum 25 ^{o}C (Ryden and Lund 1980; Ryden 1983; Goodroad and Keeney 1984; Scholefield et al. 1997; Barton et al. 1999; Swerts et al. 1997; Aulakh et al. 2001; Zaman et al. 2002). However, the most critical factors are the absence of O_2, available C and NO_3^- concentration. Thus denitrification is expected to be an important N transformation process in areas where soils and sediments are subject to water logging (making them anaerobic), contain sufficient

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organic C and intercept inputs of NO_3^- or NO_2^- in groundwater. These areas include riparian wetlands (Nguyen et al. 1999; Matheson et al. 2003), drains and ditches, and stream or river channels (Garcia-Ruiz et al. 1998; Bronson and Fillery 1998; Mcmahon and Dennehy 1999; Walker et al. 2002; Groffman et al. 2002). However, denitrification can also occur in less obviously waterlogged areas within the agricultural landscape due to the existence of anaerobic micro-sites, such as in the center of soil aggregates (Parkin 1987) or in areas of localized high O_2 consumption ("hot spots"), which are created by decaying organic C (Godde and Conrad 2000; Khalil et al. 2002; Mosier et al. 2002).

1.2.3.2 Nitrate (NO_3^-) leaching

Nitrate leaching losses in New Zealand grazed pastures occur from both fertiliser N and urine N; however the later is regarded as the major source of NO_3^- leaching (De Klein and Ledgard 2005; Di and Cameron 2002). Nitrate, because of its negative charge, is largely excluded from soil exchange sites and is therefore most likely to be lost to ground or surface waters via leaching and seepage from uplands or emitted into the atmosphere as NO, N_2O and N_2 via denitrification, if not taken up by plants or assimilated by microorganisms. Nitrate leaching losses have both economical as well as environmental implications. During leaching, NO_3^- also carries other cations (like Ca^{+2}, Mg^{+2}, K^{+1}, Na^{+1}) as counter ions and these results in permanent losses of those nutrients and lower soil fertility (Di and Cameron 2002). After entering water bodies via seepage from uplands or direct leaching, NO_3^- can have adverse effects on water quality by causing eutrophication and algal blooms (Howarth 1988). It is therefore regarded as one of the biggest water pollutants worldwide (Petrovic 1990) and also in New Zealand (Ledgard et al. 1999; Silva et al. 1999; Di and Cameron 2002). The declining water quality of Lake Taupo, Lake Rotoiti and the Rotorua Lakes has been linked to the export of N from farming and other sources in those areas. Farming has been shown to be a

major contributor to the algal blooms occurring in New Zealand surface waters (http://www.terranature.org/riversStreams.htm).

High NO_3^- concentrations (11.3 mg L^{-1}) in drinking water is also linked to health problems in young infants (methemoglobinemia or blue baby syndrome), and the World Heath Organisation (WHO) has recommended a safe upper limit (11.3 mg NO_3^--N L^{-1} or 50 mg NO_3 L^{-1}) in drinking water. Environment Waikato data suggest the quality of about 10% of the groundwater in the livestock farming area of the region is below WHO drinking water standards (Annon 2005). Although NO_3^- is not always toxic to animals, NO_3^- toxicity in grazing animals is likely to occur when they ingest water and forage that are high in NO_3^- concentration (Bolan et al. 2004). Ruminants are more susceptible to NO_3^- toxicity than simple-stomached animals because rumen microbes enhance the reduction of NO_3^- to NO_2^- in the digestive tract. The toxicity symptoms include trembling, staggering gait, rapid respiration and prostration. Affected animals cease to eat and soon collapse and may die. Losses of weight and milk production and non-infectious abortion have been noted as sub-lethal effects in dairy cattle. Uncertainty exists about the level of NO_3^- ingestion that is considered the minimal lethal dose. Studies have indicated that 7.6 - 9.0 g NO_3^--N per 100 kg body weight is lethal to animals. Therefore, proper mitigation measures for NO_3^- leaching are critical because of its economical, environmental and health risks. Factors known to affect NO_3^- leaching include: land use, fertiliser rate, form and timing, organic manure, irrigation, cover crops, crop residue management and tillage.

1.2.4 Mitigation options to reduce N losses

Nitrogen losses from applied chemical N fertilisers (especially urea) via gaseous emissions of NH_3 and N_2O, and NO_3^- leaching have both economic and environmental implications (Bolan et al. 2004). Such N losses are clearly undesirable and there is a need to enhance fertiliser N

efficiency by synchronizing plant N uptake with the available soil N (NH_4^+ or NO_3^-) concentrations to minimise such losses and increase farm profitability. Various mitigating options including avoiding heavy N application rates, applying N fertiliser at appropriate times, splitting N application rates, avoiding grazing during winter, using slow release fertiliser, incorporating urea fertiliser with either urease inhibitor or urease plus nitrification inhibitors, or using NH_4^+ based fertiliser with nitrification inhibitor alone (and/or nitrification inhibitor) to minimise N losses and improve fertiliser use efficiency.

Among these management options, the use of urease inhibitors (UIs) is a highly efficient way to reduce NH_3 losses and to increase fertiliser N efficiency (Watson and Miller 1996; Blennerhassett et al. 2006; Martin et al. 2008; Zaman et al. 2008). Urease inhibitors have the potential to retard urea hydrolysis by inhibiting the urease enzyme in the soil (Gill et al. 1997) thereby allowing more time for rain or irrigation to dilute the applied urea from the application site and hence reduce the potential for NH_3 volatilisation (Black et al.1987; Grant et al. 1996). A large number of chemicals have been tested as potential inhibitors of soil urease activity. These inhibitors are classified according to their structures or their binding modes with urease. Amtul et al. (2002) divided UIs into (i) substrate-analogue inhibitors, which have structural similarities to urea and inhibit urease by competing for the same active site on the enzyme (thio-urea, methyl-urea, hydroxyl-urea and numerous hydroxamic acids are substrate-analogue of UIs) and (ii) non-substrate-like or mechanism-based inhibitors, depending on their binding modes. Non-substrate-analogue inhibitors do not have any close structural similarity to that of urea, but they interfere with the enzyme's catalysis mechanism leading to enzyme inactivation.

Many compounds have been studied and evaluated as UIs (Mulvaney and Bremner 1981; Martens and Bremner 1984; Broadbent et al. 1985; O' Connor and Hendrickson 1987), but

few have shown to be effective. Among the many urease inhibitors, N-(n-butyl) thiophosphoric triamide (nBTPT) (Fig. 1.2) is reported to be the most effective in retarding urea hydrolysis in aerobic soils at very low concentrations ranging from 0.01-0.5% nBTPT w/w (Bremner and Chai 1986; Joo et al. 1987; Beyrouty et al. 1988; Bremner et al. 1991, Watson et al. 1994a; Bremner 1995; Rawluk et al. 2001). N-(n-butyl) thiophosphoric triamide itself is not a urease inhibitor, but after application, nBTPT in soil is quickly transformed into its oxygen analogue N-(n-butyl) phosphoric triamide (nBPTO) (Fig. 1.2), which is the actual agent responsible for the inhibition of urease activity (Christianson et al. 1990; Creason et al. 1990).

N-(n-butyl)thiophosphoric triamide
(*n*BTPT)

N-(n-butyl)phosphoric triamide
(Oxygen analogue)
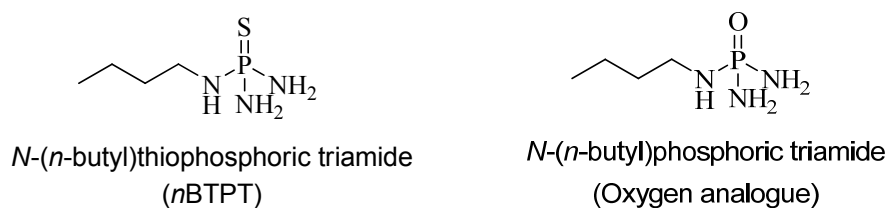
Fig. 1.2 Structure of the urease inhibitor nBTPT and its oxygen analogue

1.2.5 Bioavailability of N in plants

Nitrogen is absorbed both by roots and above ground parts including leaves and shoots (Marschner, 1995; Carrow et al., 2001; Hull and Liu, 2005). Plants take up N both as NO_3^- or NH_4^+ via the roots. Glass et al. (2002) reported that both NO_3^- and NH_4^+ share the same metabolic pathway and both ions are actively absorbed into the root cells at low external concentrations. Most plants prefer NO_3^- over NH_4^+, however the rate of uptake of NH_4^+ is often found to be greater than that of NO_3^-, especially in some poorly drained soils and at low temperatures over the winter (Clark et al. 1979). One important aspect of NH_4^+ supply is that NH_4^+ can be directly assimilated into amino acids in the presence of the enzyme glutamine synthetase (Srivastava and Singh 1987; Choi and Kwon 1998) and the rate of assimilation is

faster than that for NO_3^-, while NO_3^- has to be reduced before assimilation, which requires additional energy (Raven 1985; Ullich 1992). Plants may save energy by taking up NH_4^+ instead of NO_3^-, which may lead to reduced respiratory cost and improved carbohydrate status in the plant tissues (Heeb et al. 2005). Watson et al. (1990b) reported that many plants can take up N as urea, and some may do so preferentially. When urea is not hydrolysed due to unfavorable conditions, roots of many plants can take it up as an intact molecule (van Beusichem and Neeteson 1982; Bradley et al. 1989; Kirkby and Mengel 1970).

Granular urea, by virtue of its concentration, provides small pockets of extremely concentrated N that in some conditions will limit the opportunity for access by roots. The use of urea in suspension or "FPA" (fine particle application) form are alternative means of applying fertiliser, especially when plants need more N during periods of rapid growth or at times of critical physiological stress, to improve nitrogen uptake. In contrast to granular application, applying urea in FPA form results in much more even distribution of the applied urea on a per plant basis, and thus may provide an opportunity for plants to take up N through both leaves and roots in a more efficient way. Some overseas studies have reported increased N uptake and crop yield after application of urea in foliar form (Giroux 1984; Turley and Ching 1986; Millard and Robinson 1990; Smith et al 1991; Gooding and Davies 1992; Tejada and Gonzalez, 2004). For example Franke (1967) reported that foliar uptake of urea-N is more successful than other forms of N as the urea improves the permeability of the cuticle and so facilitates diffusion into the leaf. According to Cook and Sehgal (1970), urease is a substrate-inducible enzyme, so as urea is taken up, the ability of the plant tissue to metabolize it to amino acids is increased. Turley and Ching (1986) reported that urea can be rapidly absorbed and assimilated by leaves of barley following foliar application. Within 4 hours of application of 30 g of N kg^{-1} fresh weight, seedling leaves contained 44-fold more urea than controls. Van Keulen et al. (1989) suggested that with time, a higher N percentage in the leaf

will automatically result in increased yield. Castle et al. (2006) reported that direct uptake of urea through the leaves increases growth of clover plants; possibly because this reduces energy requirements for assimilation and it provide more time for uptake.

In New Zealand, Summit-Quinphos (NZ) Ltd., working with Helicopter Services Ltd., have pioneered the application of its urease-inhibitor (nBTPT, hereafter referred to by the name ('Agrotain') with urea in suspension form. The urease-inhibitor nBTPT is commercially available under the trade name of Agrotain. Agrotain is a clear green liquid containing a 25% w/w solution of nBTPT in stabiliser solution. In New Zealand a rate of 1L Agrotain/tonne urea is used. Recently a truck application technology has also been introduced by Quinspread (Quin 2008). Field trials have been conducted on mixed grass/clover pasture to examine the effects of granular and suspension applications of urea, with and without Agrotain (Quin et al. 2006, 2009; Zaman and Blennerrhassett 2009). They found better N use-efficiency and pasture growth after application of urea with Agrotain in FPA form under a wide range of soil and environmental conditions compared with granular form.

1.3 Rationale of the present study

Recent research shows that Agrotain-treated urea may be effective in reducing N losses and enhancing fertiliser N use-efficiency after urea application (Blennerhassett et al. 2006; Sanz-Cobena et al. 2008; Watson et al. 2008; Zaman et al. 2008). It is likely that this is through reduced urea hydrolysis but it may also enable plants to take their N up in either urea or NH_4^+ forms that require less energy for metabolism. To date, no research has been undertaken on the direct absorption of urea with urease inhibitors by pasture plants leaves and roots, its dispersion, diffusion and movement in soils and its metabolism. The focus in this present

study is an investigation of the mechanisms underpinning uptake and assimilation of urea in the presence of urease inhibitor under controlled and field conditions.

As previously mentioned, urease inhibitor has the potential to reduce urea hydrolysis and improve fertiliser N efficiency by increased N uptake. However, the mechanism by which these increases in N response and N uptake take place after application of urea with urease inhibitor has not been studied. Plants take up N both as NH_4^+ or NO_3^- as a result of a change in the N form; however, there may be benefits of NH_4^+ or urea over NO_3^- as a result of reduced energy involved in metabolism into protein (Middleton and Smith 1979; Raven 1985; Ullrich 1992). In addition, the importance of NO_3^-, NH_4^+, and urea to plants is well documented in the literature. In this study the potential of incorporating granular and FPA urea fertiliser with Agrotain and other common forms of N fertilisers to enhance N uptake efficiency, and the mechanism and movement of urea, NH_4^+ and NO_3^- in soil was investigated.

1.5 Overview

To address the questions raised, this thesis is arranged into six chapters covering the following topics:

Chapter 1

General introduction concerning the use of urea fertiliser and its potential losses and mitigation options currently presented in the literature. This chapter also outlines the aims and overview of this research.

Chapter 1

Chapter 2

An investigation was carried out to understand the potential for incorporating granular urea fertiliser with the urease inhibitor N-(n-butyl) thiophosphoric triamide (nBTPT - **"Agrotain"**), to enhance fertiliser N efficiency in ryegrass pastures. The key objectives of this chapter were to: (1) determine the response of pasture production and N uptake to granular urea fertiliser coated with Agrotain. These treatments were also compared with other common chemical N fertilisers. (2) I also quantified rates of N uptake using a ^{15}N urea tracer, with or without Agrotain, to further investigate the effect of Agrotain on N uptake and urea hydrolysis.

The findings of this chapter have been published in *Crop & Pasture Science* (2010, Vol. 61: 214-221). A copy of the paper can be found in Appendix.

Chapter 3

An investigation was carried out, if urea fertiliser in fine particle application (FPA), with or without the urease inhibitor N-(n-butyl) thiophosphoric triamide (nBTPT - **"Agrotain"**), affects N uptake efficiency under optimum soil moisture and temperature conditions. These treatments were also compared with other common forms of N fertilisers (ammonium nitrate, ammonium sulphate or sodium nitrate). I tested the hypotheses that urea without Agrotain applied in FPA form will improve the N response and response efficiency in ryegrass when compared with granular application, but that combining urea FPA with Agrotain would further improve N uptake efficiency. The mechanism of N uptake, especially direct absorption of urea by herbage leaves/roots in the presence of Agrotain, was investigated in a 2nd experiment using ^{15}N-labelled urea.

The research in this chapter has been submitted for publication in *Soil Science & Plant Nutrition*.

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Chapter 4

A glasshouse-based study was conducted to investigate the effect of urease inhibitor N- (n-butyl) thiophosphoric triamide (NBPT - **"Agrotain"**) and irrigation on urea hydrolysis and its movement through the soil profile. The objective of this study was to: (1) track urea movement after surface application (with or without urease inhibitor), and also probe the impacts of soil moisture content following simulated irrigation (2) establish the effects of applying urea with Agrotain in granular form onto the soil in terms of urea hydrolysis, and (3) investigate the extent to which retaining N in urea form allows for greater diffusion of the intact urea molecule into the dense pasture rooting system in the soil.

The findings of this chapter have been published in *Biology and Fertility of Soils.* http://dx.doi.org/10.1007/s00374-010-0515-3. A copy of the paper can be found in Appendix.

Chapter 5

Having achieved the results reported in Chapters 2, 3 and 4 under controlled-environment (optimum soil moisture and temperature) conditions which may differ from natural conditions, the key aims here were to compare and assess: (1) N losses and bioavailability of urea applied with Agrotain in granular form under field conditions; (2) test the hypotheses that urea applied in FPA form will reduce N losses and will improve response (defined as dry matter response (or N uptake response) in N-applied treatments compared to the control) when compared with granular application in the field; and (3) assess if combining urea FPA with Agrotain would further reduce N losses and will improve N uptake efficiency. Recovery of N in plant-soil system was investigated using ^{15}N-labelled urea.

Chapter 1

The research in this chapter has been submitted for publication in *Agricultural Ecosystems and Environment*.

Chapter 6

The final chapter consists of a general discussion and synthesis of the results presented in the body of this thesis, and areas of possible future research.

Chapter 2

The impact of urease inhibitor on the bioavailability of nitrogen in urea and in comparison with other nitrogen sources

2.1 Introduction

Nitrogen (N) is an essential nutrient for plant growth and development (Simpson 1987) as it plays a key role in the synthesis of protein and chlorophyll, which are essential for plant development, yield, post-grazing re-growth and reproduction (Vickery 1981). In response to high agricultural commodity prices, World N fertiliser demand is projected to grow steadily. From average N fertiliser consumption in 2004/05 and 2006/07 of 97.9 and 100.1 million tonnes, global demand in 2011/12 is predicted to increase to 107.5 million tonnes by 2011/12 (the International Fertiliser Industry Association 'IFIA' 2007).

Pastures in New Zealand are comprised predominantly of ryegrass (*Lolium perenne* L.) and some white clover (*Trifolium repens* L.), and thus can receive much of their N from biological fixation of atmospheric di-nitrogen (N_2) and excreta (urine + dung) of grazing animals (Saggar 2004). However, farmers still apply chemical fertilisers, mainly urea at small application rates (30 to 40 kg N ha^{-1}) after 1-2 grazing cycles (Blennerhassett et al. 2006), to meet feed demands of stock, especially after calving. Di-ammonium phosphate (DAP), ammonium nitrate, ammonium sulphate and sodium nitrate are applied to pasture soils, but the dominant form of fertiliser N is urea. Ammonium nitrate contains 33 to 34% N, with one-half of the N in the form of ammonium (NH_4^+) and the other half in the nitrate (NO_3^-) form. Because it is entirely available to plants as soon as it dissolves, ammonium nitrate is one of the quickest-acting N fertilisers. It is very hygroscopic, and requires extra care in storage and

handling. It can be explosive under certain conditions, and is more prone to leaching and denitrification. Ammonium sulphate is a sulfur source, which can be beneficial in some situations. However, ammonium sulphate has a relatively low N concentration (21% N) and it has a stronger acid-forming reaction in soil than other N fertilisers. Sodium nitrate (15% N) contains its entire N in the NO_3^- form, and, therefore, it is highly susceptible to leaching and denitrification losses. Urea accounts for 50 % of the total world N-consumption (IFIA 2007) and its use in New Zealand has also increased sharply. In New Zealand, 0.399 million tonnes of urea was applied in 2004. Since 2002, application of urea has increased by approximately 27 percent (Statistics New Zealand 2006).

Under some circumstances, urea application has been associated with relatively poor N-use efficiency due to heavy N losses (1.7 to 56% of the applied N) which depends on soil moisture, temperature and pH, wind velocity, soil organic C, and N fertiliser type (Black et al. 1985; Fenn and Hossner 1985; Freney et al. 1985; de Datta et al. 1989; Gioacchini et al. 2002). Granular urea may be subject to rapid hydrolysis (within 1 to 2 days) (Zaman et al. 2008) to produce NH_4^+ and hydroxyl (OH^-) ions, which temporarily raise soil pH around the urea granule (Mulvaney and Bremner 1981). The saturation of the soil mineral N pool by high NH_4^+, along with the temporary rise in soil pH, can result in increased N losses via ammonia (NH_3) volatilisation (Zaman et al. 2009). Such NH_3 losses from applied urea are clearly undesirable because, in addition to lowering the efficiency of the applied fertiliser, they pose a major threat to environmental quality, as NH_3 lost to the atmosphere from applied urea may subsequently be deposited on land or water, causing eutrophication and acidification of natural ecosystems on a regional scale (Sommer and Hutchings 2001). Ammonia volatilisation may also add to global warming by acting as secondary source of N_2O (Martikainen 1985). It is therefore essential to develop fertilisation management strategies to improve urea N uptake and decrease N losses.

Among the different management options (e.g. avoiding heavy N application rates, applying N fertiliser at appropriate time, splitting N applications, using slow release fertiliser, or coating N fertilisers with polymers or elemental S), adding urease inhibitors to urea may have the greatest potential to reduce N losses and enhance its N efficiency (Carmona et al. 1990; Chai and Bremner 1987; Blennerhassett et al. 2006; Zaman et al. 2008). Of the various urease inhibitors [including hydroxyl urea, phosphoroamides, phenyl phosphorodiamidate (PPDA)], N-(n-butyl) thiophosphoric triamide (nBTPT, 'Agrotain'), is regarded as the most effective at low concentrations (0.1%) when coated onto urea (Bremner and Chai 1986; Joo et al. 1987; Watson et al. 1994). N-(n-butyl) thiophosphoric triamide, itself is not an active urease inhibitor, but after application, it is quickly converted in the soil to its oxygen analogue N-(n-butyl) phosphoric triamide, which then slows urea hydrolysis (Christianson et al. 1990).

Increased herbage DM has been reported after applying granular urea with Agrotain to grazed pastures in New Zealand (Blennerhassett et al. 2006; Martin et al. 2008; Zaman et al. 2008). However information on the mechanism which increases N response and N uptake after application of urea with Agrotain is lacking. In this Chapter, two experiments were established to test the hypothesis that coating urea fertiliser with Agrotain would result in increased N uptake. Nitrogen uptake by herbage in response to other chemical fertilisers was also assessed in order to provide a broader comparison with other commonly used N sources. In a second experiment, rates of N uptake were measured by using ^{15}N-labelled urea with or without Agrotain.

Chapter 2

2.2 Materials and methods

2.2.1 Experiment 1 – Herbage response to fertiliser treatments

A glasshouse experiment was conducted at the University of Canterbury using topsoil (0-75 mm) which was collected from a grazed pasture site near Lincoln, Canterbury New Zealand (43° 64'32.00" S, 172° 38'58.90" E).The soil used was Paparua silt loam, Typic Haplustepts (Soil Survey Staff 1998). After removing visible plant litter and root material, the soil was sieved to 2 mm, brought to soil water content of 80% field capacity and transferred to small trays (420 mm x 300 mm) to a depth of 65 mm (6 kg tray^{-1}). The soil in each tray was treated with a basal dose of phosphorus (P) at 40 kg ha^{-1} using triple super phosphate (TSP) 2.5 g tray^{-1} and sulphur (S) elemental S 0.5 g tray^{-1}. Four soil samples, each sample comprising 10 randomly collected soil cores, were analysed for key soil properties (Table 2.1).

Table 2.1. Physical and chemical properties of the soil used in experiments.

Soil properties	Values
pH	5.65
Total N (%)	0.38
Organic matter (%)	7.0
Olsen P (µg/ml)	20
CEC (me/100g)	14
Ca_2^+ (me/100g)	6.7
K^+ (me/100g)	0.45
Mg^{++} (me/100g)	1.74

Each tray was sown (143-147 seeds per tray) with perennial ryegrass cv. 'Grasslands Nui' in four rows with a row-to-row distance of 60 mm (Plate 2.1). Trays were weighed every 3 days and soil water adjusted to 80 % of field capacity. Trays were randomised within the glasshouse every day. After two months, plants were cut to 40 mm above ground level. Three days after herbage cut, the five chemical fertiliser treatments (urea, Agrotain-treated urea, ammonium nitrate, ammonium sulphate or sodium nitrate) were broadcast evenly onto

individual trays by hand at a rate equivalent to either 25 or 50 kg N ha^{-1}. Each treatment had four replicates. The control treatment received no N.

Herbage from each tray was harvested to a standard height of 40 mm on day 21 and day 42 of the treatments application. Consistency of cutting height was maintained using a moveable metal frame. Bulk fresh weight harvested from each tray was recorded. To determine herbage moisture fraction and N uptake, small herbage sub-samples were obtained randomly from each tray, weighed fresh, transferred to pre-weighed paper bags and dried at 65 ^{o}C for 7 days as described in Zaman et al. (2008). After drying, weighed plant material was ground to <0.2 mm and analysed for total N concentration using a total carbon, nitrogen and sulphur analyser (LECO CNS-2000 elemental analyser, Australia). Herbage DM, N response and response efficiency were calculated. Nitrogen response was calculated by subtracting pasture dry matter yield of the control (no N treatment) from yield from individual fertiliser treatments. Nitrogen response efficiency (g of pasture dry matter yield produced per g of applied N) was calculated by dividing N response by the amount of applied N. Nitrogen uptake was calculated by multiplying pasture dry matter yield by N content in plant and dividing by 100.

Plate.2.1. Glasshouse set-up used for 1st experiment.

Samples of herbage from each tray were taken on a weekly basis to monitor changes in nitrate reductase activity (NRA) and ammonium-N (NH_4^+) and nitrate-N (NO_3^-) contents in the tissue. At the same time each day (between 9 and 10 am) leaf nitrate reductase activity was assessed using an *in vivo* assay previously used in nitrate-use investigations (Smirnoff and Stewart 1985; Stewart et al. 1992). Following incubation of leaf tissue in a buffer containing nitrate, a 1.0 ml sample was assayed for the enzymatic production of nitrite using a standard colorimetric method (Andrews 1986). Tissue ammonium and nitrate contents were assessed on methanol extracts also using standard colorimetric methods (Andrews 1986). A fresh herbage sample (0.2g) was placed in glass vial and extracted using 5 ml of methanol for 24 hours at room temperature. A 20 μl aliquot of the methanol extract was placed in test tube for determination of nitrate concentration following cadmium reduction to nitrite. A 1.0 ml aliquot of the same methanol extract was placed in a test tube for determination of ammonium concentration. Herbage extraction was evaporated and re-dissolves in loading buffer (pH 2.2). The amino acid content was then determined (Nutrition

Laboratory, Massey University, Palmerston North, New Zealand) on a Waters ion-exchange HPLC system (Waters WISP715, Waters Corp., Milford, MA) with postcolumn ninhydrin derivatization and detection at 570nm (440 nm for proline).

2.2.2 Experiment 2 - ^{15}N labelled pot experiment

For the ^{15}N experiment, approximately 1.5 kg field-moist soil was placed in pots (140 mm in diameter) to a depth of 150 mm. Each pot was sown with perennial ryegrass cv. 'Grasslands Nui' (7-10 seeds per pot) and watered as described previously (Plate 2.2). Three weeks after germination, the plants were well established and cut to standard height of 40 mm above ground level. After three weeks the herbage was re-cut to 40 mm above ground level. After a further two weeks of re-growth the herbage was cut to 80 mm height and ^{15}N-labelled urea (10 atom%), with or without Agrotain granules, was uniformly applied to the soil surface at the rate equivalent to 25 kg N ha^{-1} (41.25 mg N pot^{-1}), the experiment involved 3 replications per treatment plus 3 control pots with no added fertiliser. Granular ^{15}N-labelled urea (10 atom%) was obtained from Novachem (Sydney, Australia), and was coated with the urease inhibitor by Summit-Quinphos (NZ) Ltd. Pots were immediately watered with distilled water to reduce ammonia volatilisation (Watson and Miller 1996), and water content of the pots was maintained at a maximum of 80% field capacity. Replicate pots were destructively harvested at 0.5, 1, 2, 3, 5, 10 and 21 days after treatment application to determine herbage DM yield and ^{15}N uptake by herbage. After each harvest, plants roots and leaves were rinsed gently with tap water followed by distilled water and then separated into roots and leaves. Fresh weights of shoots and roots were recorded, transferred to pre-weighed paper bags and dried at 65 $^{\circ}$C for 7 days. After drying, pasture material in paper bags were weighed and ground in a ball mill for total N and ^{15}N determination. Total N and ^{15}N in herbage and air-dried soil sub-samples were analysed using a Dumas elemental analyser (Europa Scientific ANCA-SL) interfaced to an isotope mass spectrometer (Europa Scientific 20-20 Stable

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Isotope Analyser; Europa Scientific Ltd, Crewe, U.K.) at the University of Waikato Stable Isotope Analysis Unit. Calculations of ^{15}N recovery in plant were carried out as described by the International Atomic Energy Agency (1976).

The percentage nitrogen derived from fertiliser (% N dff) =

$$\left(\frac{\%^{15}N \ \ excess \ in \ sample}{\%^{15}N \ \ excess \ in \ fertiliser} \right) \times 100$$

The percentage utilisation of applied nitrogen $= \dfrac{\left(\%N \ dff \times yield \ of \ N\right)}{rate \ of \ N \ application}$

Sub-samples of soil (5 g on an oven dried basis) were also taken from each pot at every harvest to determine urea hydrolysis by measuring concentrations of mineral N in the soil. Soil was extracted with 2M KCl for 1 h and filtered through Whatman 42 filter paper. Soil extracts were immediately frozen and then analysed for NH_4^+-N and NO_3^--N concentrations by flow injection analyser (FIA; Plant and Food Research Institute, Lincoln, New Zealand).

Plate.2.2. Glasshouse set-up used for 2nd experiment.

2.2.3 Statistical analysis

Analyses of variance (ANOVA) were performed using Minitab (Version 12, Minitab Inc. USA). Least significant differences (LSD) were calculated to compare treatment means at $P<0.05$. In experiment 2, repeated-measure analysis of variance (ANOVA) was used to determine if time had a significant effect on different parameters, ANOVA was then used at individual time points when the treatment x time interaction was found to be significant.

2.3 Results

2.3.1 Experiment 1 - herbage production, fertiliser N response and total N uptake

Herbage dry matter yields in response to all fertilisers (applied at either 25 or 50 kg N ha^{-1}) were significantly higher than that of the control treatment. Agrotain-treated urea applied at either 25 or 50 N ha^{-1} produced significantly ($P<0.05$) greater herbage dry matter yield (cumulative of 2 pasture cuts) than other treatments (Table 2.2). The increase in herbage dry matter yield with Agrotain-treated urea applied at either 25 or 50 N ha^{-1} compared with urea was 16% or 19%, respectively (Table 2.2). With the exception of ammonium sulphate, fertilisers applied at 25 kg N ha^{-1} exhibited greater response efficiency than those treatments applied at 50 kg N ha^{-1}.

Nitrogen response followed a similar pattern to that of the herbage dry matter yield (Table 2.2). Agrotain-treated urea applied at 25 N ha^{-1} increased N response by 66% compared with urea alone. The improvement in N response with Agrotain-treated urea was even greater than this relative to the NH_4^+- and NO_3^--based fertilisers. Response efficiency also varied among the different treatments (Table 2.2). Agrotain-treated urea applied at 25 kg N ha^{-1} produced 13 g DM g^{-1} of applied N compared with 8 g DM g^{-1} of applied urea-N at the same rate.

Overall, Agrotain-treated urea applied at the 25 kg N ha^{-1} rate resulted in a significantly higher response efficiency than urea, ammonium nitrate and ammonium sulphate fertilisers applied at the same rate. No differences in response efficiency were observed at the higher application rate. Nitrogen uptake by the herbage was also significantly ($P<0.05$) greater when the herbage was supplied with Agrotain-treated urea at 25 or 50 kg N ha^{-1} compared with urea alone at the same rates (Table 2.2). Over the 42-days period, Agrotain-treated urea applied at 25 kg N ha^{-1} increased uptake by 26% compared with urea alone and approximately 11% in comparison with other N fertiliser treatments. At 50 kg N ha^{-1}, Agrotain-treated urea increased uptake by 38% cf urea, 22% cf ammonium nitrate, 13% cf ammonium sulphate and 16% cf sodium nitrate.

Table 2.2. Total herbage dry matter yield (g/m^2) and N response (g DM/m^2), total N content (g N/m^2), and response efficiency (g DM/g of applied N) to urea with or without Agrotain and different forms of chemical fertiliser applied in granular form (Expt 1) over a 42 day period.

Treatments	Total herbage DM	N uptake	N response	Response efficiency
	(g/m^2)	(g N/m^2)	(g DM/m^2)	(g DM/g of applied N)
Control (no N)	64^a	1.6^a		
Urea-25	83^b	2.7^b	19^a	8^{ab}
Urea-50	88^b	3.2^{bc}	23^{ab}	5^a
Urea + Agrotain-25	96^c	3.4^{cd}	31^c	13^c
Urea + Agrotain -50	104^d	4.4^e	40^d	8^{ab}
Ammonium nitrate -25	83^b	3.1^b	18^a	8^{ab}
Ammonium nitrate -50	90^{bc}	3.6^d	25^{ab}	5^a
Ammonium sulphate -25	82^b	3.1^b	18^a	7^{ab}
Ammonium sulphate -50	95^c	3.9^d	33^{bc}	7^{ab}
Sodium nitrate -25	89^{bc}	3.1^b	24^{ab}	10^{bc}
Sodium nitrate -50	94^c	3.8^d	29^{bc}	6^a

Within columns, means with the same letters are not significantly different at the P < 0.05 level.

The relative effects of N fertiliser on % changes in dry matter production are shown in Fig. 2.1 (A1 + A2) and % changes in tissue N content in Fig. 2.1 (B1 + B2). Uptake of N (and thus increases in tissue N content) exceeded growth of new tissue (i.e. negative % difference between dry matter changes and N content change, (Fig. $2.1C_1$). By cut 2 (42 days) growth increment had equalled or exceeded any increase in tissue N content (% differences between dry matter change and N content change close to zero or positive, Fig. $2.1C_2$).

2.3.2 Experiment 1 - Nitrate reductase activity and ammonium, nitrate and amino acid contents of leaf tissue

Nitrate reductase activities measured over the 42 days were not significantly affected by the added fertiliser treatments (average 450 nmol NO_2/ g fresh weight/hour, range 300-600 nmol NO_2/ g fresh weight/ hou. Ammonium and nitrate content of the leaf tissue were not significantly influenced by fertiliser treatment. Ammonium content of the leaf tissue averaged 9 nmol/ g fresh weight (range 4-14 nmol g/ fresh weight) and nitrate content averaged 700 μmol /g fresh weight (range 300-1000 μmol/ g fresh weight). Leaf amino acid concentrations (Table 2.3) were generally found to be within the range previous published for pasture grasses (Tania et al., 2000). Fertiliser addition did not significantly affect amino acid concentrations in leaf tissue (Table 2.3).

2.3.3 Experiment 2 - Soil NH_4^+ -N and NO_3^--N

Urea applied with Agrotain delayed urea hydrolysis by releasing NH_4^+ at a slower rate compared with urea alone (Fig. 2.2a). In contrast, urea applied alone exhibited more rapid hydrolysis soon after its application, as evidenced by significantly higher concentrations of soil NH_4^+ on day 1 and 2. Soil NH_4^+ concentration in the urea alone treatment reached its maximum on day 1, and decreased afterward. Soil NO_3^- concentrations were lower than NH_4^+ and were not significantly influenced by urea with or without Agrotain (Fig. 2.2b), although

during the first 3 days, urea with Agrotain treatment had slightly higher NO_3^- concentrations than that of urea alone. After day 5, NO_3^- concentrations in both treatments increased, up to day-10 and decreased thereafter.

Table 2.3. Effect of urea with or without Agrotain and different forms of N fertiliser on amino-acid contents of the shoot (nmol/g (Experiment 1)

Treatments	Aspartic acid	Threonine	Serine	Glutamic acid	Proline	Glycine	Alanine	Cysteine	Valine	Isoleucine	Leucine
Urea only	49	34	51	203	11	7	92	3	16	8	10
Urea+Agrrotain	63	39	62	232	9	8	90	3	14	7	8
Ammonium nitrate	57	41	56	207	6	6	72	2	11	5	7
Ammonium sulphate	60	35	56	291	8	9	80	3	13	6	8
Sodium nitrate	64	31	50	207	6	7	77	3	12	6	7
LSD(p<0.05)	23	17	19	51	5	8	23	2	5	3	4
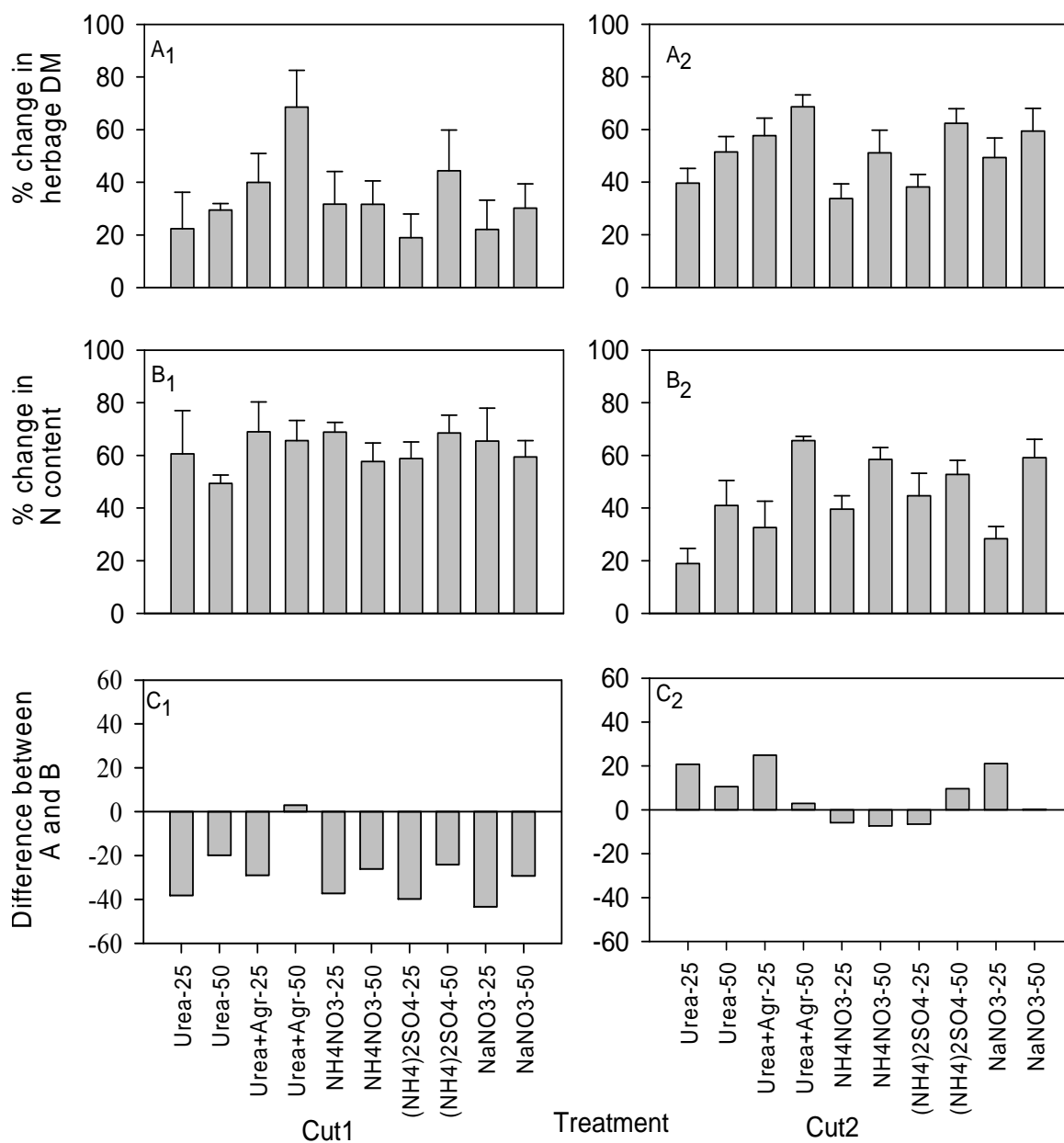
Fig. 2.1. The relative effects of N fertilisation on (A_1, A_2) % change in herbage dry matter (relative to controls), (B_1, B_2) % changes in N content (relative to controls) and (C_1, C_2) the difference between A and B (Expt 1). Bars are means ± SEM where n=4.
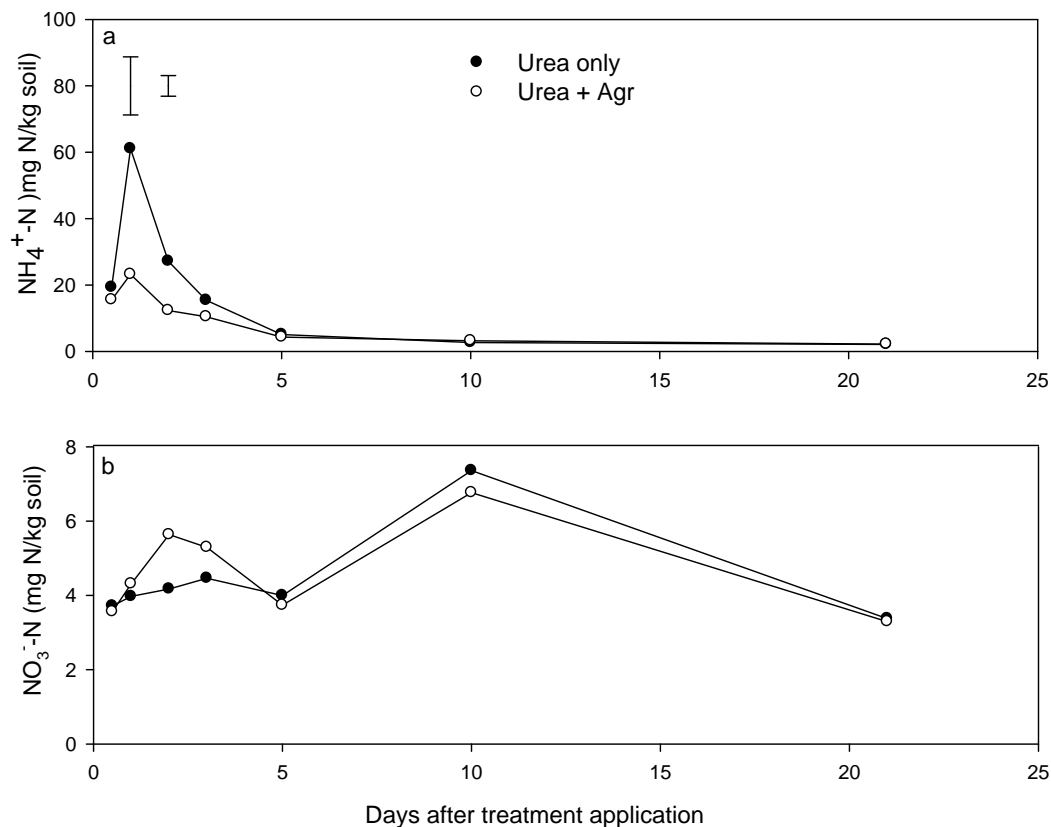
Fig. 2.2. Effect of ^{15}N urea with or without Agrotain on soil mineral-N after application (Expt 2). Vertical bars represent l.s.d. values where treatment means are significantly different at *p*= 0.05.

2.3.4 Experiment 2 – herbage production, N uptake and ^{15}N recovery in plant

Herbage dry matter, N uptake and %^{15}N recovery measured at 0.5, 1, 2, 3, 5, and 10 and 21 days after ^{15}N urea application with or without Agrotain are shown in Fig. 2.3. Herbage dry matter during the first 10 days of fertiliser application increased slowly and was not significantly affected by the applied treatments (Fig. 2.3a). After 10 days, urea with Agrotain produced significantly more herbage dry matter (144 g DM/m^2) compared with urea alone (118 g DM/m^2). Herbage N uptake was not significantly different between the fertiliser treatments during the first 3 days after treatment application (Fig. 2.3b). After day 3, herbage treated with urea plus Agrotain exhibited significantly greater N uptake than that treated with

urea alone. Overall, total N uptake of herbage treated with urea plus Agrotain was 35.7 % greater than herbage treated with urea alone (Table 2.4). In the shoots there was no significant difference in ^{15}N recovery between the two treatments during the first 3 days after application (Fig. 2.3c). Thereafter, urea plus Agrotain treatment resulted in significantly higher ^{15}N recovery compared with urea alone. The ^{15}N contents of the roots measured at different times were also influenced by urea with or without Agrotain. Agrotain-treated urea resulted in roots with higher ^{15}N content than those roots from the urea treatment alone (Fig. 2.3d).

Table 2.4. Herbage dry matter yield and N response (g DM/m^2), total N content (g N/m^2), and response efficiency (g DM g^{-1} of applied N) in response to addition of ^{15}N-urea with or without Agrotain applied in granular form (Expt 2).

Treatments	Herbage dry matter	N response	N uptake	Response efficiency
	(g DM/m^2)		(g N/m^2)	(g DMg^{-1} of applied N)
Control (no N)	32.1^a		0.4^a	
Urea only	118^b	85.9^a	1.4^b	34.3^a
Urea + Agrotain	144.6^c	112.5^b	1.9^b	45.0^b

Within columns, means with the same letters are not significantly different at the P < 0.05 level.

2.4 Discussion

All forms of chemical fertilisers applied at either 25 or 50 kg N ha^{-1} produced a significantly (*P*<0.05) higher herbage DM, N uptake and fertiliser N response compared with the control treatment in Experiment 2 (Figs. 2.3a and b). Urea with Argotain increased further herbage DM, N uptake and fertiliser N response compared with urea alone in and other fertiliser treatments. The N responses occurred despite the fact that the optimal soil moisture conditions (75–80% of field capacity) maintained in these experiments was favourable for urea hydrolysis and nitrification but prevented leaching losses (nitrate) and reduced volatilisation (urea). The studies conducted in Chapter 2 do not measure NO$_3^-$ leaching loss

or volatilisation loss. Under these circumstances we anticipated that the physical conditions in the experiment would allow the various fertilisers to perform equally well.
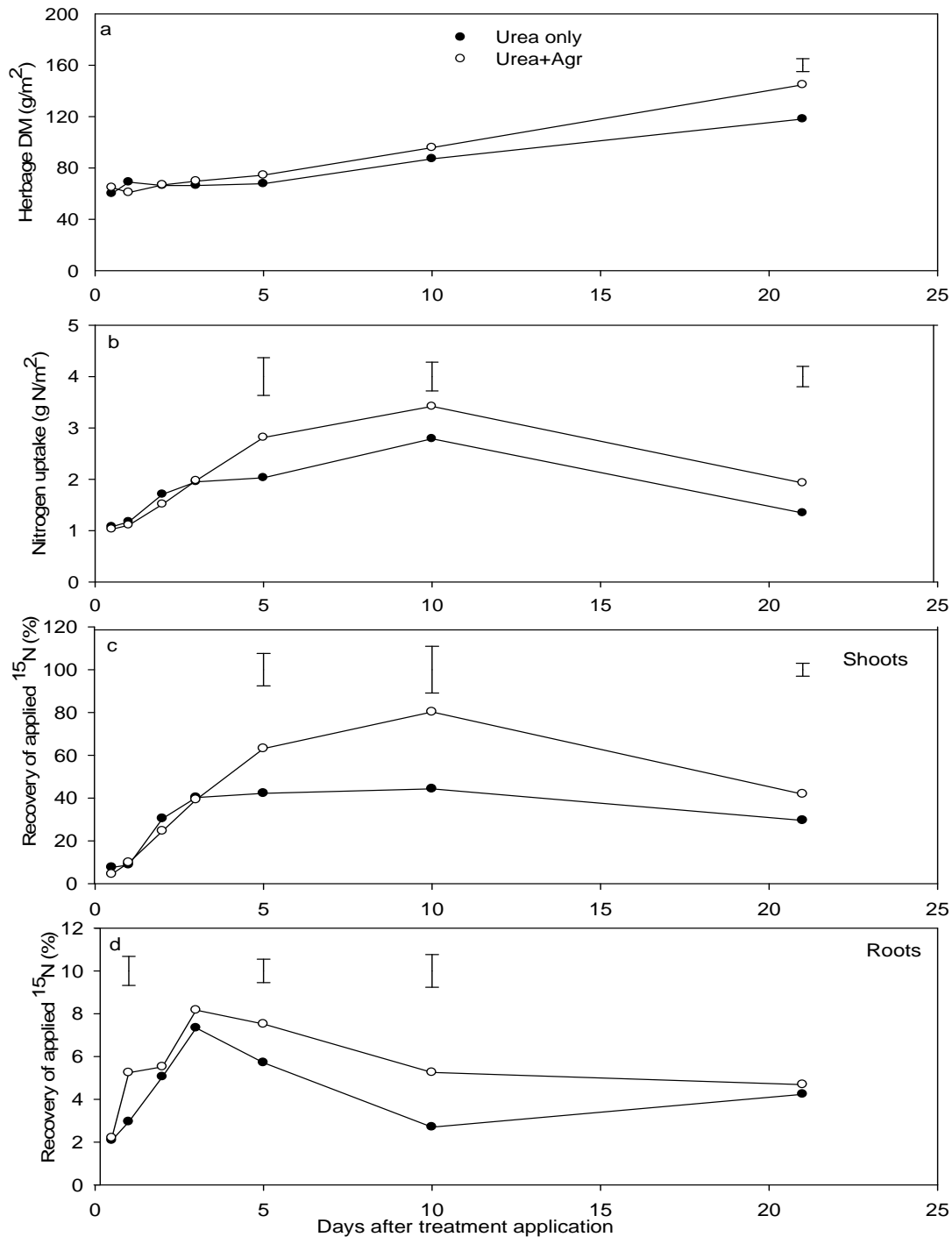
Fig. 2.3. Effect of ^{15}N urea with or without Agrotain on **(a)** herbage dry matter, **(b)** nitrogen uptake, **(c)** percentage recovery of applied ^{15}N by shoots, and **(d)** percentage recovery of applied ^{15}N by roots (Expt 2). Vertical bars represent l.s.d. values where treatment means are significantly different at $p = 0.05$.

These results have important implications for fertiliser application under field conditions, and are consistent with results from previous field studies. Agrotain-treated urea applied at a much higher rate (150 kg N ha^{-1}) in a field study produced 17% more pasture dry matter compared with urea alone (Zaman et al. 2008). Similarly, a trend of increased pasture dry matter and improved N response has also been observed in other field trials, where application rates similar to the present study (25 to 50 kg N ha^{-1} of Agrotain-treated urea) were applied to grazed pastures on different soil types and climatic conditions (Blennerhassett et al. 2006; Martin et al. 2008).

It is important to consider the relative significance of the potential mechanisms underpinning the growth responses observed in this and previous studies. Being an uncharged particle, urea is likely to diffuse easily into the rooting zone at moderate-high soil moisture and this tends to minimise NH$_3$ losses. Ammonia volatilisation losses from urea fertiliser in New Zealand have been reported to range from 5 – 25% of the applied fertiliser N (Theobald and Ball 1984; Black et al. 1985; Ledgard et al. 1999), especially under low soil moisture (30-50% FC), and this tends to indicate that the primary mechanism for improved yield in response to Agrotain-treated urea is reduced volatile losses. However, a recent trial using Agrotain-treated urea (Zaman et al. 2008) indicated that reductions in volatilisation may not be the only factor improving fertiliser efficiency. Increases in N response of 80 - 93% were measured, despite decreases in volatilisation representing only about 5% of the total N applied. This supports the notion that increases in herbage dry matter, N response and N uptake by Agrotain-treated urea over urea alone in the present study could be attributed to a number of factors in conjunction with reduced losses of volatile N e.g. delayed urea hydrolysis, direct urea uptake by ryegrass and improved N bioavailability.

In experiment 2, soil NH_4^+ production was low in Agrotain-treated urea compared with urea alone, but NO_3^- content was not significantly different. This suggests that urea hydrolysis was delayed by Agrotain (Watson 2000; Zaman et al. 2009) and that the urea may be taken up directly by ryegrass from the soil (Watson and Miller 1996). These results are in line with those of Zhengping et al. (1996), who also observed slow urea hydrolysis and a lower accumulation of soil NH_4^+ after applying Agrotain-treated urea to soils under controlled conditions. The importance of the study by Zhengping et al. (1996) is that it was conducted in the absence of plants. This precludes the possibility that plant uptake of NH_4^+ could explain reduced soil NH_4^+ concentration in the presence of Agrotain. We therefore, conclude that the greater recovery of applied N in the presence of Agrotain (Fig. 2.3) is a result of uptake of urea. Solution culture studies with rice have shown that, under conditions where urea is not hydrolysed, it can be taken up by roots as an intact molecule (Matsumoto et al. 1966; Bollard et al. 1968; Harper 1984).

The extension of the retention of N either in the urea form in the soil by 5 to 10 days as a result of the action of Agrotain (Zaman et al. 2009) or in NH_4^+ form (Fig. 2.2a) is important for subsequent plant uptake. The uncharged urea does not adhere to soil particles or organic matter, and the higher ^{15}N content of the root after 12 h of treatment application suggests that urea diffused into the root-zone, largely because of the optimum soil moisture content (75–80% FC). This diffusion of urea may result in improved N bioavailability by providing plants an opportunity to take up N in the urea form which is more efficient than other forms of N. Like herbage dry matter production, N uptake in both experiments in the present study exhibited significant improvements in response to Agrotain-treated urea compared with urea alone (Table 2.2 and Fig. 2.3b). In the ^{15}N experiment, the lack of any significant difference in N uptake during the first 3 days of treatment application highlights the fact that plant roots

had equal opportunity to take up applied nitrogen (either as urea or ammonium), with or without Agrotain (Fig. 2.3b). After the progressive disappearance of urea as a result of fast hydrolysis in the absence of Agrotain (Fig. 2.2a), Agrotain-treated urea resulted in significantly higher N uptake probably due to delayed urea hydrolysis.

Although not directly tested in this study, an added potential advantage of the action of Agrotain is an energetic one. Slow urea hydrolysis by Agrotain enable pastures to uptake N in either urea or NH_4^+ forms which may be incorporated into organic compounds and finally into plant protein at less energy cost compared to NO_3^-, suggesting that the pasture plant may be left with extra energy to allocate to growth. Castle et al. (2007) also demonstrated in a growth cabinet experiment that clover plants at 8°C took up significantly more N in urea form than NO_3^-, particularly through the leaves and this resulted in an increase in photosynthesis and increased dry matter production.

It is important to note that we found that fertiliser response efficiency was greater at the lower application rate (25 kg N ha^{-1}) than at the higher application rate (50 kg N ha^{-1}). Overall, Agrotain-treated urea applied at the lower rate resulted in a much higher response efficiency (>63%) than those of the other treatments. This higher response efficiency was possibly due to the fact that Agrotain maintains urea in the urea form for an extra 5-10 days. During this period, N uptake may be maximised at the lower application rate, but the extra N added at the higher rate may undergo greater rates of transformation without conferring a yield advantage. Blennerhassett et al. (2006) also observed a much higher improvement in N response efficiency by Agrotain-treated urea when it was applied to grazed pastures at a lower rate of 30 kg N ha^{-1} compared with higher rate of 60 kg N ha^{-1}.

To determine whether nitrogen taken up by the herbage was translated into dry-matter yield, the percentage change in each in response to fertiliser addition was calculated. At the first cut (21 days), there was significant fertiliser effect on % change in herbage dry matter yield (Fig. 2.1 A_1) but no significant fertiliser effect on % change in N content (Fig. 2.1 B_1). Calculation of the difference between the growth response and the N content response (Fig. 2.1 C_1) suggests that N consumption was greater in the early stages of the fertiliser response, but translation into dry-matter yield was low. However, this additional N may be beneficial later, when demands for N are higher. By cut 2 (42 days) there was a significant fertiliser effect on change in herbage dry matter yield (Fig. 2.1 A_2), and hence in N uptake (Fig. 2.1 B_2). Significantly, the difference between these two responses (Fig. 2.1 C_2) was close to parity or positive, indicating that the growth response had at least equalled the uptake of N. Clearly, for all forms of applied N, uptake of N precedes initiation of growth by some time. Alternatively, the lack of a strong growth response early in the pasture cycle may limit the efficient use of applied N, and may in part explain the reduced N efficiency under the high addition regime (50 kg N ha^{-1}). Another important implication of this lag in tissue growth response is an apparent reduction in calculated recovery of applied N in the latter stages of this 21 day experiment. Overall % recovery was found to decrease at day 21 because of the diluting effect of additional shoot growth on tissue N content. This impact was only made apparent by the extended length of this experiment compared to previous ^{15}N recovery experiments (e.g. Watson and Miller 1996).

 The concentration of a range of leaf tissue amino acids was found to be within the previously published range for pasture grasses (Streeter et al. 2000) and was not affected by Agrotain-treated urea compared with other fertiliser types after 21 days. Watson and Miller (1996) found that the concentration of a number of amino acids (e.g. γ amino-butyric acid, threonine,

serine, glycine, ornithine) in the shoot was significantly lower in tissues in response to urea + urease inhibitor compared with urea alone. However, their assays were carried out 4-10 days after the treatments were applied, suggesting a possible effect on transformation reactions. Watson and Miller (1996) also suggested "that urea-N within the plant is not… [used] in the same way as N taken up in the NH_4^+-N form". Our findings indicate that fertilisation with Agrotain-treated urea is unlikely to have detrimental effects on herbage N quality.

2.5 Summary

These findings in this chapter have indicated that the delay in urea hydrolysis is an important mechanism underpinning the benefit of Agrotain-treated urea, in addition to the reduction in ammonia losses reported elsewhere. However, we cannot exclude the possibility that there are other mechanisms, such as direct absorption of urea by ryegrass leaves/roots in the presence of Agrotain, which could result in improved N responses from Agrotain-treated urea compared with urea alone or other chemical fertilisers. Hence, the study described in the next chapter focusses on the potential for direct absorption of urea by ryegrass leaves/roots in the presence of Agrotain.

Chapter 3

Comparison of plant-availability of urea fertiliser in fine particle application or granular form and with urease inhibitor

3.1 Introduction

Although granular urea is becoming an increasingly important source of nitrogen (N) in pasture-based systems, its application has been reported to have low N efficiency (10 to 15 kg of dry matter produced per kg of applied N) compared with other chemical fertilisers (Watson et al. 1990; Harrison and Webb 2001; Blennerhassett et al. 2006). After application to the soil, urea is rapidly hydrolysed by soil urease enzymes, which temporarily increases soil pH around the urea granule (Mulvaney and Bremner 1981) and provides hot spots for N losses via NH_3 volatilisation. The literature reports a range of NH_3 losses from 4% to 36% of the applied N (Vertregt and Rutgers 1987; Lockyer and Whitehead 1990; Zaman et al. 2008). Such NH_3 losses from applied urea are clearly undesirable because, in addition to reducing the nutritional efficacy of the fertiliser, they can result in formation of the greenhouse gas nitrous oxide (N_2O) and acidification of soil and surface waters (Martikainen 1985; Janzen 1999). It is therefore essential to develop fertilisation management strategies that improve fertiliser N efficiency and decrease N losses.

A number of previous field and glasshouse trials have showed that coating granular urea with the urease inhibitor N-(n-butyl) phosphorothioic triamide (nBTPT, "Agrotain") had considerable potential for improving the N response of pasture after application (e.g. Blennerhassett et al. 2006; Chen et al. 2008; Martin et al. 2008; Zaman et al. 2008; this thesis, Chapter 2; Dawar et al. 2010a). Once applied to the soil, Agrotain is quickly converted to its

oxygen analogue N-(n-butyl) phosphoric triamide (NBPTO), which then forms a tridentate ligand with the urease enzyme (Manunza et al. 1999), delaying urea hydrolysis and reducing the concentration of NH_3 near the surface, thus decreasing the potential for volatilisation and increasing N uptake and yield (Carmona et al. 1990; Watson 2000; Gioacchini et al. 2002). As discussed in Chapter 2, treating urea with Agrotain may also provide plants an opportunity to take up more N in either urea or NH_4^+ forms and to convert N into plant protein more efficiently than NO_3^- (Middleton and Smith 1979).

The efficiency of urea may be improved further if urea is applied in suspension or fine particle application (FPA) form (Quin et al. 2006; Zaman et al. 2009). Fine particle application refers to a fluid made by mixing fertiliser ingredients which have been finely ground to 100-200 microns (0.1-0.2mm), with 30-40% water by weight. Fine particle application of urea has the potential advantages of low application rates, uniform distribution of fertiliser and quick plant response to applied nutrients, which are likely to minimise localised hot spots for N losses and may also provide plants an opportunity to take up the applied N directly in the urea form (Zaman et al. 2009). However, limited information is available on the application of urea with Agrtain in the FPA form, the factors affecting N uptake and yield in ryegrass pastures and the mechanisms underpinning observed responses.

In this chapter, the extent of N uptake efficiency under optimum soil moisture and temperature conditions of urea fertiliser in FPA, with or without Agrotain was examined. The impact of FPA on uptake of other common forms of N fertilisers (ammonium nitrate, ammonium sulphate or sodium nitrate) was also examined. The previous chapter investigated the potential of incorporating urea fertiliser in granular form with Agrotain to enhance fertiliser N uptake efficiency under controlled conditions (Chapter 2; Dawar et al. 2010a).

This showed that treating urea with Agrotain has the significant potential to increase N use efficiency and herbage production. The objective of this research was to investigate the impacts of applying urea, with or without Agrotain, to ryegrass in FPA form on herbage dry matter and N uptake. We tested the hypotheses that urea without Agrotain applied in FPA form will improve N response and response efficiency in ryegrass when compared with granular application, and that combining urea FPA with Agrotain would further improve N uptake efficiency. The mechanism of N uptake, especially direct absorption of urea by herbage leaves/roots in the presence of Agrotain, was investigated in a 2nd experiment using ^{15}N-labelled urea.

3.2 Materials and methods

3.2.1 Experiment 1: FPA and granular applications of ammonium and nitrate fertilisers

A glasshouse experiment was conducted at the University of Canterbury using topsoil (0-75 mm) from a grazed pasture site was collected near Lincoln, Canterbury New Zealand (43$^{°}$ 64$^{'}$32.00$^{"}$ S, 172$^{°}$ 38'58.90$^{"}$ E). The soil used was free-draining Paparua silt loam, Typic Haplustepts (Soil Survey staff 1998). After removing visible plant litter and root material, the soil was sieved to 2 mm, brought to soil water content of 80% field capacity and transferred to small trays (420 mm x 300 mm) to a depth of 65 mm (6 kg tray^{-1}). The soil in each tray was treated with a basal dose of phosphorus (P) at 40 kg ha^{-1} using triple super phosphate (TSP; 2.5 g tray^{-1}) and sulphur (S) at 0.5 g tray^{-1}. Four soil samples, each comprising 10 randomly collected soil cores, were analysed (Hill laboratories Ltd, Hamilton, New Zealand) for key soil properties (Table 3.1).

Each tray was sown (145-150 seeds per tray) with perennial ryegrass cv. 'Grasslands Nui' in four rows with a row-to-row distance of 60 mm. Trays were weighed every 3 days and soil

water adjusted to 80% of field capacity. Tray position was randomised every day. After 8 weeks establishment, plants were cut to 60 mm above ground level. Three days after herbage cut, the five chemical fertiliser treatments (urea, Agrotain-treated urea, ammonium nitrate, ammonium sulphate or sodium nitrate) were applied either in FPA (through spray) or granular (2-4 mm) forms at a rate equivalent to 25 kg N ha^{-1} (Plate 3.1). Each treatment had four replicates. The control treatment received no N.

Table 3.1. Physical and chemical properties of the soil used in experiments.

Soil properties	Values
pH	5.65
Total N (%)	0.38
Organic matter (%)	7.0
Olsen P (μg/ml)	20
CEC (me/100g)	14
Ca_2^+ (me/100g)	6.7
K^+ (me/100g)	0.45
Mg^{++} (me/100g)	1.74

Herbage from each tray was harvested to a standard height of 40 mm on day 21 and day 42 after treatment application. Consistency of cutting height was maintained using a moveable metal frame. Bulk fresh weight harvested from each tray was recorded. To determine herbage moisture fraction and N uptake, small herbage sub-samples were obtained randomly from each tray, weighed fresh, transferred to pre-weighed paper bags and dried at 65 ^{o}C for 7 days. After drying, weighed plant material was ground to <0.2 mm and analysed for total N concentration using a total carbon, nitrogen and sulphur analyser (LECO CNS-2000 elemental analyser, Australia). Herbage DM, N response and response efficiency were calculated. Nitrogen response was calculated by subtracting herbage DM yield of the control (no N treatment) from yield from individual fertiliser treatments. Nitrogen response efficiency was calculated by N response divided by the amount of applied N.
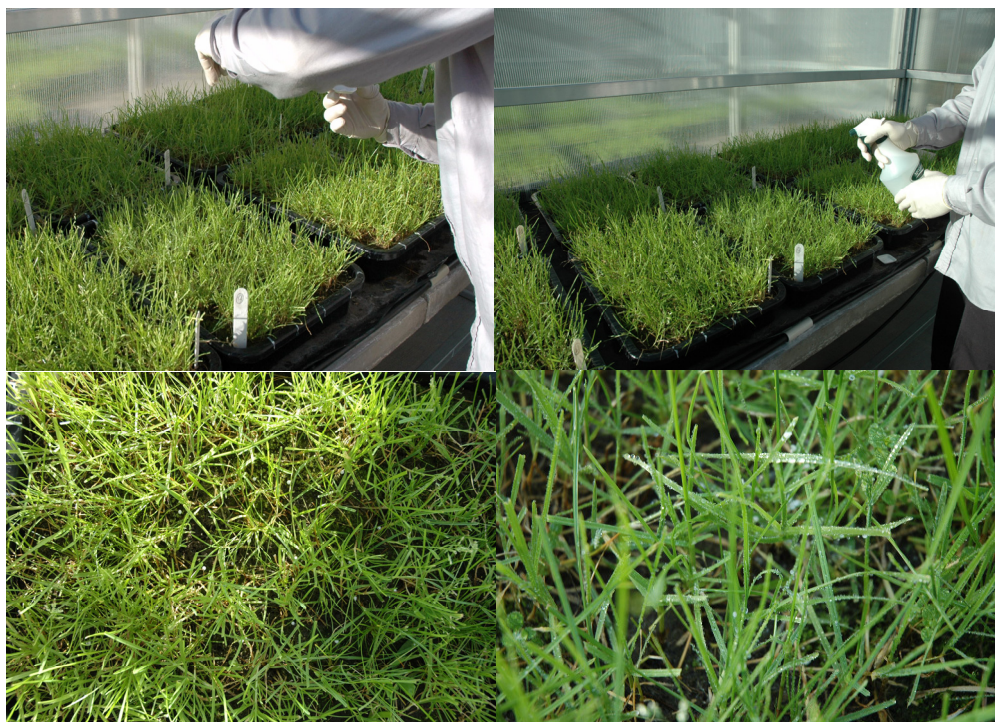
Plate 3.1. Glasshouse set-up used for 1st experiment.

Samples of herbage from each tray were also taken before each harvest to monitor changes in nitrate reductase activity (NRA) and ammonium (NH_4^+) and nitrate (NO_3^-) contents in the tissue. At the same time each day (between 9 and 10 am) leaf nitrate reductase activity was assessed using an *in vivo* assay previously used in nitrate-use investigations (Smirnoff and Stewart 1985; Stewart et al. 1992). Following incubation of leaf tissue in a buffer containing nitrate, a 1.0 ml sample was assayed for the enzymatic production of nitrite using a standard colorimetric method (Andrews 1986). Tissue ammonium and nitrate concentration were assessed on methanol extracts also using standard colorimetric methods (Andrews 1986). A fresh herbage sample (0.2g) was placed in glass vial and extracted using 5 ml of methanol for 24 hours at room temperature. A 20 μl aliquot of the methanol extract was placed in test tube for determination of nitrate concentration following cadmium reduction to nitrite. A 1.0 ml aliquot of the same methanol extract was placed in a test tube for determination of

ammonium concentration. Herbage extraction was evaporated and re-dissolved in loading

buffer (pH 2.2). The amino acid content was then determined (Nutrition Laboratory, Massey

University, Palmerston North, New Zealand) on a Waters ion-exchange HPLC system

(Waters WISP715, Waters Corp., Milford, MA) with postcolumn ninhydrin derivatization

and detection at 570nm (440 nm for proline).

3.2.2 Experiment 2: ^{15}N labelled pot experiment

For the ^{15}N experiment, approximately 1.5 kg field-moist soil was placed in pots (140 mm in

diameter) to a depth of 150 mm. Each pot was sown with perennial ryegrass cv. 'Grasslands

Nui' (7-10 seeds per pot) and watered as described previously. 56 days after sowing the

herbage was cut to 80 mm height and ^{15}N-labelled urea (10 atom %), with or without

Agrotain, was applied in solution (0.5 ml) form (through a syringe) at a rate equivalent to 25

kg N ha^{-1} either to the shoots and leaves-only or to the soil surface (avoiding the shoots and

leaves) (Plate 3.2). Plants in the shoots and leaves-only treatment had a circular piece of

Whatman® polythene-backed Benchkote covering the surface of the pot under the shoots, to

prevent N in runoff reaching the roots or the soil surface. Replicates pots were destructively

harvested at 0.16, 0.33, 1, 2, 3, 5, 10 and 21 days after fertiliser application to monitor ^{15}N

uptake by herbage and herbage dry matter yield. After each harvest, herbage leaves and roots

were washed gently with tap water followed by distilled water and then separated into roots

and leaves. Fresh weights of shoots and roots were recorded, and then tissue was transferred

to pre-weighed paper bags and dried at 65 $^{\circ}$C for 7 days. After drying, material in paper bags

was weighed, ground in a ball mill for total N and ^{15}N determination. Total N and ^{15}N in

herbage was analysed using a Dumas elemental analyser (Europa Scientific ANCA-SL)

interfaced to an isotope mass spectrometer (Europa Scientific 20-20 Stable Isotope Analyser;

Europa Scientific Ltd, Crewe, U.K.) at the University of Waikato Stable Isotope Analysis

Unit. Calculations of ^{15}N recovery in plant were carried out as described by the International Atomic Energy Agency (1976).

The percentage nitrogen derived from fertiliser (% N dff) =

$$\left(\frac{\%^{15}N \;\; excess \;\; in \;\; sample}{\%^{15}N \;\; excess \;\; in \;\; fertiliser} \right) \times 100$$

The percentage uptake of applied nitrogen = $\dfrac{\left(\%N \; dff \times yield \; of \; N \right)}{rate \; of \; N \; application}$

Soil sub-samples (5 g on oven dry basis) were also taken out from each pot after every harvest to determine urea hydrolysis by measuring concentrations of mineral N in the soil. Moist soil (5 g oven dry basis) was extracted with 25 mL 2M KCl for 1 h and filtered through Whatman 42 filter paper. Soil extracts were immediately frozen and then analysed for NH_4^+-N and NO_3^--N concentrations by flow injection analyser (FIA; New Zealand Plant and Food Research Institute, Lincoln, New Zealand).

Plate 3.2. Glasshouse set-up used for 2nd experiment.

3.2.3 Statistical analysis

Analyses of variance (ANOVA) were performed using Minitab (Version 12, Minitab Inc. USA) to test for the effects of fertiliser type and method of application. Least significant differences (LSD) were calculated to compare treatment means at P<0.05. For the time-based measures in experiment 2, repeated-measure analysis of variance was used to determine if time had a significant effect on different parameters. One-way ANOVA was subsequently used, when the treatment x time interaction was found to be significant.

3.3 Results

3.3.1 Experiment 1: herbage production, fertiliser N response and total N uptake

All forms of chemical fertilisers applied in FPA form produced significantly ($P<0.05$) greater herbage dry matter yield (cumulative of 2 pasture cuts) than with application in granular form (Fig. 3.1A). Agrotain-treated urea in FPA produced significantly greater herbage dry matter yield (112 g/m^2) than other treatments. The increase in herbage dry matter yield with Agrotain-treated FPA urea compared with granular urea and FPA urea was 35% and 14%, respectively. All chemical fertilisers applied in FPA form exhibited greater N use efficiency than those treatments applied in granular form. Herbage dry matter yields from ammonium and nitrate fertilisers, applied in FPA form, were significantly higher than those of their respective granular treatments.

Nitrogen response (herbage DM in excess of controls) followed a similar pattern to that of the herbage dry matter (Fig. 3.1B). All forms of chemical fertilisers applied in FPA form significantly (P<0.05) improved overall N response compared with application in the granular form (Fig. 3.1B). Agrotain-treated FPA urea increased N response by 31% and 96% respectively compared with FPA urea or granular urea. All forms of chemical fertilisers

applied in FPA form resulted in significantly (P<0.05) higher N-use efficiency (dry matter production per kg of N applied) compared with application in granular form (Fig. 3.1C). Agrotain-treated FPA urea produced 24 g DM g^{-1} of applied N compared with 18 and 12 g DM g^{-1} of N applied in response to FPA urea or granular urea, respectively. Overall, Agrotain-treated FPA urea produced a significantly higher response efficiency than that of the other forms of fertilisers applied in either FPA or granular form. Total N uptake by the herbage was also significantly (P<0.05) greater when herbage was supplied with N in FPA than in granular form (Fig. 3.1D). Agrotain-treated FPA urea resulted in greater total-N uptake than when herbage was treated with other forms of N fertilisers. Over the 42-day period, Agrotain-treated FPA urea resulted in 23% and 59% more total N uptake over FPA urea and granular urea, respectively. The total increase in N uptake in response to Agrotain-treated FPA urea relative to other forms of fertilisers (applied in FPA form) ranged from 27 to 42%.
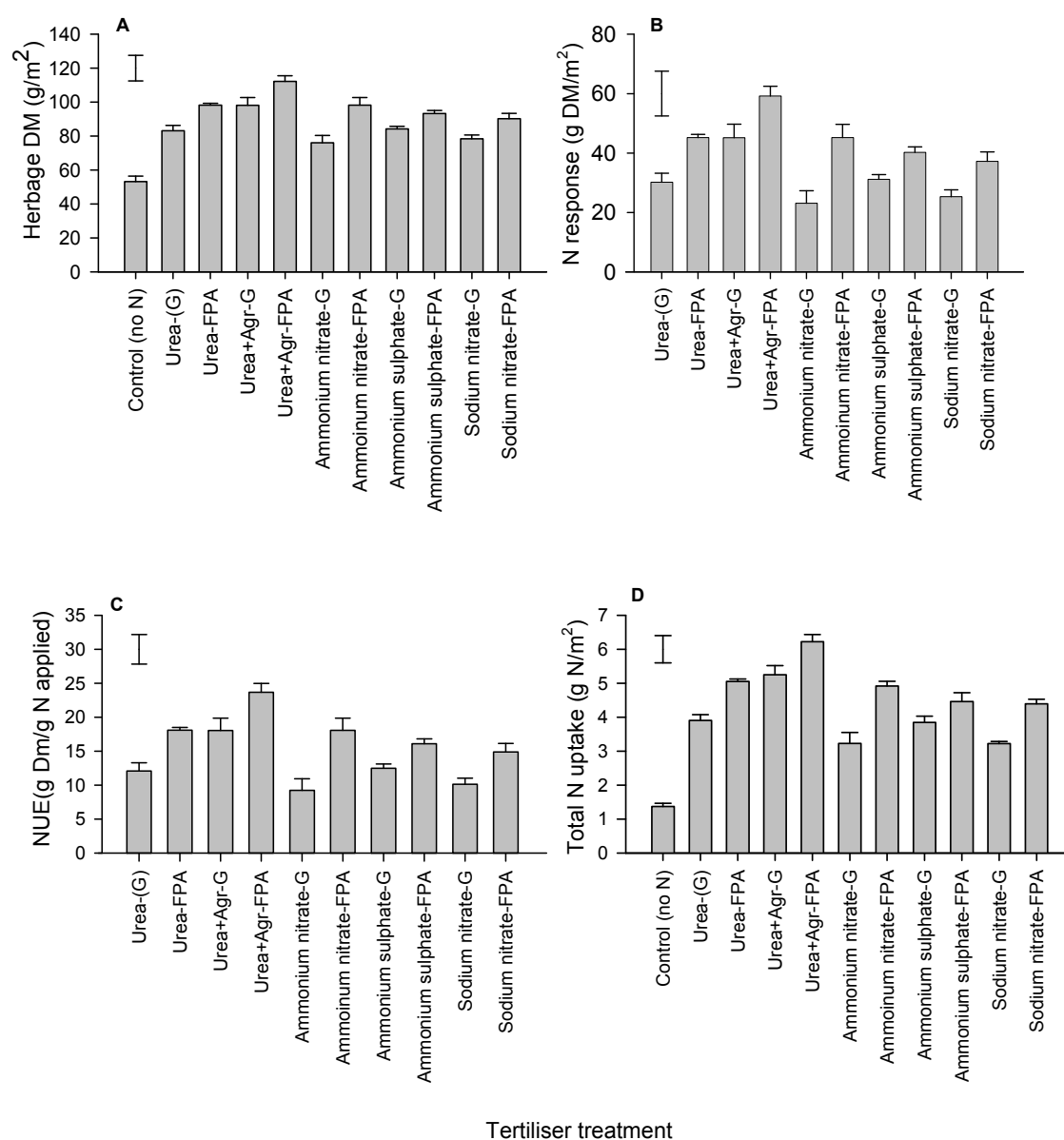
Fig. 3.1. Total herbage dry matter yield (A), N response (B) (g DM/m^2), response efficiency (C) (g DM/g of applied N), and total N (D) (g N/m^2) to urea with or without Agrotain and different types of chemical fertiliser applied in fine particle application (FPA) and granular (G) form (Expt 1). Values are for the combination of two cuts at 21 and 42 days after treatment application. Bars are means ± SEM where n=4. Vertical bar represent l.s.d. values where treatment means are significantly different at *p*= 0.05.

The relative effects of N fertiliser on % changes in dry matter production are shown in Fig. 3.2 (A1 + A2) and % changes in tissue N content in Fig. 3.2 (B1 + B2). At cut 1 (21 days), growth increment generally equalled or exceeded increases in tissue N content (% differences between dry matter change and N content change close to zero or positive, Fig. 3.2, C$_1$).

Growth increment exceeded increases in N content more strongly when fertilisers were applied in FPA form than when applied in granular form. Agrotain-treated FPA urea resulted in a significantly (P<0.05) greater % increase in dry matter production compared with other forms of fertilisers applied in FPA form. By cut 2 (42 days), % increase in tissue N content over controls exceeded the % increase in dry matter production (i.e. negative % difference between dry matter changes and N content change, Fig. 3.2, C_2) and the improved growth increment effect of FPA had disappeared.

3.3.2 Experiment 1: Nitrate reductase activity and ammonium, nitrate and amino acid contents of leaf tissue

Nitrate reductase activities measured at 21 days and 42 days were significantly affected by the added fertiliser treatments and generally decreased with time (Fig. 3.3). Nitrate reductase activities at cut 1 (21 days) were higher than controls in response to all chemical fertilisers, however NRA was significantly lower (P<0.05) in FPA urea, Agrotain-treated FPA urea and ammonium nitrate FPA than those of their respective granular treatments. Ammonium and nitrate contents of the leaf tissue were not significantly influenced by fertiliser treatment. Ammonium content of the leaf tissue averaged 12 nmol/g^{-1} fresh weight (range 9-22 nmol g^{-1} fresh weight) and nitrate content averaged 300 umol g^{-1} fresh weight (range 200-400 μmol g^{-1} fresh weight). Fertiliser application did not significantly affect amino acid concentrations in leaf tissue (Table 3.2).
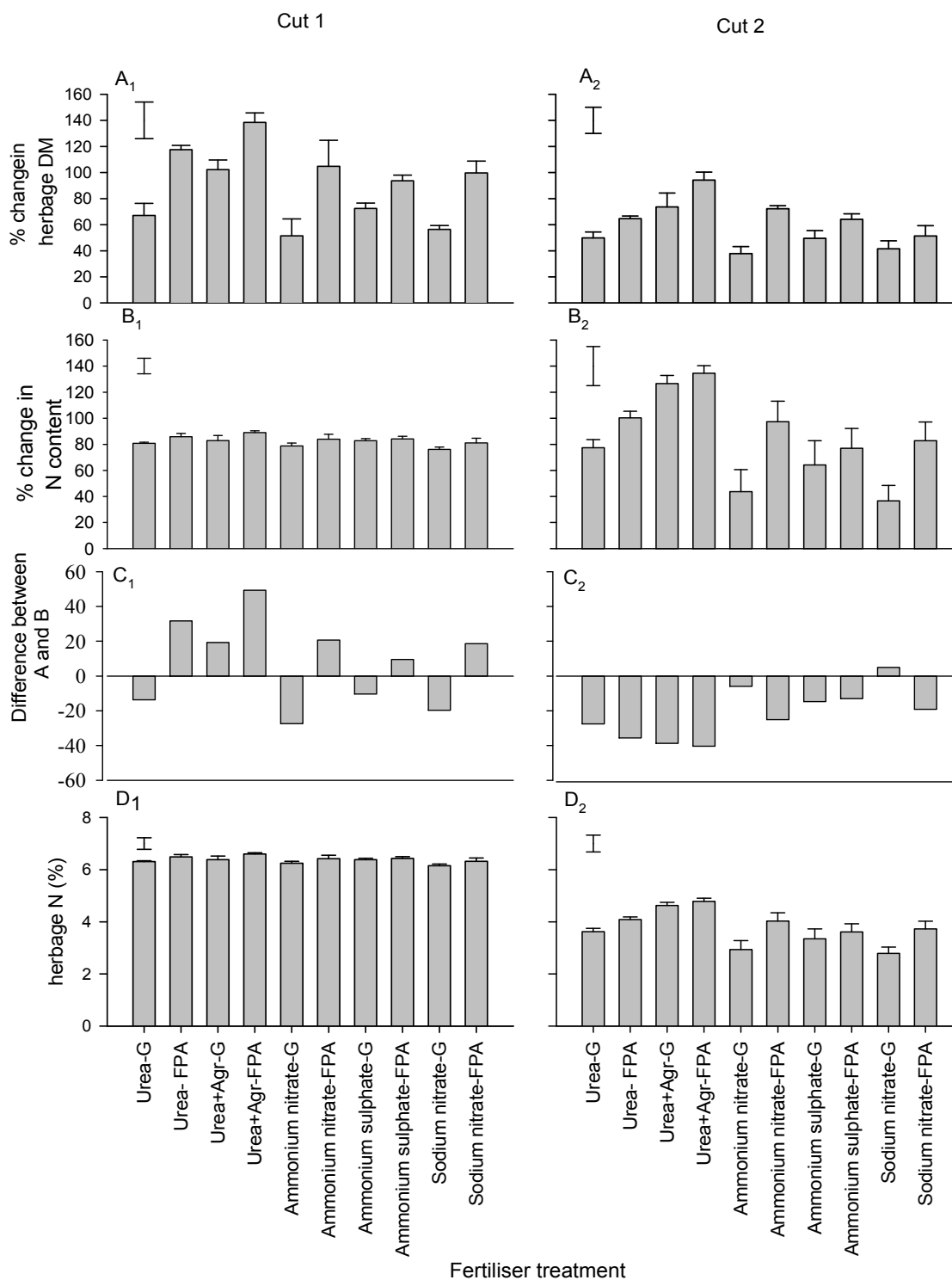
Fig. 3.2. The relative effects of N fertilisation on (A1 – cut 1, A2 – cut 2) % change in herbage dry matter (relative to controls), (B1, B2) % changes in N content (relative to controls), (C1, C2) the difference between A and B and (D1, D2) herbage N concentration. Bars are means ± SEM where n=4. Vertical bar represent l.s.d. values where treatment means are significantly different at *p*= 0.05.
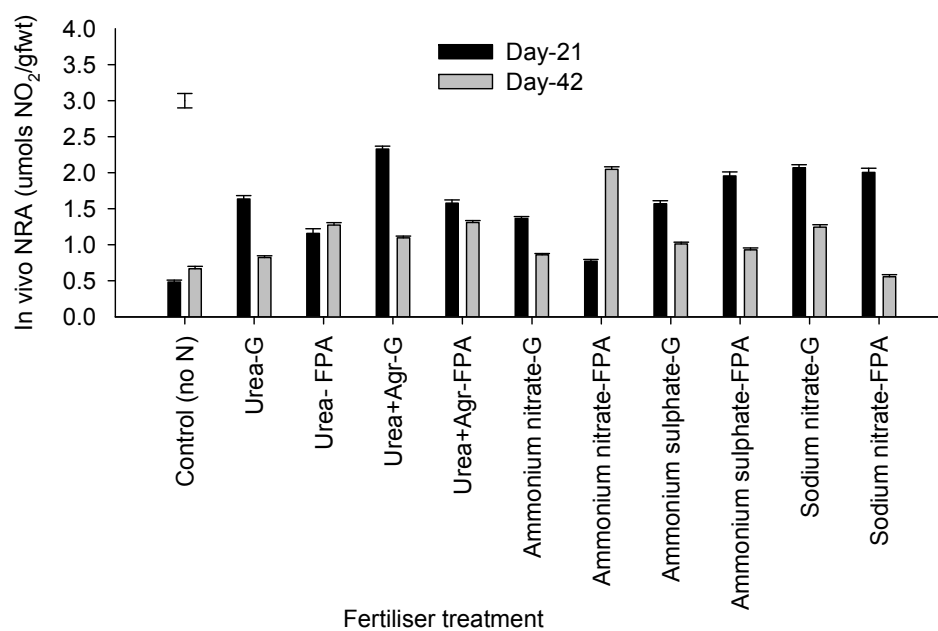
Fig. 3.3. Effect of urea, with or without Agrotain, and different types of N fertiliser applied in fine particle application (FPA) and granular form (Experiment 1) on nitrate reductase activity (μmol NO2/gfwt) in leaf tissue. Bars are means ± SEM where n=4. Vertical bar represent l.s.d. values where treatment means are significantly different at *p*= 0.05.

3.3.3 Experiment 2: Soil NH_4^+-N and NO_3^--N

Urea applied with Agrotain in soil application delayed urea hydrolysis by releasing NH_4^+ into the soil solution at a slower rate (Fig. 3.4a) than urea without Agrotain. In contrast, urea applied alone exhibited more rapid hydrolysis soon after its application, as evidenced by significantly higher concentrations of soil NH_4^+ at 4 hours, 8 hours, 1 and 2 days. Soil NH_4^+ concentration in the urea treatment reached its maximum on day 1, and decreased thereafter. Soil NO_3^- concentrations were lower than NH_4^+ and were not significantly influenced by urea addition with or without Agrotain (Fig. 3.4b).

3.3.4 Experiment 2 - herbage production, N uptake and ^{15}N recovery in plant

Results for herbage dry matter, N uptake and %^{15}N recovery measured at 0.16, 0.33, 1, 2, 3, 5, 10 and 21 days after ^{15}N urea application with or without Agrotain to soil or leaves are

shown in (Fig. 3.5). Herbage dry matter during the first 5 days of fertiliser application increased slowly and was not significantly affected by the applied treatments (Fig. 3.5a). By 10 days, urea (with or without Agrotain) applied directly to the leaves had produced significantly more herbage dry matter than urea applied to the soil surface. At the end of the experiment (at day 21) urea with Agrotain applied to the leaves produced significantly more herbage dry matter (total = 227 g DM/m^2) than all other treatments (Table 3.3); urea alone applied to leaves resulted in 196 g DM/m^2. After day 1, herbage treated with urea plus Agrotain directly to the leaves exhibited significantly greater total N uptake than with urea applied to the soil (Fig. 3.5b). This provides further evidence that Agrotain delayed urea hydrolysis by releasing NH_4^+ at a slower rate (see also Fig. 3.4 and results in Chapter 2). Overall, herbage total N uptake was significantly greater in response to the direct leaf application than the soil application (Table 3.3). At day 21, N uptake of herbage treated with urea plus Agrotain was 28.7% and 70.8 % (applied to leaves and soil, respectively) greater than that treated with urea alone. N response efficiency was also greater in response to shoot-applied urea (Table 3.3). There was a significant difference in ^{15}N recovery (percentage utilisation of applied N) between the soil and foliar application (Fig. 3.5c). There was no significant difference between urea added with or without Agrotain directly to leaves during the first 2 harvests after application. From day 1 onwards, urea plus Agrotain applied to leaves resulted in significantly higher ^{15}N recovery compared with urea alone applied to leaves or urea (with or without Agrotain) applied to the soil, although the difference between urea + Agrotain applied to the soil and to the shoots diminished somewhat towards the end of the 21 day experiment.

Table 3.2. Effect of urea, with or without Agrotain, and different N fertilisers applied in granular form (G) or as fine particle application (FPA) on amino-acid concentration of the shoot (nmol/g).

Treatments	Alanine	Aspartic acid	Cysteine	Glutamic acid	Glycine	Isoleucine	Leucine	Proline	Serine	Threonine	Valine
Urea-G	141	63	6	199	6	13	16	9	53	37	26
Urea-FPA	163	51	6	171	5	24	29	48	57	35	45
Urea+Agr-G	135	61	4	218	9	9	9	19	65	41	22
Urea+Agr-FPA	126	45	5	176	6	10	12	61	51	29	22
Ammonium nitrate-G	141	35	5	153	5	11	11	15	41	24	26
Ammonium nitrate-FPA	131	72	4	187	5	18	20	60	55	36	37
Ammonium sulphate-G	169	59	5	207	6	14	17	34	51	41	33
Ammonium sulphate-FPA	153	70	5	208	7	17	20	135	64	45	36
Sodium nitrate 3-G	134	66	4	210	6	12	15	16	52	33	26
Sodium nitrate -FPA	123	47	4	158	3	15	16	40	37	23	29
l.s.d($p<0.05$)	41	23	2	49	2	9	11	42	22	17	17
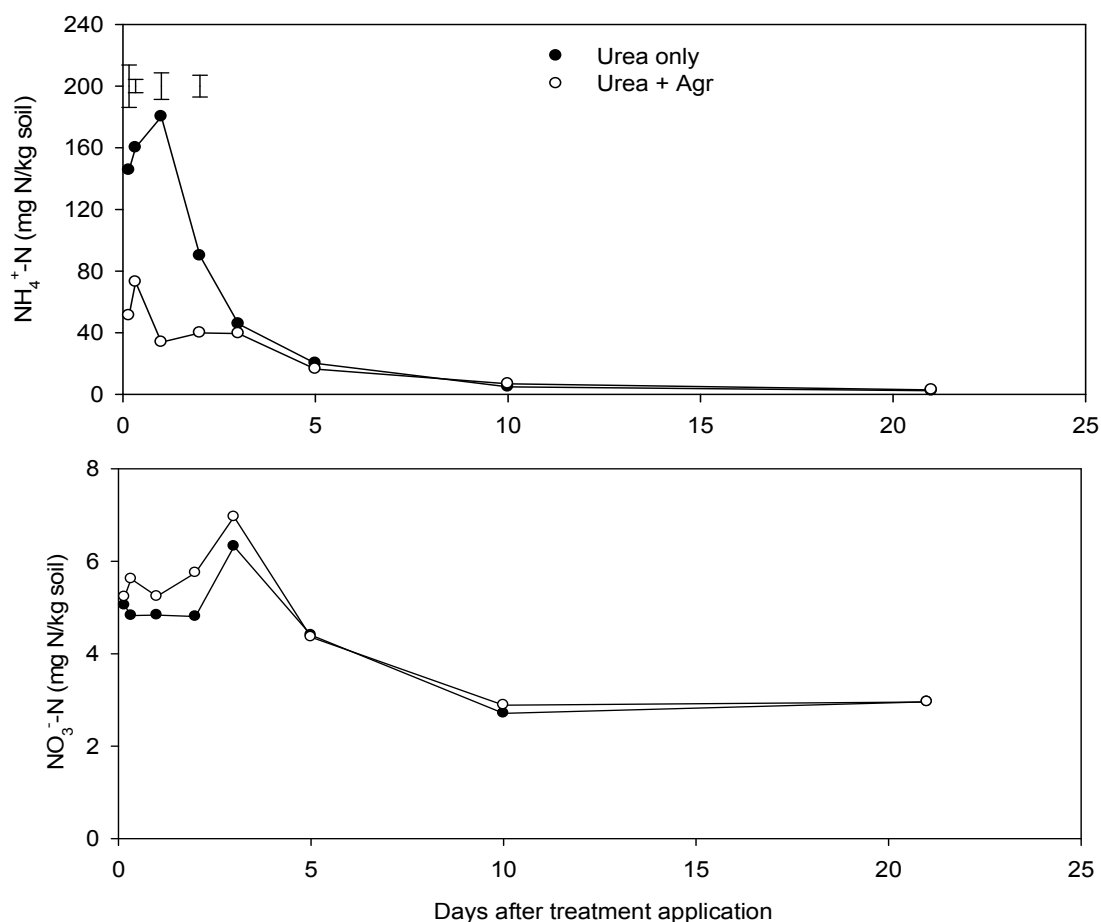
Fig. 3.4. Effect of urea, with or without Agrotain on soil mineral-N (Experiment 2). Vertical bars represent l.s.d. values where means are significantly different at $p= 0.05$.

Table 3.3. Herbage DM yield, N response total N content and response efficiency in response to addition of ^{15}N- urea, with or without Agrotain, applied in fine particle application (FPA) to the soil or directly to shoots (Expt 2). The harvest took place 21 days after fertiliser application.

Treatments	Herbage dry matter (g DM/m^2)	N response (g DM/m^2)	N uptake (g N/m^2)	Response efficiency (g DM/g of applied N)
Control (No N)	71^a		1.1^a	
Ur-Soil application	161^b	90^a	2.5^b	36^a
Ur+Agr-Soil application	171^b	100^a	2.6^c	40^a
Ur-Shoot application	196^c	124^b	3.3^d	49^b
Ur+Agr-Shoot application	227^d	156^c	4.2^e	62^c

Within columns, means with the same letters are not significantly different at the P < 0.05 level.
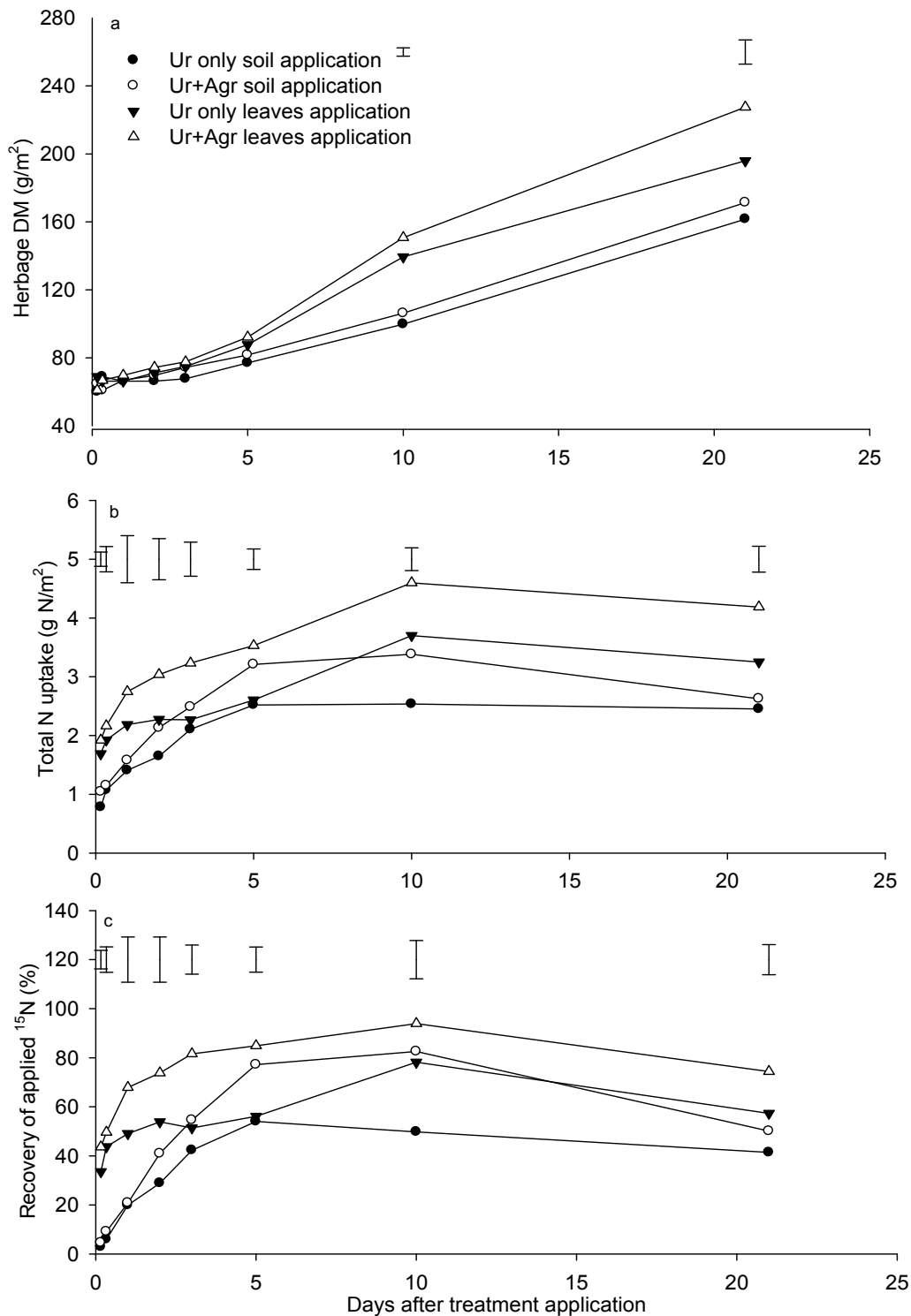
Fig. 3.5. Effect of urea, with or without Agrotain and applied to the soil or to leaves on **a**) herbage dry matter **b**) nitrogen uptake **c**) percentage recovery of applied ^{15}N by shoots (Experiment 2). Vertical bars represent l.s.d. values where means are significantly different at *p*= 0.05.

3.4 Discussion

Fine particle application of urea, urea + Agrotain and other nitrogen fertilisers resulted in significant improvements in herbage DM and N response compared with their corresponding granular treatments (Expt 1 - Fig. 3.1; Expt 2 - Fig. 3.5a). These results are in line with others who have also found that applying N fertilisers in FPA form increases herbage dry matter and fertiliser N efficiency by increased N uptake (Quin et al. 2006; Zaman and Blennerhassett 2009), but are higher than reports from other researchers for granular urea applied in the autumn to pastures under field conditions in New Zealand (Theobald and Ball 1984; Ledgard et al. 1999; Blennerhassett et al. 2006). This difference is likely to be a reflection of the optimal soil moisture and warmer temperature conditions in this glasshouse experiment. Low soil temperature (8°C) is reported to limit N movement from plant roots to the shoots and thus reduces DM production (Castle et al. 2006).

There are a number of mechanisms involved in the high N response to FPA urea + Agrotain in this experiment. Unlike granular application, FPA results in a uniform distribution of applied fertiliser on a per plant basis, and therefore a significant proportion of the applied fertilisers were intercepted by the pasture leaves. (This is also likely to be a major mechanism in the improvement in N-response observed following FPA treatment with non-urea fertilisers.). Further, urea improves the permeability of the cuticle and thus facilitates diffusion into the leaf (Franke 1967). Thus, the deposited urea particles could have provided pasture plants an opportunity to absorb N directly through their leaves/cuticles (Watson et al. 1990). Finally, NH_3 volatilisation is a major cause of the often low recoveries of fertiliser N after foliar urea application (e.g. Vasilas et al. 1980;

Gooding and Davies 1992). Agrotain treatment would result in a reduced rate of conversion of urea on and in the plant, thus providing plants an opportunity to convert the absorbed urea into plant protein more efficiently. It is via this combination of effects that Agrotain-treated FPA urea exhibited improvement in N response compared to FPA urea and other fertilisers.

The improvements in herbage DM and fertiliser efficiency could also in part be due to a lower energy requirement for assimilation of N into protein in plants when they take up NH_4^+ or urea than when NO_3^- is the primary N source. Urea and NH_4^+ are known to require less energy to metabolize and to convert them to plant protein (Middleton and Smith 1979), while NO_3^-- N has to be reduced before assimilation, which requires additional energy (Raven 1985; Ullrich 1992) meaning that the pasture plant may be left with extra energy to allocate to growth. Therefore, use of ammonium and nitrate fertilisers may result in relatively lower pasture yield. Castle et al. (2006) have proposed that the direct uptake of urea through leaves could reduce energy requirements and avoids the influence of cold temperatures in the root environment. The improvements in herbage dry matter and N response by Agrotain-treated FPA urea over FPA urea could also be attributed to delayed urea hydrolysis, which generally takes place within 1 to 2 days of application. Witte et al. (2002) have shown that any major N losses are most likely to occur in the first 36 to 48 h after urea application, and when considerable excess amounts of urea and ammonium are present in the leaves. A delay in urea hydrolysis by the action of Agrotain has the potential not only to minimise the risk of N losses via NH_3 volatilisation from the plant (Schjoerring et al. 2000) but also to improve the bioavailability of applied N (Zaman et al. 2008).

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In experiment 2, NH_4^+ production was low in soil supplied with Agrotain-treated urea compared with soil treated with urea alone, but NO_3^- content was not significantly different between treatments (Fig. 3.4). This confirms the findings of the previous chapter that Agrotain delayed urea hydrolysis and produced NH_4^+ at slow rate (Chapter2; Dawar et al 2010a). These results are comparable with the findings of other researcher which suggest that urea hydrolysis is delayed by Agrotain (Watson 2000) and that the urea may be taken up directly by ryegrass from the soil (Watson and Miller 1996). Zhengping et al. (1996) also observed slow urea hydrolysis and a lower accumulation of soil NH_4^+ after applying urea with Agrotain to soils under controlled conditions. The importance of the study by Zhengping et al. (1996) is that it was conducted in the absence of plants. This precludes the possibility that plant uptake of NH_4^+ could explain reduced soil NH_4^+ concentration in the presence of Agrotain. We therefore, conclude that the greater recovery of applied N in the presence of Agrotain (Fig. 3.5) is a result of uptake of urea.

Like herbage dry matter production, N uptake in both experiments exhibited significant increases in response to Agrotain-treated FPA urea (Fig. 3.1b) or Agrotain-treated urea direct shoot application (Fig. 3.5b). In the ^{15}N experiment, the lack of any significant difference in N uptake during the first day of treatment application highlights the fact that leaves had equal opportunity to take up applied nitrogen (either as urea or ammonium), with or without Agrotain (Fig. 3.5b). After the progressive disappearance of urea as a result of rapid hydrolysis in the absence of Agrotain, herbage supplied with Agrotain-treated urea showed a significantly higher N uptake than urea alone. Agrotain may also improve the bioavailability of urea-N by delaying plant urease activity, thus providing plants with an opportunity to convert the absorbed urea into plant protein more efficiently. Similarly, direct absorption of urea through the leaves/cuticles may save the plant some

energy in uptake and transport of urea/NH_4^+ from roots to shoots, thus enhancing plant growth. Castle et al. (2007) also demonstrated in a growth cabinet experiment that clover plants at $8^{\circ}C$ took up significantly more N in urea form than NO_3^-, particularly through the leaves, and this resulted in 10% more dry matter production.

To determine whether N taken up by the pasture was translated into dry-matter yield, we calculated the percentage change (relative to controls) in response to fertiliser addition. At the first cut (21 days), there was a significant difference in the response between granular and FPA fertiliser addition (in favour of a positive % change in herbage dry matter yield in response to FPA; Fig. 3.2 A_1) but there was very little difference between application type and fertiliser form in terms of their effect on % change in N content (Fig. 3.2 B_1). The resulting calculation of the difference between the growth response and the N content response (Fig. 3.2 C_1) clearly indicates that in the early stages of the pasture response, application of fertiliser via FPA results in a greater relative response of growth than uptake of N. Clearly, N in FPA form elicits a strong growth response early in the pasture cycle and may increase the uptake of applied N. This may, in part, explain the reduced N efficiency under the granular application. By cut 2 (42 days) the FPA effect on % change in pasture dry matter yield was still evident (Fig. 3.2 A_2), but it had been subsumed by an increased % change in N content (Fig. 3.2 B_2). Thus, the % difference between these two responses (Fig. 3.2 C_2) was close to parity or negative. The relative effects of granular and fine particle application on the processes of N uptake and subsequent herbage growth may have important implications for pasture fertiliser management. A greater understanding of the physiological mechanisms underpinning these related, but independent, processes is clearly required.

Nitrate reductase activities at day 21 (cut 1) were higher than the control in response to all chemical fertilisers, however, foliar NRA was significantly lower (P<0.05) following treatment with FPA than following granular application. By 42 days, NRA had decreased significantly (P<0.05) in all treatments. Foliar NRA was significantly lower (P<0.05) in responses to FPA urea, Agrotain-treated FPA urea and ammonium nitrate FPA than it was in the respective granular treatments, while there was no such effect of application on other fertilisers. Castle et al. (2003) also reported that NRA decreased with time and was greater in a high-N treatment than a low-N treatment. The concentration of a range of leaf tissue amino acids in response to granular application was found to be within the published range for pasture grasses (Streeter et al. 2000) and was not affected by Agrotain-treated urea compared with other fertiliser types after 21 days. However, the concentration of a number of amino acids was lower in tissues in response to Agrotain treated FPA urea compared with other treatments. Watson and Miller (1996) reported similar results in response to urea + urease inhibitor compared with urea alone. However, their assays were carried out 4-10 days after the treatments were applied, suggesting a possible effect on transformation reactions. Watson and Miller (1996) have suggested that urea-N within the plant is not used in the same way as N taken up in the NH_4^+-N form. In the present study, the most likely explanation for the reduction in amino acid concentration is the dilution effect of a greater increase in dry matter production relative to N uptake (Fig. 3.2) in the early stages of the response.

3.5 Summary

In conclusion, these experiments show that urea, with or with out Agrotain, and other chemical fertilisers applied in FPA form, may significantly improve herbage growth, N-response and response efficiency compared with application in the granular form. FPA is therefore likely to provide efficiency gains regardless of the type of fertiliser being used. However, applying urea + Agrotain in the FPA form resulted in even higher herbage growth, N-response and response efficiency compared with urea alone or other fertilisers. This suggests that if urea hydrolysis is delayed by Agrotain then urea could be taken up by ryegrass leaves/roots. Agrotain improves plant-availability of urea-N through reductions in soil and plant urease activity, thus providing plants an opportunity to convert the absorbed urea into protein more efficiently. Previous findings of a low N-response and response efficiency from urea applied in FPA form are probably because of additional factors such as high application rates and extreme soil and environmental conditions. In addition, urea might be lost from leaves because of wind or because it is not intercepted by the leaves in the first place. Applying urea in FPA form is a good management strategy and combining FPA urea with Agrotain has the potential to increase N use efficiency and herbage production further. In Chapter 4, the mechanisms underlying this benefit are further investigated.

Chapter 4

Urea hydrolysis and lateral and vertical movement in the soil: effects of urease inhibitor and irrigation

4.1 Introduction

Urea is the predominant chemical fertiliser applied to grazed pastures in New Zealand and to arable crops worldwide. However, a major potential disadvantage associated with the use of urea is gaseous loss of N via ammonia (NH_3) emission, especially when applied under less than optimum conditions (e.g. soil water content and rainfall, temperature, cation exchange capacity (CEC), pH and wind (Black et al. 1985; Fenn and Hossner 1985; Freney et al. 1985; de Datta et al. 1989; Gioacchini et al. 2002; Kissel et al. 2004; Ahmed et al. 2006)). A number of options have been proposed to improve urea efficiency. These include physical (altering the rate, timing and method of application and producing large-granule urea) and chemical (coating urea with different materials and mixing urea with chemicals) approaches. The application of urease inhibitors has been considered a promising way to reduce N losses and enhance urea N efficiency. Several urease inhibitors have been identified and tested but N-(n-butyl) phosphorothioic triamide (nBTPT, "Agrotain") has been shown to retard urea hydrolysis at a very low concentration (Mulvaney and Bremner 1981; Chai and Bremner 1987; Bremner and Chai 1989; Carmona et al. 1990).

In previous studies (see Chapter 2 and 3), I have found that treating urea with Agrotain has the potential to increase N use efficiency and herbage production in pasture grass (Dawar et al. 2010a). Other researchers have also reported increased herbage dry matter and N uptake after application of Agrotain-treated urea (Blennerhassett et al. 2006; Martin et al. 2006;

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Zaman et al. 2008). The mechanisms underlying such responses have yet to be fully elucidated. I have suggested that a delay in urea hydrolysis by Agrotain provides an opportunity for direct plant uptake of an increased proportion of the applied urea-N than is the case with urea alone (Chapter2; Dawar et al. 2010a). However, there is also a possibility that there is another benefit of retaining N in the urea form - that is, being an uncharged particle, urea is likely to diffuse easily into the rooting zone at moderate-high soil moisture. Such diffusion of urea may provide herbage an extended opportunity to take up added urea through a greater proportion of the root system. This could be added to the previously established benefit of direct uptake via the leaves (Watson et al. 1996; Bollard et al. 1968; Harper 1984; Matsumoto et al. 1966; Chapter 3).

Urea-use efficiency may be improved through reduced gaseous losses of NH_3, especially if it is moved into the soil with small amounts of irrigation. Zhengping et al. (1996) previously studied the effect of urease inhibitor on the movement and transformation of urea and its hydrolysis products in the soil following sub-surface application (3 cm deep). Similarly, earlier chapters (Chapter 2 and 3) investigated urea hydrolysis and production of NH_4^+ after surface application of urea with Agrotain (Dawar et al. 2010a). These investigations have showed that Agrotain delayed urea hydrolysis by releasing NH_4^+ into the soil solution at a slower rate – we currently lack an understanding of urea-N and NH_4^+-N movement in the soil profile. Based on the findings of Chapter 2 and 3, the objective of the present study was to track urea movement after surface application (with or without urease inhibitor).

I also investigated the impacts of soil moisture content following simulated irrigation, to mimic the conditions that might be faced by growers in the field if a rainfall event occurs soon after fertiliser application. I tested the hypothesis that applying urea with Agrotain in granular form to the soil surface will delay urea hydrolysis, and thus allow far greater diffusion of the intact urea molecule into the dense pasture rooting zone in the soil.

4.2 Materials and methods

4.2.1 Experimental location and procedure

A glasshouse experiment was conducted at the University of Canterbury using topsoil (0-75 mm) collected from a grazed pasture site near Lincoln, Canterbury New Zealand (43° 64'32.00" S, 172° 38'58.90" E). The soil used was Paparua silt loam, Typic Haplustepts (Soil Survey Staff 1998). The soil was passed through a 2mm sieve, and then brought to the two moisture levels (80% FC and 50% FC) either by air drying or adding additional water. Sieved soil (1.6 kg/bag) was packed in 100 mm long and 140 mm diameter plastic bags, which were then placed into standard planting pots. The repacked columns were watered every 2-3 days for 4 weeks prior to the experiment to allow for soil particles to settle. Four soil samples, each comprising 10 randomly collected soil cores, were analysed for key soil properties (Table 4.1). The bulk density of the packed soil core was 0.88 g cm^{-3}. The experiment consisted of five treatments (urea only; urea + Agrotain; urea + irrigation on day 1; urea + Agrotain + irrigation on day 1; and a control receiving no N. Urea granules (2-4 mm), with or without Agrotain, were placed on the soil surface in the centre of each core (a diameter of 40 mm at a rate equivalent to 100 kg N/ha or 240 mg N per bag (Plate 4.1). After 1 day of treatment application, soil cores of the 50% FC were adjusted to 80% FC by applying surface irrigation equivalent to 8 mm with a hand mist sprayer. Soil cores were then incubated at 20°C under glasshouse conditions.

Plate. 4.1. Pot set-up and placement of urea granules used for this experiment.

4.2.2 Analysis of soil

Three soil cores per treatment were destructively sampled at 1, 2, 3, 4, 7 and 10 days after treatment application as the transformation of urea was found to be complete by this time. The soil in each core was divided into 10 layers, each 10 mm thick, to measure vertical movement of N, and the soil mass recorded. In addition, the soil in each layer was divided into three concentric sub-samples using rings of radius 20, 40 and 60 mm from the centre of the core to the pot to the outer edge of the pot to monitor lateral movement of N (Plate 4.2). A sub-sample of each soil sample was used for moisture determination and an additional sub-sample was extracted with 2M KCl for 1 h and filtered through Whatman 42 filter paper. Soil extracts were immediately frozen and then used to determine the concentration of different N forms (i.e. urea-N, NH_4^+-N and NO_3^--N) in the soil. Urea-N was analyzed using quantitative colorimetric urea determination (QuantiChorm^{TM} urea assay kit) by bioassay systems Hayward United State of America (USA). NH_4^+-N and NO_3^--N concentrations were determined by flow injection analysis (FIA).

4.2.3 Statistical analysis

Analyses of variance (ANOVA) were performed using Minitab (Version 12, Minitab Inc. USA). Least significant differences (LSD) were calculated to compare treatment means at

P<0.05. Repeated-measure analysis of variance (ANOVA) was used to determine if time had a significant effect on the concentration of the N species, ANOVA was then used at individual time points when the treatment x time interaction was found to be significant.

Table 4.1 Physical and chemical properties of soil used in this experiment.

Soil properties	Values
pH	5.75
Total N (%)	0.36
Organic matter (%)	6.0
Olsen P (µg/ml)	20
CEC (me/100g)	15
Ca_2^+ (me/100g)	6.6
K^+ (me/100g)	0.44
Mg^{++} (me/100g)	1.73

4.3 Results

4.3.1 Recovery of urea-N in soil

Fig. 4.1A shows the urea-N remaining in the soil, expressed as a % of the N applied to the total soil core. Urea hydrolysis of urea-alone treatments was rapid with little urea remaining two days after treatment application in the soil. Contrary to this, Agrotain was highly significantly ($P<0.05$) effective up to 7 days in delaying urea hydrolysis (Fig 4.1A). At day-2, 60-65 % of the applied N remained as urea in Agrotain-treated urea (with or without irrigation) treatments. After 7 days, 8-9 % of N applied was recovered as urea within Agrotain treatments (with or without irrigation). There was no significant effect of irrigation on total urea-N retention in soil when urea was added with or without Agrotain.

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

Decreases in urea-N concentration were paralleled by concomitant increase in the NH_4^+-N concentration in the soil core (Fig 4.1B). Agrotain-treated urea (with or without irrigation) delayed urea hydrolysis and released NH_4^+ into the soil at a slower rate (Fig. 4.1B) than with urea alone with or without irrigation. In contrast, urea applied alone, with or with out irrigation, exhibited more rapid hydrolysis soon after its application, as evidenced by significantly ($P<0.05$) higher concentrations of soil NH_4^+-N at 1 and 2 days.
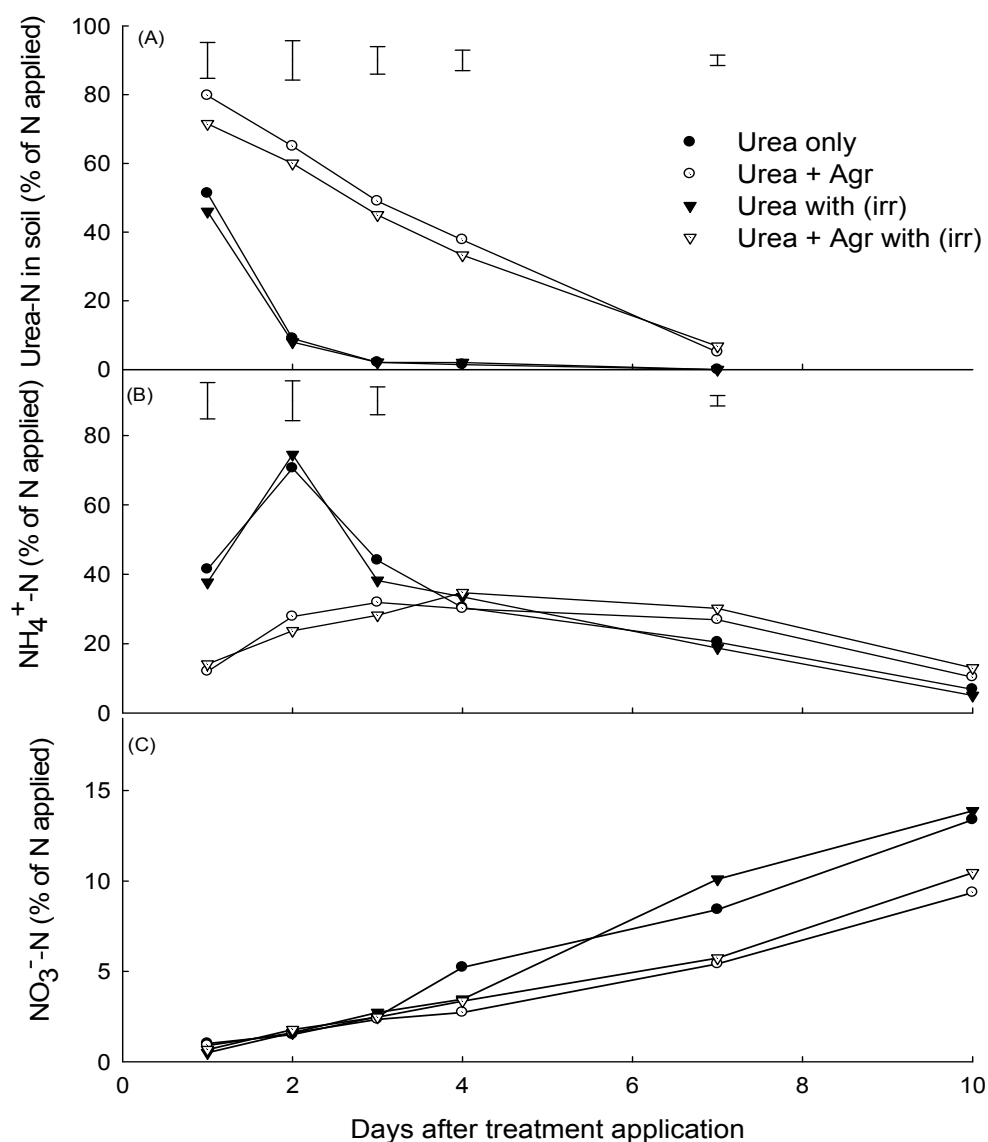
Fig. 4.1. Recovery of urea-N (A), NH_4^+-N (B) and NO_3^- -N (C) as a percentage of total applied N to the soil core. Vertical bars represent LSD values where treatment means are significantly different at $p= 0.05$

In the urea alone treatment, with or without irrigation, more NH_4^+-N was recovered from the soil on day 1; this increased on day 2 when it reached its maximum, and decreased thereafter. Soil NO_3^--N concentrations were much lower than NH_4^+-N and were not significantly ($P<0.05$) influenced by urea addition, with or without Agrotain and irrigation (Fig. 4.1C).

4.3.3 Downward movement of urea-N and NH_4^+-N in soil

The vertical distribution of urea-N in the soil layers after treatment application is shown in Fig. 4.2. After day 1, in all treatments most of the urea-N remained in the surface soil layer, and did not diffuse below the 10-30 mm soil layer. In contrast, the application of irrigation after day 1 produced a significantly ($P<0.05$) greater urea-N concentration at 20-40 mm depth (with or without Agrotain). Regardless of irrigation, Agrotain treatment retained more N in the urea form and this moved down through the soil layer up to day 7.

Irrigation increased the movement and concentration of urea to the sub-surface soil layers (30-50 mm), particularly from day 2 to day 4 (Fig. 4.2). At day 2, Agrotain-treated urea with irrigation moved below 20 mm and represented a significantly ($P<0.05$) greater proportion of urea-N - i.e., 25% urea of recovered N remained at 30-50 mm depth compared with 15% of Agrotain-treated urea without irrigation (Table. 4.2). After day 3, the urea concentration increased in the sub-surface soil layer; 37% of recovered N remained as urea in the 30-50 mm layer following treatment with Agrotain-treated urea with irrigation compared with 29% of Agrotain-treated urea without irrigation. Four days after addition of Agrotain treated-urea with irrigation, 48% of the recovered N remained as urea at 30-50 mm compared with 20% without irrigation.

Fig. 4.2. Effect of urease inhibitor and irrigation on downward movement of urea-N in soil, expressed as a percentage of the total added urea N. Bars are means ± SEM where n=3.

Changes in the distribution and concentration of NH_4^+-N in different soil layers after N application are presented in Fig. 4.3. After day 1, in all treatments most of the NH_4^+-N remained at the surface, and did not diffuse below the 10-20 mm soil layer. However, the concentration of NH_4^+-N following application of urea alone was significantly ($P<0.05$) greater than that following application of urea with Agrotain. Importantly, the hydrolysed

urea in the absence of irrigation was primarily in the upper surface soil layer and was not more evenly distributed down the sub-surface soil layers (a result which is similar to that of Agrotain-treated urea without irrigation). At day 1 after fertiliser treatment, a significantly ($P<0.05$) greater proportion of hydrolysed urea was found 30-50 mm below the soil surface up to day 7 (Fig. 4.3). At day 2, 28% of recovered N remained as NH_4^+-N at 30-50 mm in response to the urea with irrigation treatment, compared with 8% in the urea without irrigation treatment. After day 2, the concentration increased in the sub-surface soil layers, and 36% and 49% of recovered N remained as NH_4^+-N at 30-50 mm in the urea with irrigation treatment compared with 20% and 24% in the urea without irrigation treatment at day 3 and 4, respectively. A similar trend, but of lower magnitude, was observed for Agrotain-treated urea (with and without irrigation).

Table 4.2 The amount of (a) urea-N and (b) NH_4^+-N which moved downward out of the upper layer of soil to which it was applied (i.e. into the 30-50 mm soil horizon). This is quoted as a percentage of the total amount of each N form recovered.

Treatments	Day-1	Day-2	Day-3	Day-4
(a) Urea-N				
Urea only	17	11	-	-
Urea+Agr only	7	15	29	20
Urea with (irr)	15	23	-	-
Urea+Agr with (irr)	7	25	37	48
(b) Ammonium-N				
Urea only	-	8	20	24
Urea+Agr only	-	13	22	33
Urea with (irr)	-	28	36	44
Urea+Agr with (irr)	-	35	48	49

4.3.4 Lateral movement of urea-N and NH_4^+-N in soil

In all treatments, soil urea-N concentrations at day 1 were very high in the inner ring (0-20 mm radius) close to the granule placement position and decreased laterally (Fig. 4.4).

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Addition of Agrotain-treated urea resulted in a significantly ($P<0.05$) greater proportion of urea being found in the outer rings of the soil up to day 4 compared with other treatments (Fig. 4.4; Table 4.3). At day 2 (after irrigation at day 1), 28% of recovered N was found in urea form in the middle ring (20-40 mm radius) in Agrotain-treated urea compared with 23% without irrigation. In the outer ring (40-60 mm radius) 18% of recovered N was found as urea following application of Agrotain-treated urea with irrigation, while urea did not move to the outer ring in the corresponding without-irrigation treatment. After day 2, the concentration of urea increased in the outer ring, so that by day 3, 15% of recovered N was found in the outer ring (40-60 mm) following addition of Agrotain-treated urea with irrigation compared to 8% without irrigation. In Agrotain-treated urea with irrigation, 27% of the recovered N remained as urea in the outer ring (40-60 mm radius) even after day 4, compared with 5% in the same treatment without irrigation. During the experiment, urea-N moved through the soil away from the central placement zone (0-20 mm radius). In the outer ring (40-60 mm radius), urea-N concentration following addition of Agrotain-treated urea without irrigation was 8% on day 3 and had decreased to 5% by day 4. Over the same time period, the proportion of applied N recovered as urea in soils following addition of Agrotain-treated urea with irrigation increased from 15 to 27 %.

The recovery of NH_4^+-N tended to mirror that of urea-N (Fig. 4.5). After day 1 in all treatments, most of the NH_4^+-N remained in the inner ring (0-20 mm radius) and did not diffuse to the outer ring. However, the concentration of NH_4^+-N in the inner ring was significantly ($P<0.05$) greater in the urea-alone treatments than in those of urea with Agrotain treatments. When irrigation was applied after day 1, a significantly greater proportion of NH_4^+-N was found in the middle and outer rings up to day 4 (Table 4.3). At day 2, 52% of recovered N was found as NH_4^+-N in the middle ring (20-40 mm radius) of the urea alone

with irrigation treatment compared with 29% without irrigation. In the outer ring (40-60 mm radius), 7% of recovered N was found as NH_4^+-N in the urea alone with irrigation treatment, while no NH_4^+-N was found in the corresponding without irrigation treatment. At day 3, 17 % of recovered N was found as NH_4^+-N in the outer ring in the urea alone with irrigation treatment, compared with 6% without irrigation. At day 4, the concentration was increased further in the outer ring, and 22 % of recovered N was found as NH_4^+-N in the urea alone with irrigation treatment compared with 9% without irrigation.
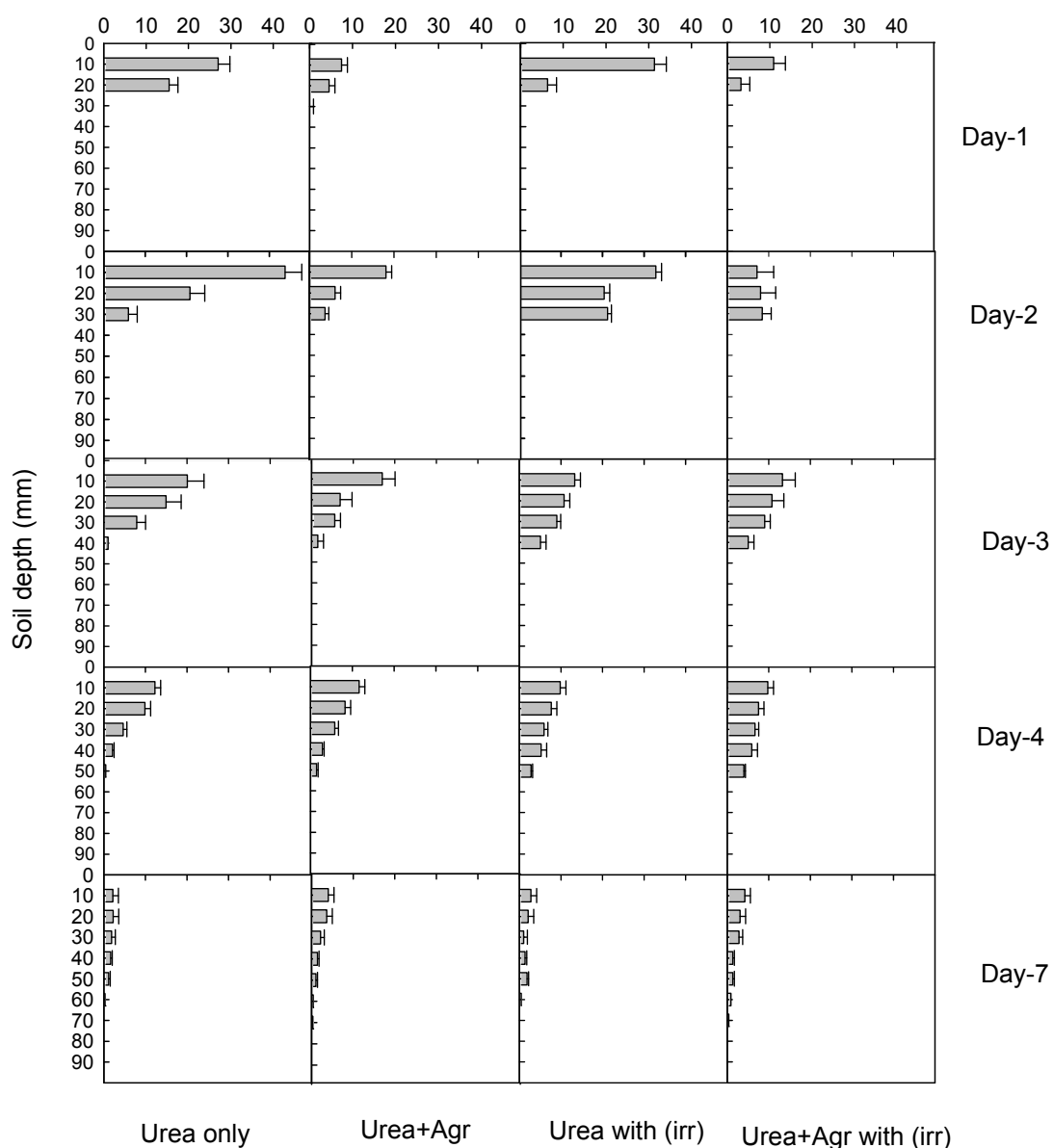
Fig. 4.3. Effect of urease inhibitor and irrigation on downward movement of NH_4^+-N in soil, expressed as a percentage of the total added urea N. Bars are means ± SEM where n=3.
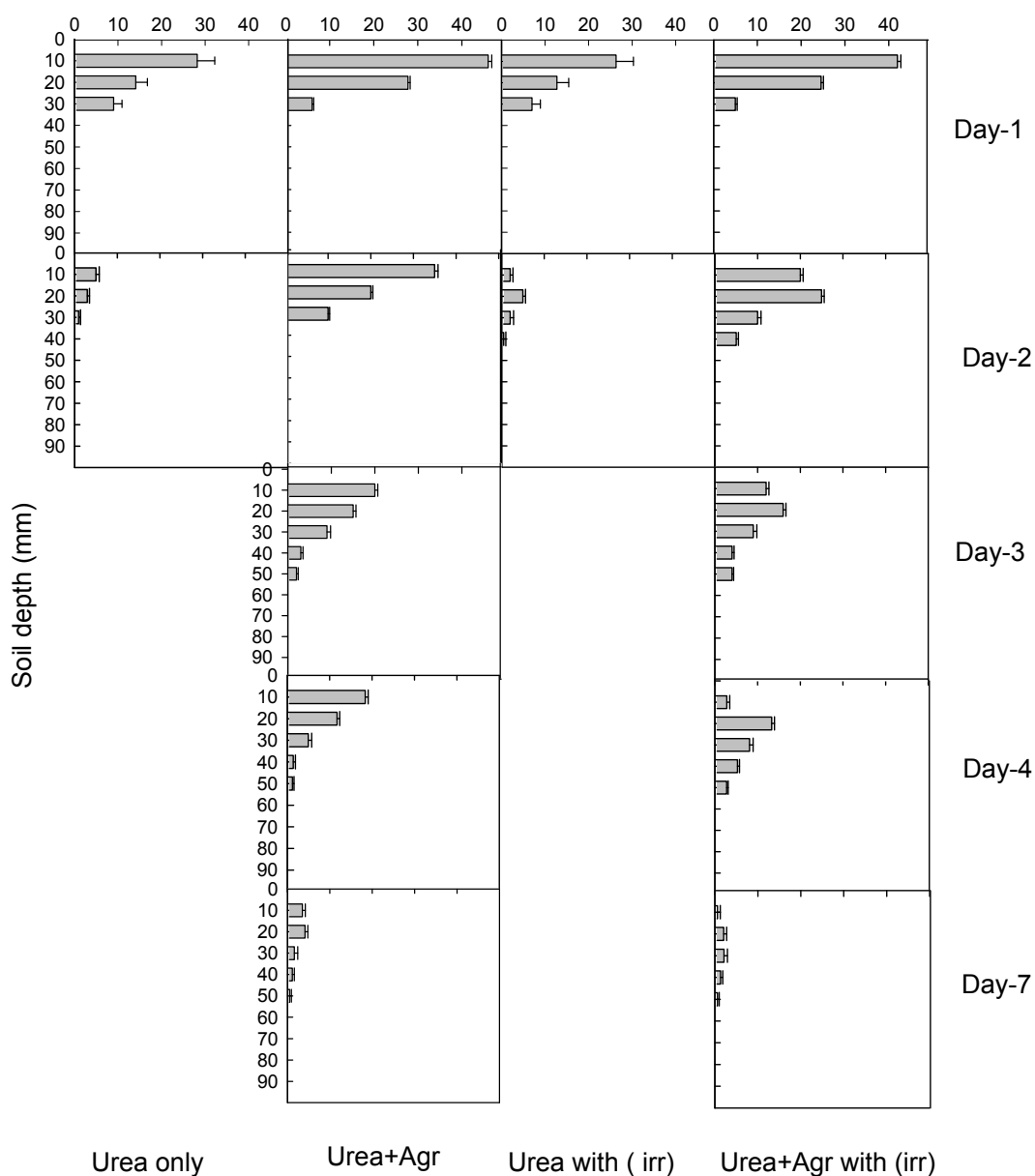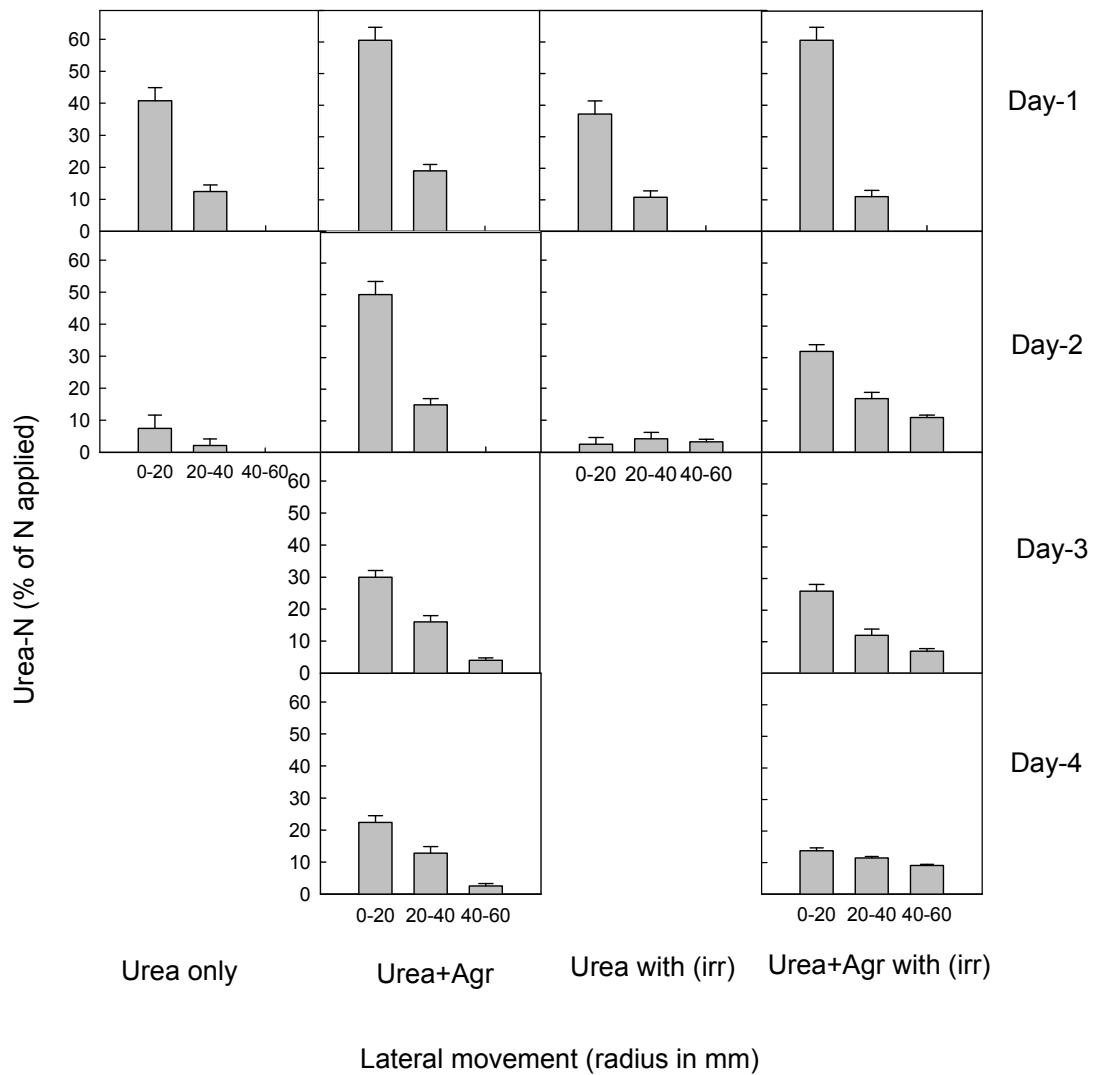
Fig. 4.4. Effect of urease inhibitor and irrigation on lateral movement of urea-N in soil, expressed as a percentage of the total added urea N. Bars are means ± SEM where n=3.
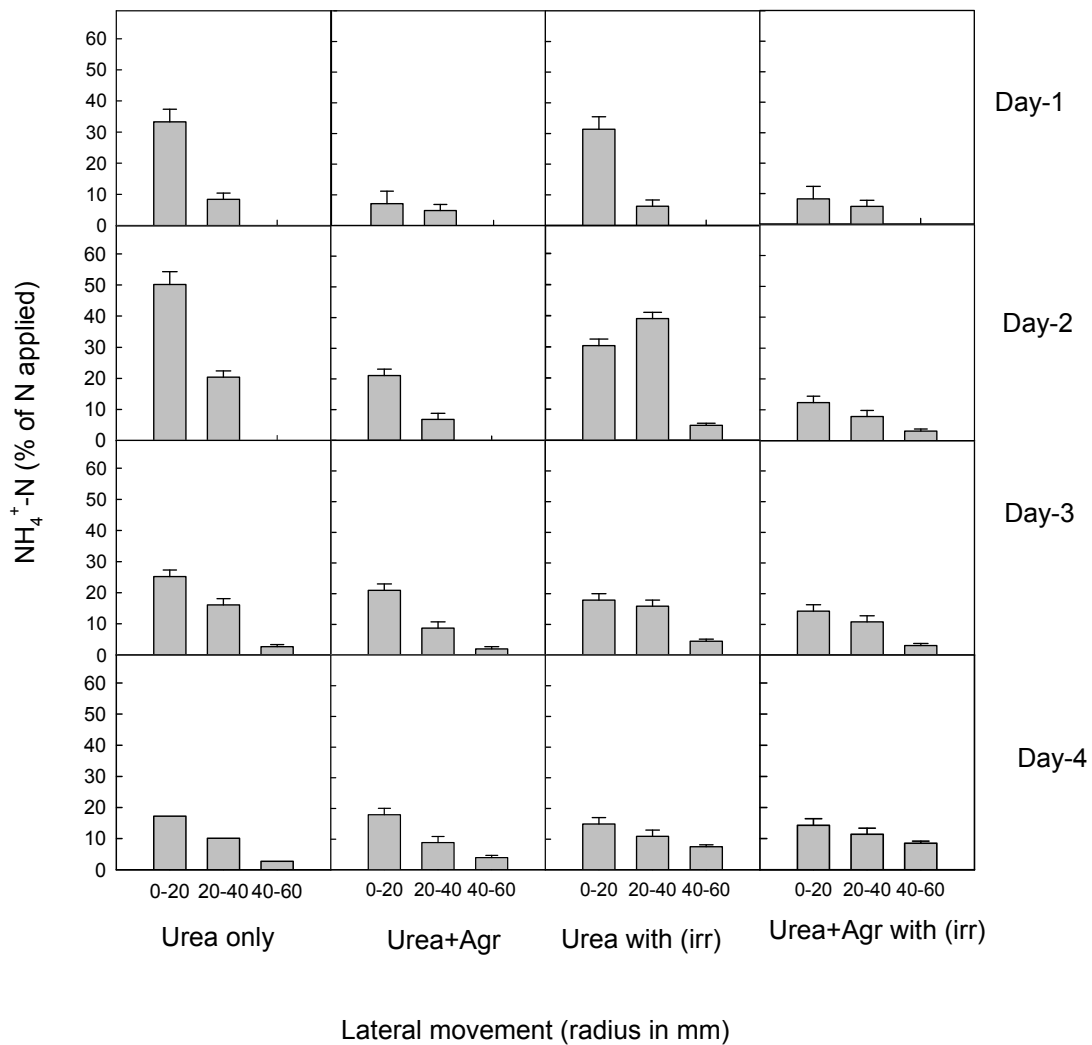
Fig. 4.5. Effect of urease inhibitor and irrigation on lateral movement of NH_4^+-N in soil, expressed as a percentage of the total added urea N. Bars are means ± SEM where n=3.

Table 4.3 The amount of (a) urea-N and (b) NH_4^+-N which moved outward from the inner ring to which it was applied (i.e. out to the 20-40 mm and 40-60 mm rings). This is quoted as a percentage of the total amount of each N form recovered.

Treatments	Day-1		Day-2		Day-3		Day-4	
	20-40 (mm)	40-60	20-40 (mm)	40-60	20-40 (mm)	40-60	20-40 (mm)	40-60
(a) Urea-N								
Urea only	23	-	22	-	-	-	-	-
Urea+Agr only	24	-	23	-	32	8	33	5
Urea with (irr)	21	-	42	32	-	-	-	-
Urea+Agr with (irr)	15	-	28	18	26	15	33	27
(b) Ammonium-N								
Urea only	20	-	29	-	36	6	34	9
Urea+Agr only	40	-	24	-	27	9	28	13
Urea with (irr)	17	-	52	7	36	17	32	22
Urea+Agr with (irr)	41	-	33	13	31	25	33	24

4.4 DISCUSSION

This experiment provides us with important insights into urea hydrolysis and its movement in soil as influenced by urease inhibitor (Agrotain) under optimum soil moisture and temperature. Both Agrotain and irrigation 1 day after urea application affected urea hydrolysis and its movement in the soil. Despite optimum soil moisture (80% FC) and temperature ($25^{\circ}C$) under glasshouse conditions, urea hydrolysis was delayed up to 7 days by adding Agrotain as can be seen by high urea-N and low NH_4^+ concentrations in different soil layers (Fig. 4.2 & 4.3) compared with urea alone. Other researchers have found that Agrotain delayed urea hydrolysis up to 2 weeks (Hendrickson and Douglass 1993; Rawluk et al. 2001); the optimum soil moisture and high temperature in our glasshouse could have accelerated the decomposition of NBPT thereby making it ineffective after day 7. The duration of NBPT activity is shorter at optimum soil moisture and high temperatures because of increased urease activity (Clay et al. 1990; Bremner et al. 1991). Such delay in urea hydrolysis by Agrotain has implications for N losses via NH_3 volatilisation (Zaman et al. 2008). Slow urea hydrolysis and lower accumulation of soil NH_4^+ by Agrotain under laboratory and field conditions have also been observed by other researchers (Zhengping et al. 1991; Watson, 2000; Zaman et al. 2008; Dawar et al. 2010). Delayed urea hydrolysis by Agrotain allows more time for rainfall or irrigation to wash the applied urea from surface soil to sub-surface soil layers as evident from the vertical and lateral movement of urea in our experiment under 80% FC as well as in 50% FC after 1 day of irrigation (Fig. 4.2 & 4.4). Such lateral and downward movements of urea could be due to the fact that urea is an uncharged particle, and therefore unlikely to be adsorbed by soil organic matter or by exchange sites, therefore applying irrigation after 1 day of treatment application accelerated urea movement: 25-48 % of the total recovered urea-N was below 20 mm depth and 18-27 % to the outer rings, particularly from day 2 and day 4. Similar results of rapid movement with flood irrigation

have been observed in field and laboratory experiments using urea (Stillwell and Woodmansee 1981) or other soil tracers (Bowman and Rice 1986). Zhengping et al. (1996) also observed urea movement through soil profile after applying Agrotain-treated urea to soil under controlled conditions. Applying irrigation after 1 day of treatment application brought back the moisture content to 80% FC, therefore the pattern of urea movement was similar in the 2 moisture treatments (Zhengping et al. 1996).

The decrease in urea-N concentration in the urea-alone treatment (with or without irrigation) was paralleled by a concomitant increase in the NH_4^+-N concentration in the soil layers (Fig. 4.1b). The concentration of NH_4^+-N formed from the hydrolysis of urea alone (without irrigation) treatment was significantly ($P<0.05$) higher around the placement ring (0-20 mm diameter) in the surface layer of the soil. In the absence of Agrotain, the majority of urea hydrolysis occurred in the topsoil layer, which led to the production of high concentrations of NH_4^+ in the surface soil layer (Fig. 4.3). Ammonium is considered to be a relatively less mobile ion in soils than urea (Haynes 1985; Wang and Alva 2000; Wang and Zhang 2004), as it is strongly held by soil exchange sites because of its positive charge therefore it tends to accumulate near the soil surface.

Irrigation after 1 day enabled NH_4^+-N to move from surface soil layer into sub-surface soil layers (Fig. 4.3) as 28-44 % of the total recovered NH_4^+-N was below 20 mm depth and 13-24 % to the outer rings, from day 2 to day 4 (Table 4.2 & 4.3). The increase in NH_4^+-N in sub-surface soil layers could be due to its macro-pore leaching and movement of un-hydrolysed urea. These results support the idea that urea-use efficiency may be improved through reduced gaseous losses of NH_3 if urea is moved into the soil with small amounts of irrigation (16 mm) (Black et al. 1987). Irrigation facilitates the transport of added urea into

the root-zone of sub-surface soil layers, dilutes surface NH_4^+ concentration, reduces NH_3 partial pressure and thereby minimizes NH_3 losses (Whitehead and Raistrick 1993). The distribution and movement of applied N during an irrigation event will depend on N form (urea versus NH_4^+). The source of NH_3 is mainly the exchangeable NH_4^+ present in the soil. We suggest that, although soil colloids adsorb NH_4^+ ions, applying irrigation of up to 10 mm could reduce the higher concentration of NH_4^+-N in the surface soil layer, thereby resulting in its even distribution down the soil profile and laterally away from the application point.

4.5 Summary

Although conducted under artificial conditions (repacked soil columns), this experiment has shown that Agrotain not only delays urea hydrolysis by inhibiting the urease enzyme in the soil, but it also results in greater movement of urea both vertically and laterally in the soil. This may partly explain the previous findings in Chapter 2 (Dawar et al. 2010a) and 3 of an improved yield response in pasture herbage in response to fertilisation with Agrotain-treated urea. In addition, irrigation allows for both greater movement of urea to the potential rooting zone of the pasture from the application point and also a reduction in the concentration of NH_4^+ present in the surface soil layer. In combination, these effects may have important implications for the effective management of N-addition under field conditions. It is to the field that this thesis now turns.

Chapter 5

Urease inhibitor reduces N losses and improves plant-bioavailability of urea applied in fine particle application or granular form under field conditions.

5.1 Introduction:

Application of different N fertilisers such as urea, ammonium nitrate, ammonium sulphate and sodium nitrate to increase agricultural yields has led to increasely intensive livestock operations in many regions of the world. However, this increase in N use, with N use efficiency (NUE) reported to be between 33 to 50%, is contributing to higher worldwide N losses via ammonia (NH_3) volatilisation and nitrate (NO_3^-) leaching that impact air and water quality (Raun and Johnson 1999; Baligar et al. 2001; Follett et al. 2001; IPCC 2007). Granular urea application after 1-2 grazing cycles is a common practice in grazed pastures in New Zealand (Blennerhassett et al. 2006). However, the fertiliser use efficiency of granular urea is reported to be relatively low (i.e. 10:1, meaning that every kg of applied N produces 10 kg of dry matter) (Quin et al. 2006). Such a low efficiency of granular urea means that a large percentage of the applied fertiliser N is not being used for productive purposes and is lost to recipient ecosystems (Harrison and Webb 2001; Blennerhassett et al. 2006). After application, urea is quickly hydrolyzed to ammonium (NH_4^+), which in turn induces gaseous N losses via NH_3 volatilisation (Mulvaney and Bremner 1981; Zhengping et al. 1991). High NH_3 emission from soil lowers the efficiency of applied urea and has potentially negative environmental effects like acidification of soil and eutrophication of water if deposited on vulnerable recipient ecosystems (Martikainen 1985; Janzen et al. 1999).

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There is a need to improve the efficiency of urea-based fertilisers through new technologies and management approaches. In a previous study under optimum moisture and temperature conditions in glasshouse (Chapter 2), I found significant improvement in herbage dry matter production when urea was applied with the urease inhibitor Agrotain compared with that following application of urea alone or other ammonium- and nitrate-based fertilisers (Dawar et al. 2010a). Another application method involves adding urea in a fine particle application (FPA). This results in a more even distribution of urea, much of which (70% to 80%; Chapter 3; Dawar et al. 2010b) is intercepted by pasture leaves, enabling them to take up N in urea form directly through the large leaf surface. In Chapter 3, I also described how application of a range of N fertilisers (including urea) in FPA form improves bioavailability of N and produces more herbage dry matter compared with application in granular form (Dawar et al. 2010b).

Although several studies have been published on N losses from granular urea applied to grazed pastures and other cropping systems worldwide (Martikainen 1985; Janzen et al. 1999; Cookson et al. 2001; Abdalla et al. 2010), there is a lack of information on N losses from urea applied with Agrotain under field conditions. In addition, to our knowledge, no study has reported on N losses from urea applied with Agrotain in FPA form in a grazed pasture system. The objectives of the research in this Chapter were to compare and assess N losses and bioavailability of urea applied with Agrotain in both FPA and granular forms under field conditions. I tested the hypotheses that urea applied in FPA form will reduce N losses and will improve the pasture response efficiency when compared with granular application. I also hypothesised that combining FPA urea with Agrotain would further reduce N losses and improve N uptake efficiency. Recovery of N in the plant-soil system, and losses

via gaseous emissions, were investigated using ^{15}N-labelled urea applied to a field lysimeter experimental system.

5.2 Materials and methods

5.2.1 Field site

A field experiment was conducted on a permanent grazed pasture site near Lincoln, Canterbury, New Zealand (43$^°$ 64'32.00" S, 172$^°$ 38'58.90" E). The soil used was free-draining Paparua silt loam, Typic Haplustepts (Soil Survey Staff 1998). The soils occur on nearly flat landscape and have derived from gravelly parent material transported by rivers and deposited over the last 10,000 to 3000 years. The gravels are of greywacke origin and are mainly composed of quartz and feldspars. This is a well drained, shallow soil having 250 mm thick, very dark gray coloured, silty clay loam topsoil with strongly developed fine to medium nuts and granular structure underlain by dark gray coloured, silty clay loam, gravelly subsoil having moderate to weekly developed medium to coarse nutty structure with gleying and mottling.

Morphological properties indicate that rooting depth is the major limitation of this soil. Roots mainly confine to the 300 mm of the topsoil. Strongly developed nutty and granular structure of the topsoil facilitates better drainage and aeration. Dark colour in the topsoil is an indication of the accumulation of organic matter (humus). Good aeration and organic matter help increase microbial activities in the soil. Moist and wet soil consistency properties show that use of agricultural implements for land preparation is easy within a vast range of moisture levels. Soils are friable when moist and not sticky when wet. Reddish brown mottling below 300 mm in the subsoil is an indication of reduction and oxidation of iron oxides present in the soil due to fluctuating water table. Dark gray colours of the AB and

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Bwg (300-480+ mm) horizons indicates more reducing conditions due to long term saturation. This is due to slow hydraulic conductivity properties of the gravelly, Bwg horizon.

Four soil samples, each comprising 10 randomly collected soil cores, were analysed for key soil properties. The soil is free draining and had a pH 5.7, total N of 0.40%, organic matter of 7%, Olsen P of 25 µg mL^{-1} and cation exchange capacity (CEC) of 18 cmol$_c$ kg^{-1}. The pastures were predominantly ryegrass (*Lolium perenne* L.) and some white clover (*Trifolium repens* L.). The experimental area was fenced off one year prior to treatment application to avoid N deposition from grazing cows. Soil moisture and temperature probes were inserted at 0-10 cm soil depth to monitor moisture contents and temperatures. The amount of rainfall/irrigation applied was also monitored by installing a rain gauge at the experimental site.

5.2.2 Lysimeters collection and installation

A total of 25 undisturbed soil lysimeters (30 cm inner diameter and 40 cm deep), were collected from the field site. Each lysimeter made of polyvinyl chloride (PVC) tube (with a sharpened bevel at the bottom end) was first placed on the ground surface and pasture roots and soil around the perimeter were cut using a serrated knife. A clamping block (i.e. consisting of two pieces of timber, with the semi-circle cut on each side, held together by two pieces of threaded rod) was placed around each lysimeter to facilitate the insertion. A square piece of heavy plywood was then placed on top of each lysimeter and they were slowly pushed down using a 4.5- tonne digger (Plate 5.1). Once the lysimeters had reached required the depth (40 cm), the tubes were carefully pulled out, and a base plate with an L-shaped drainage nozzle in the center was attached to the bottom of each lysimeter with a water

resistant glove. A 5 mm diameter tube was then attached to each nozzle to collect leachate in a 5 L plastic bottle. The gap between the soil core and the lysimeter wall was sealed using hot Vaseline to minimise edge flow effects on water movement. In the same field, lysimeters were placed 0.5m above ground level. Two wooden strips (30 cm long and 4 cm high) were placed beneath each lysimeter to protect the nozzle and the leachate collection tube from being damaged. After levelling each lysimeter, a wooden retaining wall was built on both sides and the side gaps between lysimeters and the wall were filled carefully with soil up to the same level as that of the lysimeters (Plate 5.2).

Plate. 5.1. Lysimeter insertion.

Plate 5.2. Lysimiters installed in field

5.2.3 Treatment applications

All lysimeters received a basal dose of phosphorus (P) and sulphur (S), each applied at 40 kg ha^{-1} using triple super phosphate (TSP) and elemental S, respectively. The herbage was cut to a height of 6 cm above ground level to ensure uniformity before treatments application. Five replicates of the following treatments were prepared: ^{15}N-labelled urea (10 atom %), with or without Agrotain, applied either in granular form to the soil surface or in FPA form (through a spray) at a rate equivalent to 100 kg N ha^{-1}; an additional control treatment received no N. Granular ^{15}N-labelled urea was obtained from Novachem (Sydney, Australia), and was coated with the urease inhibitor by Summit-Quinphos (NZ) Ltd.

5.2.4 Plant and soil analysis

Herbage from each lysimeter was harvested 3 times, on day 21, day 42 and day 63 following treatment application. Bulk fresh weight harvested from each lysimeter was recorded. Harvested material was placed in pre-weighed paper bags and dried at 65 ^{o}C for 7 days. After drying, herbage was weighed and representative sub-samples of each sample were finely ground in a ball mill for total N and ^{15}N determination using a Dumas elemental analyser (Europa Scientific ANCA-SL) interfaced to an isotope-ratio mass spectrometer (Europa Scientific 20-20 Stable Isotope Analyser; Europa Scientific Ltd, Crewe, U.K.) at the University of Waikato Stable Isotope Analysis Unit. Calculations of ^{15}N recovery in herbage were carried out as described by the International Atomic Energy Agency (1976):

The percentage nitrogen derived from fertiliser (% N dff) =

$$\left(\frac{\%^{15} N \quad excess \ in \ sample}{\%^{15} N \quad excess \ in \ fertiliser} \right) \times 100$$

The percentage utilisation of applied nitrogen $= \dfrac{\left(\%N \, dff \times yield \ of \ N \right)}{rate \ of \ N \ application}$

At the end of the experiment each lysimeter was removed from the trench and carefully sliced into 0-10, 10-20 and 20-40 cm depth increments for analysis. The plant roots were removed by hand, rinsed gently with tap water followed by distilled water, and placed in pre-weighed paper bags and dried at 65 ^{o}C for 7 days. For total N and ^{15}N analyses, representative sub-samples were ground very finely in a ball mill and analysed as described above. Bulk density, moisture content, total N and ^{15}N of the soil were determined for each depth. For total soil N and soil ^{15}N analyses, representative sub-samples from each depth were air-dried, then ground very finely in a ball mill and analysed as described earlier. Sub-samples of soil (5 g on an oven dried basis) were also analysed for concentrations of mineral N in the soil. Soil was

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extracted with 2*M* KCl for 1 h and filtered through Whatman 42 filter paper. Soil extracts were immediately frozen and later analysed for NH_4^+-N and NO_3^--N concentrations by flow injection analysis (FIA).

5.2.5 Gaseous emissions of NH_3 and N_2O, and NO_3^- leaching

Mini plots (4 replicates receiving the same 5 treatments as above) were established in the same field to measure NH_3 and N_2O emissions. A quantitative active flow method described by Kissel et al., (1977) was modified and used to measure NH_3 emission. A PVC chamber (0.0398 m^2 area with a 2 mm hole in the middle and a tight sealed transparent lid to allow photosynthesis) was inserted on the perimeter of each mini field plot. Air from each chamber was sucked at a constant flow rate (2-3 L min^{-1}) through a manifold using a pump (Plate 5.3). Each manifold allowed us to suck air from four mini plots. A small fraction of that air was then passed through 0.05N H_2SO_4 in 100 mL plastic bottles using a sparging frit. The acid solution in each plastic bottle was replaced with fresh solution every 24 h and analyzed for NH_4^+-N concentration by a flow injection analyzer (FIA). The temperature inside each chamber was checked periodically with a thermometer and no rise in temperature was observed during the measurement period because of continuous air suction from each chamber. Any rainfall that occurred during the NH_3 measurement was compensated by spraying an equivalent volume of water.

Nitrous oxide emission was monitored by taking gas samples from each plot using the static chamber method developed by Saggar et al., (2004, 2007) (plate 5.4). Two gas samples were taken at times t_0 and t_{60} (60-min intervals) from each chamber with 60 mL polypropylene syringes fitted with three-way stopcocks. Gas samples were immediately transferred to pre-evacuated 12 mL exetainers and analysed on a Shimadzu GC-17A gas chromatograph

equipped with a [63]Ni-electron capture detector operating at column, injector and detector temperature of 65, 100 and 280 $^{\circ}$C, respectively. Basal N_2O emissions were measured 1 day before treatment application.

The sample of ambient air collected directly after closing the chambers (t_0), was used as a reference for calculating N_2O gas fluxes. Accuracy of the gas chromatographic data at ambient concentrations was 1% or better. The increases in N_2O concentrations within the chamber headspace were generally linear ($R^2 > 0.90$) with time (Zaman et al., 2009). The average rate of change in gas concentration was, therefore, determined using linear regression, and gas fluxes are then calculated from the following equation using the ideal gas law:

$$F = \rho\, \frac{V}{A}\, \frac{\Delta c}{\Delta t}\, \frac{273}{T + 273}$$

Where F is the N_2O flux (mg m^{-2} h^{-1}); ρ is the density of N_2O (mg m^{-3}); V is the volume of the chamber (m^3); A is the base area of the chamber (m^2); $\Delta c/\Delta t$ is the average rate of change of concentration with time (ppmv h^{-1}); and T is the temperature ($^{\circ}$C) in the chamber.

Nitrate leaching was monitored by collecting any leachate from each lysimeter (plate 5.2). After each leaching event, the volume of leachate collected was recorded and a sub-sample of 30 mL was taken for NH_4^+-N and NO_3^--N analyses by flow injection analysis (FIA).

Plate 5.3. Experimental site used for NH_3 emission measurements

Plate 5.4. Experimental site used for N_2O emission measurements

5.2.6 Herbage production and N uptake

Separate field plots were established adjacent to the lysimeter to measure herbage production and N uptake in intact pasture (Plate 5.2). Each plot was 1 m^2 in area, separated by a 1-m buffer zone. All plots received a basal dose of phosphorus (P) and sulphur (S), each applied at 40 kg ha^{-1} using triple super phosphate (TSP) and elemental S, respectively. The herbage was cut to a height of 6 cm above ground level to ensure uniformity before treatments application. The 5 treatments (7 replicates each) were identical the lysimeter experiment: urea with or without Agrotain, applied by hand in either granular form to the soil surface or in FPA form (through a spray) at a rate equivalent to 100 kg N ha^{-1} and a control treatment receiving no N.

Herbage from each plot was harvested 3 times to a standard height of 4 cm on day 21, day 42 and day 63 after treatment application. Bulk fresh weight harvested from each plot was recorded. To determine herbage moisture fraction and N uptake, small herbage sub-samples were obtained randomly from each plot, weighed fresh, transferred to pre-weighed paper bags and dried at $65^{o}C$ for 7 days. After drying, plant material was weighed, ground to <0.2 mm in a ball mill and analysed for total N concentration (LECO CNS-2000 elemental analyser, Australia). Herbage DM, N response and fertiliser response efficiency were calculated. Nitrogen response was calculated by subtracting herbage DM yield of the control (no N treatment) from the yield of individual fertiliser treatments. Nitrogen response efficiency was calculated as the N response divided by the amount of applied N.

5.2.7 Statistical analyses

Repeated measure analysis of variance (ANOVA) was carried out to determine the effect of time on measured parameters using Minitab (Version 12, Minitab Inc., USA). General linear model (GLM) was carried out at individual times when specific time treatment interactions

were statistically significant. Least significant differences (LSD) were calculated only when the treatment effect was significant at $P < 0.05$.

5.3 Results

5.3.1 Rainfall, irrigation, soil temperature and moisture content

Total amount of water inputs (rainfall + irrigation) and soil temperature and moisture contents in the top 10 cm soil depth during October 2010 to December 2010 are shown in Fig. 5.1A. Total water input from rainfall and irrigation events during the experimental period was 374 mm. Daily average soil temperature in 0-10 cm soil depth was approximately 18 $^{\circ}$C during October to December (Fig. 5.1B). Soil moisture contents in the top 10 cm showed temporal variations with the rainfall/ irrigation events (Fig. 5.1C).
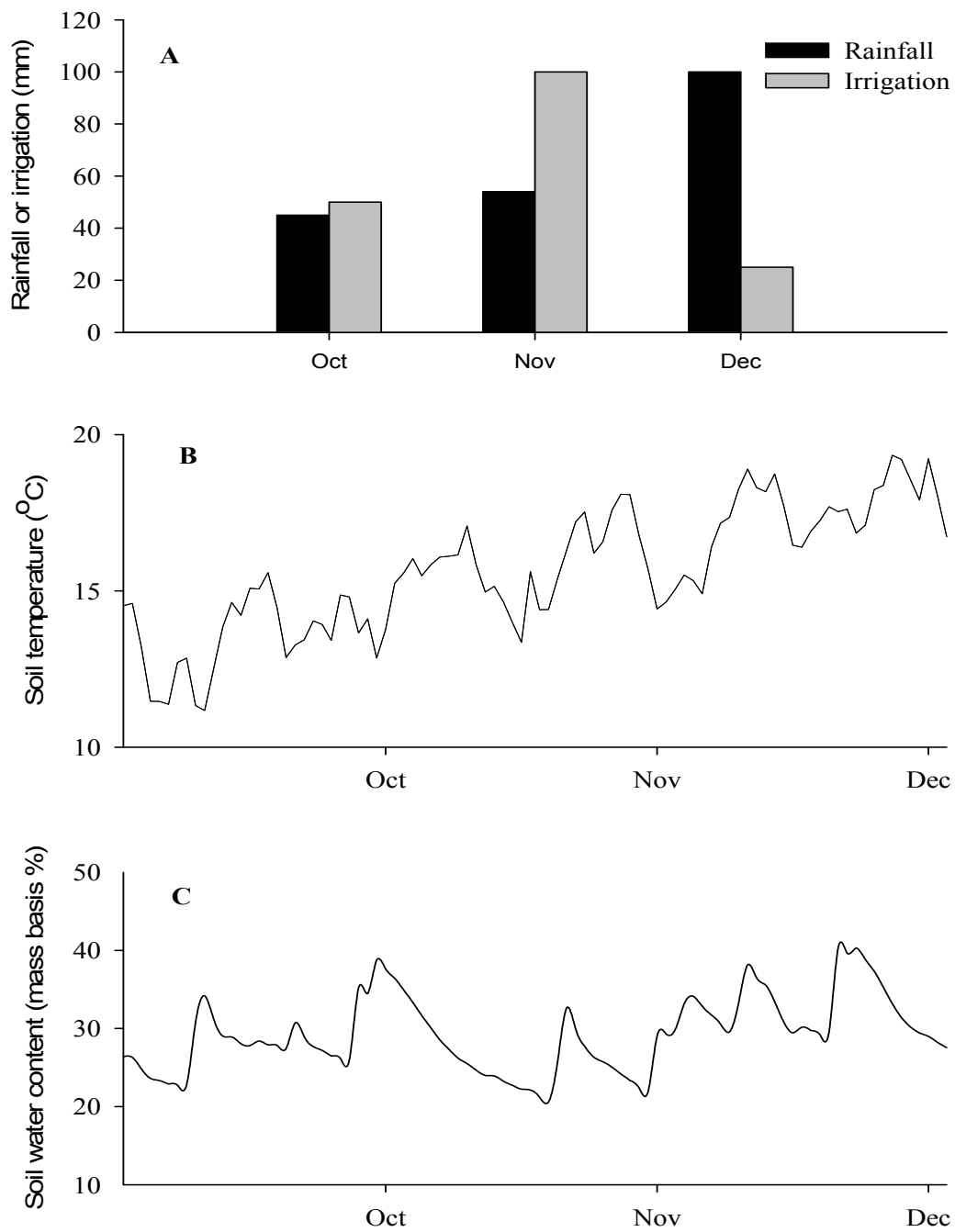
Fig. 5.1. Amount of rainfall or irrigation (mm), soil temperature and moisture contents (0-10 cm soil depth) during the experimental period.

5.3.2 Gaseous emissions of NH_3 and N_2O

NH_3 losses from applied urea did not differ between granular and FPA treatments in the absence of Agrotain (Fig. 5.2, Table 5.1). Daily, as well as cumulative, NH_3 losses were significantly ($P < 0.05$) reduced only when urea was applied with Agrotain, either in granular or in FPA form. For example cumulative NH_3 losses measured over 14 days were 6.8 kg N ha^{-1} from Agrotain-treated granular urea and 19.3 kg N ha^{-1} from granular urea-alone treatment. Similarly, NH_3 losses were 6.0 kg N ha^{-1} from the Agrotain-treated FPA urea treatment and 17.5 kg N ha^{-1} from the FPA urea-alone treatment. This represents a reduction of 65% and 69% by Agrotain-treated urea over urea alone. In the absence of Agrotain, the majority of NH_3 losses occurred during the first 2 days following urea application as can be seen by high NH_3 peaks exhibited by granular urea or FPA urea treatments (Fig. 5.2). NH_3 emissions returned to the level of the control on day-4 after treatment application (Fig. 5.2). Total NH_3 losses were 18.7% and 16.9% of the applied N in granular urea and FPA urea treatments, respectively. In contrast, urea applied with Agrotain in granular or in FPA form lost only 6.2% and 5.4% of the applied N as NH_3, respectively.
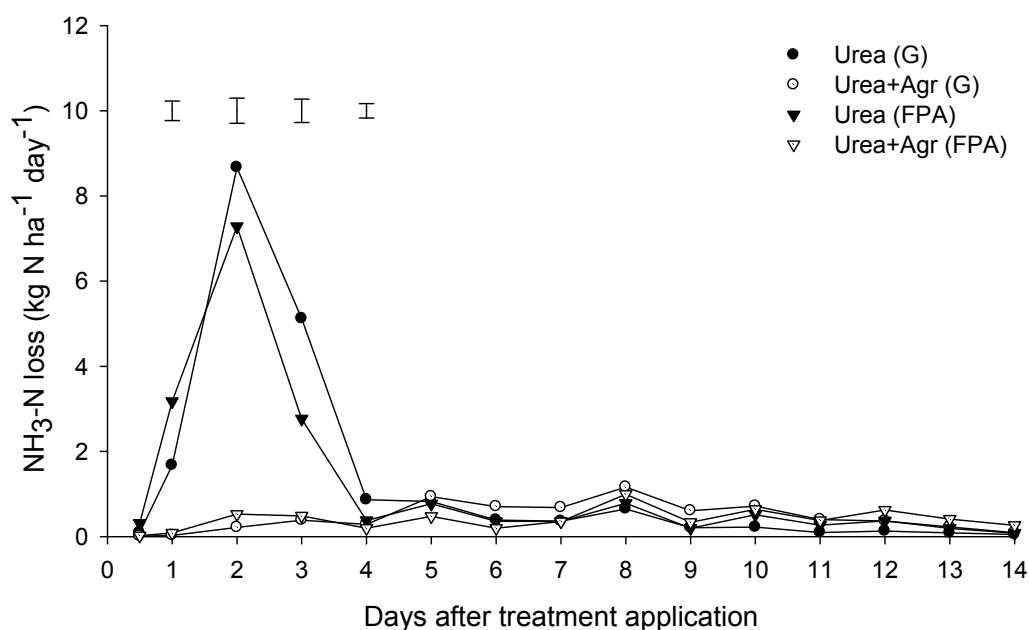
Fig. 5.2. Ammonia volatilization losses after application of urea with or without urease inhibitor (Agrotain) in granular or FPA form. Vertical bars indicate LSD values at P<0.05.

Table 5.1 Cumulative NH_3-N loss, the proportion of applied N lost as NH_3-N and % changes during 14 days of the experiment from plots treated with urea, with or without urease inhibitor, applied in granular or FPA form. Within columns, means with the same letters are not significantly different at the P<0.05 level where n= 4.

Treatment	NH_3-N losses (kg ha^{-1})	N lost as NH_3 (% of the applied N)	% Changes in NH_3 relative to urea (G)
Control (no N)	0.6[a]		
Urea (G)	19.3[b]	18.7[a]	
Urea+Agr (G)	6.8[c]	6.2[b]	-65
Urea (FPA)	17.5[b]	16.9[a]	-9
Urea+Agr (FPA)	6.0[c]	5.4[b]	-69

Nitrous oxide emission displayed temporal variations after application of urea (with or without Agrotain) in FPA and granular forms (Fig. 5.3). Soon after treatment application, a small peak of N_2O appeared on day-5, followed by another on day-14. More substantial N_2O

peaks appeared on day-35 and on day-49. Like NH_3 emission, urea applied in FPA form exhibited a similar trend in N_2O emission as corresponding granular treatments. In the absence of Agrotain, on day-35, N_2O losses were 22.0 g N_2O-N ha^{-1} from the granular urea and 20.3 g N_2O-N ha^{-1} from the FPA urea treatments, respectively. Similarly, on day-49, N_2O losses were 12.0 g N_2O-N ha^{-1} from the granular urea and 10.4 g N_2O-N ha^{-1} from the FPA urea treatments. Addition of Agrotain-treated urea, applied in either FPA or granular forms, significantly (P < 0.05) reduced cumulative N_2O emission compared with urea alone (Table 5.2). Over the 63-day experimental period, lysimeters with Agrotain-treated FPA urea emitted 0.37 kg N ha^{-1} compared with 0.42 kg N ha^{-1} of granular urea alone - representing a total reduction of 12% by Agrotain-treated FPA urea over granular urea. The amount N_2O lost as % of the applied N was 0.20% and 0.21% for Agrotain-treated urea in granular and FPA form, respectively. Such N losses were 0.26% and 0.24% for granular urea and FPA in urea-alone treatments, respectively.
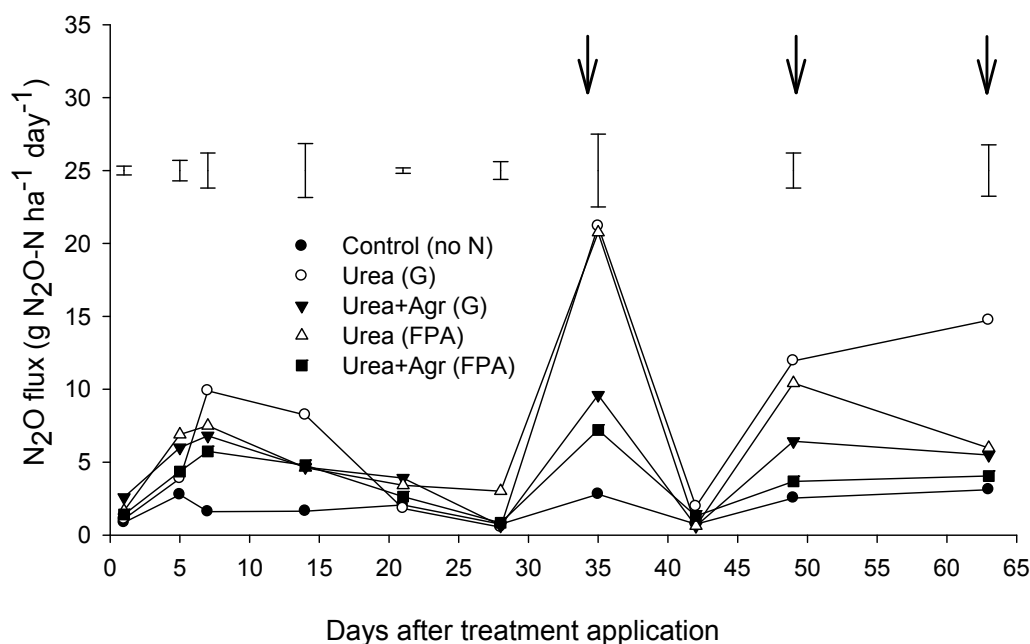
Fig. 5.3. N_2O flux after application of urea with or without urease inhibitor (Agrotain) in granular or FPA form. Vertical bars indicate LSD values at P<0.05. Arrows indicate rain or irrigation event.

Table 5.2 Cumulative N_2O-N loss, the proportion of applied N lost as N_2O-N and % changes during 63 days of the experiment from plots treated with urea, with or without urease inhibitor, applied in granular or FPA form.

Treatment	N_2O-N losses (kg ha^{-1})	N lost as N_2O (% of the applied N)	% difference in N_2O loss relative to urea (G)
Control (no N)	0.16^a		
Urea (G)	0.42^b	0.26^a	
Urea+Agr (G)	0.39^b	0.20^b	-7
Urea (FPA)	0.40^b	0.24^a	-5
Urea+Agr (FPA)	0.37^c	0.21^b	-12

Within columns, means with the same letters are not significantly different at the P<0.05 level where n= 4.

5.3.3 Nitrate leaching

Leaching events coincided with irrigation or rainfall. A total of 3 leaching events occurred during the experimental period on days 35, 49 and 60 after treatment application. Nitrate-N was the predominant form of N in the 3 leachate samples collected, with only trace amount of NH_4^+ (ranging from 0.004-0.035 kg N ha^{-1}). Nitrate varied significantly (P<0.05) with time and N treatments (Table 5.3). Cumulative NO_3^- leaching losses during the 63 days were significantly (P < 0.05) reduced when urea was applied in FPA form (2% of applied N) compared with granular form (1% of applied N). NO_3^- leaching losses reduced even further when urea in FPA form was applied with Agrotain (Table 5.3). NO_3^- losses from Agrotain-treated granular urea and FPA urea were 0.78% and 0.04% of the applied N. Over the 63-day leaching period, Agrotain–treated FPA urea reduced NO_3^- leaching losses by 55% over granular urea.

Table 5.3 Individual and cumulative NO_3^--N output (kg N ha^{-1} per leaching event) and % difference in NO_3^--N output relative to granular urea from plots treated with urea, with or without urease inhibitor, applied in granular or FPA form. Concentration of NH_4^+ in the leachate was low (in the range of 0.004-0.035 kg N ha^{-1}).

Treatment	D-35	D-49	D-60	Cum NO_3^--N losses (kg h^{-1})	% difference in NO_3^--N loss relative to urea (G)	N lost as NO_3^--N (% of the applied N)
Control (no N)	0.76[a]	0.56[a]	0.36[a]	1.68[a]		
Urea (G)	1.67[b]	1.36[b]	0.80[b]	3.82[b]		2.1[a]
Urea+Agr (G)	1.14[c]	0.80[c]	0.52[c]	2.46[c]	-36	0.78[b]
Urea (FPA)	1.07[c]	0.96[c]	0.57[c]	2.60[c]	-31	1.0[b]
Urea+Agr (FPA)	0.75[a]	0.50[a]	0.48[c]	1.72[a]	-55	0.04[c]

Within columns, means with the same letters are not significantly different at the P<0.05 level where n=5.

5.3.4 Herbage growth, N response efficiency and total N uptake

A total of 3 herbage cuts were obtained after application of treatments. Cumulative herbage dry matter, N response efficiency and N uptake varied significantly with urea application method and with addition of Agrotain (Table 5.4). Cumulative herbage dry matter was significantly (*P*<0.05) higher when urea was applied in FPA form than it was when urea was applied in granular form. Herbage production was improved even further when urea in FPA form was applied with Agrotain (a 38% increase over the granular urea-alone treatment).

Like herbage dry matter, N-use efficiency (kg dry matter production per kg of N applied) was also significantly (P<0.05) higher when urea (with or without Agrotain) was applied in FPA form than it was when applied in granular form (Table 5.4). N-use efficiency of FPA application was improved further when urea was applied with Agrotain. Overall, Agrotain-

treated FPA urea produced a significantly higher N-use efficiency than other treatments applied in either FPA or in granular form. For example N response efficiencies were 23, 19, 16 and 10 $kg^{-1}N$ for Agrotain-treated FPA urea, FPA urea-alone, Agrotain-treated granular urea and granular urea-alone, respectively (representing an average increase of 55, 89 and 126% for the first three treatments over granular urea alone). Total nitrogen uptake by the herbage was also significantly ($P<0.05$) greater when herbage was supplied with N in FPA form than in granular form (Table 5.4). Over the 63-day period, herbage recovered 148 kg N ha^{-1} and 136 kg N ha^{-1} in response to Agrotain-treated FPA urea and FPA urea alone compared with 107 kg N ha^{-1} in response to granular urea (Table 5.4). The total recovery of applied N in herbage differed significantly ($P<0.05$) amongst treatments, and was 26%, 48%, 56% and 67% for granular urea, Agrotain-treated granular urea, FPA urea and Agrotain-treated FPA urea, respectively.

Table 5.4 Total herbage dry matter (DM) (kg DM ha^{-1}), total N uptake (kg N ha^{-1}), % difference relative to urea-G, and response efficiency (kg DM kg^{-1} of applied N) from plots treated with urea, with or without urease inhibitor, applied in granular or FPA form.

Treatments	Total herbage DM (kg DM ha^{-1})	% difference relative to urea-G	Total N uptake (kg N ha^{-1})	% difference relative to urea-G	Response efficiency (kg DM kg^{-1}of applied N)	Total N Recovery (%)
Control (no N)	2412[a]		81 [a]			
Urea (G)	3439[b]		107 [b]	10[a]		26
Urea+Agr (G)	4003[c]	16	129 [c]	16[b]	20	48
Urea (FPA)	4359[d]	27	136 [c]	19[c]	27	56
Urea+Agr (FPA)	4731[e]	38	148 [d]	23[d]	38	67

Within columns, means with the same letters are not significantly different at the P<0.05 level where n= 7

5.3.5 ^{15}N recovery in plants and soil

Total ^{15}N recovery in plants was significantly ($P<0.05$) higher when urea (with or without Agrotain) was applied in FPA form than in granular form (Table 5.5). In the herbage there was a significant difference in ^{15}N recovery (as a percentage of applied N) between the granular and FPA application. Urea (with or without Agrotain) applied in FPA form resulted in significantly ($P<0.05$) higher ^{15}N recovery in the shoots compared with granular urea treatment. Shoot ^{15}N recovery was improved further when urea in FPA form was applied with Agrotain (56% more shoot ^{15}N recovery in herbage over granular urea; Table 5.6). A small amount of N (3-8%) was recovered in the roots. A significant proportion of applied N (24-33%) was recovered in the soil, particularly in the 0-20 cm depth. There was no significant difference between granular and FPA application on ^{15}N recovery in the soil at the 0-10 and 10-20 cm depths. However, FPA urea (with or without Agrotain) significantly ($P<0.05$) lowered ^{15}N recovery in the 20-40 cm soil depth compared to the granular urea treatment (Table 5.6). The remainder of ^{15}N-labelled fertiliser was not accounted for ('unaccounted' in Table 5.5) and is assumed to have been lost by gaseous emissions (via volatilisation and denitrification) and NO_3^- leaching losses. Total NH_3-N losses showed that FPA urea lost about 17% N as a NH_3 of the applied N (Table 5.1), which would amount to 77% of the unaccounted ^{15}N-labelled fertiliser. Addition of Agrotain-treated FPA urea significantly ($P<0.05$) reduced the total NH_3^-N losses to 5% of the applied N (Table 5.1).

5.3.6 Soil mineral N with depth

Soil NH_4^+ concentration in 0-10, 10-20, and 20-40 cm soil depths after day 63 were also significantly ($P<0.05$) different between the granular and FPA treatments (Table 5.7). The remaining mineral N was in the form NO_3^--N (Table 5.7). The total mineral N present in soil in the granular urea treatment after 63 days was significantly ($P<0.05$) higher (17 kg N ha^{-1}) then the Agrotain-treated FPA urea treatment (11 kg N ha^{-1}).

Table 5.5 Percentage recovery of ^{15}N in the plant and soil from plots treated with urea, with or without urease inhibitor, applied in granular or FPA form.

Treatment	Plants (shoot+root)	Soil (depth in cm)			Soil N sub total	Total ^{15}N accounted for	Total ^{15}N unaccounted for
		(0-10)	(10-20)	(20-40)			
Urea (G)	43^a	15^a	10^a	7^a	32^a	74	26
Urea+Agr (G)	55^b	18^a	11^a	4^b	33^a	88	12
Urea (FPA)	53^b	15^a	7^a	3^b	25^b	78	22
Urea+Agr (FPA)	65^c	17^a	5^b	2^{bc}	24^{bc}	89	11

Within columns, means with the same letters are not significantly different at the $P<0.05$ level where n= 5.

Table 5.6 Percentage recovery of ^{15}N in shoot, root and % difference in relative to granular urea from plots treated with urea, with or without urease inhibitor, applied in granular or FPA form.

Treatment	Shoot	% difference in relative to urea (G)	Roots
Urea (G)	39^a		3^a
Urea+Agr (G)	47^b	+21	8^b
Urea (FPA)	49^b	+26	4^a
Urea+Agr (FPA)	61^c	+56	4^a

Within columns, means with the same letters are not significantly different at the $P<0.05$ level where n= 5.

Table 5.7 Amount of ammonium-N and nitrate-N (kg ha^{-1}) in different treatments at different soil depths after experiment completion from plots treated with urea, with or without urease inhibitor, applied in granular or FPA form.

Soil depth	Urea (G)	Urea+Agr (G)	Urea (FPA)	Urea+Agr (FPA)
		Ammonium-N		
(0-10 cm)	3.2^a	4.3^a	2.3^b	0.5^c
(10-20 cm)	1.6^a	1.2^a	1.1^a	0.5^b
(20-40 cm)	1.0^a	0.2^a	0.0	0.4^a
Sub total	5.8^a	5.6^a	3.4^b	1.4^c
		Nitrate-N		
(0-10 cm)	4.9^a	3.0^b	6.4^a	3.0^a
(10-20 cm)	3.4^a	2.8^a	2.6^a	3.0^a
(20-40 cm)	3.0^a	2.2^a	1.5^b	1.1^b
Sub total	11.3^a	8.1^b	10.5^a	7.1^b
Total inorganic N	17.1^a	13.8^b	14.0^{bc}	8.5^d

Within rows, means with the same letters are not significantly different at the $P<0.05$ level where n=5.

5.4. Discussion

5.4.1 Gaseous emissions of NH_3 and N_2O

The percentages of applied N lost as NH_3-N from granular or FPA urea-alone treatments in our study were 18.7% and 16.9% (Table 5.1). These values are in the range of previously published NH_3 losses from 4% to 36% of the applied N after urea fertiliser, urine-N or animal slurry applications (Sherlock and Goh 1984; Vertregt and Rutgers 1987; Lockyer and Whitehead 1990; Bouwman et al. 1997; Eckard et al. 2003; Zaman et al. 2008; Menendez et al. 2009; Rochette et al. 2009; Sherlock et al. 2009). These NH_3 losses have both environmental and economic implications. Ammonia itself is not a greenhouse gas; however it acts as a secondary source of N_2O (Martikainen 1985) and thus contributes indirectly to global warming. It has been estimated that the direct NH_3 emissions from urea and urine applied to dairy pastures in New Zealand account for 30% and 15.9% (Sherlock et al. 2009) of total losses, respectively. Emitted NH_3 is chemically active; therefore it reacts with nitric acid (HNO_3) and sulphuric acid (H_2SO_4) in cloud droplets, extending its existence and impacts on atmospheric quality (Barthelmie and Pryor 1998). Ammonia emissions may also lead to other environmental problems like eutrophication (Bobbink et al. 1992) and soil acidification (van der Eerden et al. 1998) upon deposition on water and soil, respectively.

A number of researchers have attributed high NH_3 losses after granular urea application to the high pH 'hotspots' that urea granules produce after application (Mulvaney and Bremner 1981; Zaman et al. 2008). However, despite uniform distribution, we did not observe any reduction in NH_3 losses when urea was applied in FPA form (Table 5.1). However, NH_3 emissions were significantly lower from Agrotain-treated urea plots (in both granular and FPA form) compared with urea alone. Such a reduction in NH_3 emission could be due to decreased urea hydrolysis by Agrotain which thus results in the release of NH_4^+ at a slow rate

(Chapter 2, 3, 4; Dawar et al. 2010a and b). The majority the total NH_3 emissions occurred during the first 2 days of granular urea or FPA urea application (Fig. 5. 2) probably because of the fast urea hydrolysis rate which produces more NH_4^+ and OH^- ions (Chapter 4; Zhenping et al. 1991; Watson 2000; Singurindy et al. 2006; Zaman et al. 2008; 2009; Rochette et al. 2009). Maximum losses of N have been shown to occur when excess amounts of NH_4^+ are present in the leaves (Witte et al. 2002) or in the soil surface (Chapter 4; Dawar et al. 2010a, b). In addition to reducing the production of NH_4^+, Agrotain-mediated reduction in urea hydrolysis would likely also have the added effect of minimising the likelihood of a sudden rise in soil pH (Zaman et al. 2009), both of which are known to accelerate NH_3 emission rate. Overall, urea applied with Agrotain in granular or in FPA form reduced the total NH_3 emission by 65% and 69%, respectively, which is within the range of 42-70% achieved by other workers (Sanz-Cobena et al. 2008; Watson et al. 2008; Zaman et al. 2008).

The amount of N_2O emitted as a percentage of the applied N in our study (0.20-0.26%) is within the range estimated by the Intergovernmental Panel on Climate Change, which uses a default global emission factor of 1.25% (0.25-2.25%) for fertiliser-induced emission (Bouwman et al. 2002). Temporal variation in N_2O emission in urea-treated soils during the first 14 days of treatment application are likely due to nitrification (Zaman and Nguyen 2010), since nitrification produces N_2O as a byproduct (Inubushi et al. 1996). In contrast, the later N_2O emissions peaks on day-35 and day-49 I attribute to the abundant NO_3^- concentration (Table 5.7) and high soil moisture from rainfall events (Fig. 5.3), as denitrification produces N_2O through enzymatic reduction of NO_3^- for ATP synthesis (Tiedje 1988; Bremner 1997; Scholefield et al. 1997; Delaune et al. 1998). Agrotain-treated granular and FPA urea reduced the total N_2O losses by 7% and 12% over granular urea. Other researchers (Dobbie and Smith 2003; Zaman et al. 2008; Menéndez et al. 2009; Khalil et al.

2009) have also observed lower N_2O emission from urea treated with Agrotain. The higher

N_2O emissions from granular urea (0.42 g N_2O-N ha^{-1}) compared with Agrotain-treated FPA

urea (0.37 g N_2O-N ha^{-1}; Table 5.2) highlight the fact that Agrotain slowed urea hydrolysis,

thereby limiting nitrification rate, NO_3^- supply and ultimately N_2O production. These results

show that the use of Agrotain with urea-based fertiliser, applied either in granular form or in

FPA form may be a potential management strategy to lower N2O emissions from chemical

fertilisers applied to pasture soil. Interestingly, in this lysimeter study, uniform application of

urea in FPA form did not affect N_2O emission compared to corresponding granular

treatments. Overall, these findings indicate that a wider range of field studies at different

times of the year (spring versus, summer and autumn) are required to further assess the

potential of Agrotain-treated urea as a mitigating tool for N_2O emissions from grazed

pastures.

5.4.2 Nitrate leaching losses

Total NH_4^+ leached during the 63 day experimental period was lower than that of NO_3^-

because the positively charged NH_4^+ ions usually clay particles or soil organic matter, and

thus are largely protected against leaching in wetter soils. Nitrate is repelled from clay

exchange site because of its negative charge; therefore NO_3^- was the major form of N in the

leachate collected after fertiliser application (Table 5.3). The changes in NO_3^- concentration

in the leachate with time after application are related to the sequence of N transformation

events (Nannipieri et al. 1990; Zaman et al. 2008) after applying urea (with or without

Agrotain) to grassland. Overall, nitrate leaching losses over a 63-day period in our study were

much smaller than those reported in other studies (Ledgard et al. 1999; Cookson et al. 2000,

2001). The lower NO_3^- leaching loss is likely due to lower fertiliser application rates and

different soil moisture conditions (low rainfall/irrigation).

The amount of NO_3^- leached from the granular urea treatment was significantly (P<0.05) higher than that from the FPA urea treatment (Table 5.3), reflected in the greater amount of NO_3^--N present in the soil under granular urea treatment (Table 5.7). However, urea applied in FPA form reduced NO_3^- leaching losses by 31% compared to granular urea. Such reduction in NO_3^- leaching losses may provide the combined benefits of improved N fertilisation effects and simultaneous reduction of the risks of NO_3^- leaching and denitrification (Gooding and Davies 1992; Kettlewell and Juggins 1992; Readman et al. 1997) that impact soil and ground water. Reduction in NO_3^- leaching losses under FPA application was even greater (i.e. 55%) when urea was applied with Agrotain (Table 5.3) highlighting the fact that Agrotain slows urea hydrolysis, thereby limiting nitrification rate and NO_3^- supply. The majority of previous studies have reported on NO_3^--N leaching losses after granular application of urea (Prakash et al. 1999; Zaman et al. 2008). However, no studies to date have reported NO_3^--N leaching losses from urea or any other chemical fertiliser applied in FPA form. Therefore our findings make a significant contribution to our understanding of how agricultural N losses might be reduced by application of urea in FPA form (with or without Agrotain).

5.4.3 Herbage growth and N uptake

Fine particle application of urea and urea + Agrotain resulted in significant (P<0.05) improvements in herbage DM, N response efficiency and total N uptake compared with corresponding granular treatments (Table 5.4). These results agree with our earlier findings when urea with or without Agrotain and other chemical fertilisers applied in FPA form increased herbage DM and fertiliser N efficiency by increased N uptake (Chapter 3; Dawar et al. 2010b). Such improvement in response efficiency by urea and urea + Agrotain in FPA

form could be attributed to a number of factors including: uniform distribution of applied urea, easier N uptake through both leaves and roots, and efficient N metabolism. FPA technology results in uniform distribution of applied urea on a per plant basis, therefore a significant proportion (approximately 70%) of the applied urea in small particles is seen on pasture leaves during the first 12 hours of application (Chapter 3; Dawar et al. 2010b). These deposited urea particles could have enables pasture plants to absorb urea directly through their leaves/cuticles (Franke 1967; Watson et al. 1990) and this facilitates efficient conversion of urea into plant protein. Other researchers also found that urea is more easily absorbed through leaves/cuticles than NO_3^- and NH_4^+ (Bowman and Paul 1989; Bowman and Paul 1990; Bowman and Paul 1992; Riederer and Müller 2006).

Under field conditions, pastures plants take up the majority of their N in NO_3^- form because of the ubiquitous presence of urease and nitrifying bacteria. Being an uncharged particle, urea can be also be taken up by roots as an intact molecule without releasing any charge (H^+ or OH^-) to the rhizosphere. Direct absorption of urea and its subsequent conversion to plant protein leaves plant with extra energy (Middleton and Smith 1979), which could be used for additional growth. In contrast, NO_3^--N must be reduced before assimilation, which requires additional energy (Raven 1985; Ullrich 1992) meaning that the pasture plant may be left with extra energy to allocate to growth.

The higher response efficiency of Agrotain-treated FPA urea over FPA urea alone indicates that urea hydrolysis was delayed by Agrotain – this gives a longer opportunity for plants to take up urea through the leaves and/or roots. Agrotain treatment would also result in a reduced rate of conversion of urea on and in the plant, thus providing plants an opportunity to convert the absorbed urea into plant protein more efficiently. Urea hydrolysis generally takes

place within 1 to 2 days of application (Chapter 2, 3, 4; Dawar et al. 2010a, b) and the major

N losses via NH_3 volatilisation are most likely to occur within the same time period (Fig.

5.2), when excess amounts of urea and NH_4^+ are present in the leaves (Witte et al. 2002) or

on the soil surface (Chapter 4). A delay in urea hydrolysis by the action of Agrotain has the

potential not only to minimise the risk of N losses via NH_3 volatilisation from the plant and

soil (Fig. 5.2) but also to improve the bioavailability of urea-N. Most previous studies

undertaken by applying urea in granular form have reported that urease inhibitor may

considerably improve fertiliser N efficiency by increased N uptake (Liantie et al. 1993;

Watson et al. 1998; Xu et al. 2000; Zaman et al. 2008). Similarly, we also found significant

improvement in herbage DM and N uptake after applying urea with Agroatin under

glasshouse conditions (Chapter 2, 3; Dawar et al. 2010a, b). The present study provides

evidence for a higher N response efficiency of urea applied with Agrotain in FPA form under

variable field conditions (daily temperature, soil moisture, sunlight) and is therefore a

significant step toward improved urea N use efficiency.

5.4.4 ^{15}N recovery in plants and soil

Plants recovered 43-65% of the ^{15}N applied in the current study – this is in the range of

previously reported recovery rates (33-68%) for fertiliser applied to ryegrass seed crops

(Rowarth et al. 1998; Cookson et al. 2001; Williams et al. 2001). In the shoots a significant

greater (P<0.05) proportion of applied ^{15}N was recovered when urea was applied in FPA

form compared with granular application (Table 5.6). This increase in N uptake by the

herbage was a significant contributor to the increase in herbage yield (Table 5.4). Castle et al.

(2007) also observed more N through the leaves, and resulted in more dry matter production

in clover plants. A lower recovery of soil ^{15}N in FPA treatments (with or without Agrotain)

(Table 5.6) may have been due to by the increased plant uptake of N (Table 5.4, 5.6). At the

20-40 cm soil depth, recovery of ^{15}N was significantly ($P<0.05$) lower in FPA urea (with or without Agrotain) compared with their corresponding granular treatments, reflect the lower amount of nitrate-N we measured at this depth in these treatments (Table 5.7).

5.5 Summary

This experiment has shown that urea, with or with out Agrotain and applied in FPA form, is more effective in reducing N losses and improving herbage growth, N response efficiency, N uptake and recovery of applied ^{15}N in herbage than application in granular form. Urea in combination with Agrotain and applied in FPA form resulted in further reductions in N losses via NH_3 and N_2O emissions and nitrate NO_3^- leaching and higher herbage production, N response efficiency, N uptake and recovery of applied ^{15}N. The delay in urea hydrolysis by the action of Agrotain has the potential not only to minimise the risk of N losses from the plant and soil system, but also to allow for direct absorption of urea by ryegrass leaves/roots. This direct uptake, in addition to reductions in soil and plant urease activity, provides plants an opportunity to assimilate the absorbed urea into protein more efficiently. Applying urea in FPA form may well be a good management strategy under variable field conditions (soil moisture, pH, daily temperature and sunlight) and I conclude that combining FPA urea with Agrotain is a significant step toward improved N use efficiency and herbage production. In addition, this type of N application may significantly reduce N losses via NH_3 and N_2O emissions to the atmosphere and nitrate NO_3^- leaching to soil and ground water if applied at the right time and under the right environmental conditions. Further field research is required under different environmental conditions to evaluate and better understand FPA versus granular fertilisation of grazed pasture systems.

Chapter 6

General discussion and conclusions

6.1 Schematic illustration of principal findings

This research was undertaken to investigate the potential of incorporating urease inhibitor (Agrotain) with urea fertiliser to enhance its N-use-efficiency, and particularly the mechanisms underpinning uptake and assimilation of urea as influenced by Agrotain under glasshouse and field conditions. This project includes detailed studies of granular as well as FPA application of urea (with or with Agrotain) and other chemical fertilisers on herbage DM yield, N uptake, urea hydrolysis and its movement in controlled conditions. By contrast, the field observations facilitated the study of N uptake and N losses from urea applied with Agrotain in granular and FPA form in a grazed pasture system.

Fig. 6.1 highlights main results of the experimental work reported in Chapters 2, 3, 4, and 5:

(1) Urea applied in granular form with Agrotain resulted in significantly higher herbage DM yield (B1) and N uptake (B2) compared with urea alone (A1, 2) or compared with other forms of ammonium- and nitrate-based fertilisers. A lower N application rate (25 kg N ha^{-1}) was more effective than the higher rate (50 kg N ha^{-1}) as evident by the higher N-use-efficiency by the former (Chapter 2).

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(2) Urea applied in FPA form produced a greater herbage DM yield (C1) and exhibited a higher N response and N-use-efficiency (C2) than in granular form. Similarly, N-use-efficiency was improved in other forms of ammonium- or nitrate-based fertilisers in comparison with their corresponding granular application (Chapters 3 and 5).

(3) Urea applied in FPA form *and* in the presence of Agrotain produced the greater herbage DM yield (D1) and N-use-efficiency (D2) (Chapters 3 and 5).

(4) The ^{15}N studies conducted under glasshouse conditions clearly indicated that urea applied with Agrotain not only delayed urea hydrolysis but also increased N uptake through roots (B3) as well as through leaves (D4) (Chapters 2 and 3).

(5) The uncharged urea molecule exhibited both downward and lateral movements in the soil after surface application under glasshouse conditions (A5), and such movements were facilitated by the presence of Agrotain (B5), which retained N in urea form, and applying light irrigation after 1 day of urea application (Chapter 4).

(6) Nitrogen losses via gaseous emissions of NH_3 and N_2O, and NO_3^- leaching from applied urea varied with the urea application and with the application method (Chapter 5). Both granular and FPA applications exhibited similar levels of NH_3 losses (A6 and C6), but these losses were significantly reduced only when urea was applied with Agrotain, either in granular (B6) or in FPA form (D6). Both Agrotain and FPA reduced N_2O emission, however emission levels were low and such reductions were not statistically significant at

P<0.05. Nitrate-N leaching losses were significantly reduced when urea was applied in FPA form (C8). These losses were further reduced when urea was applied in FPA form with Agrotain (D8) compared with urea alone treatments.
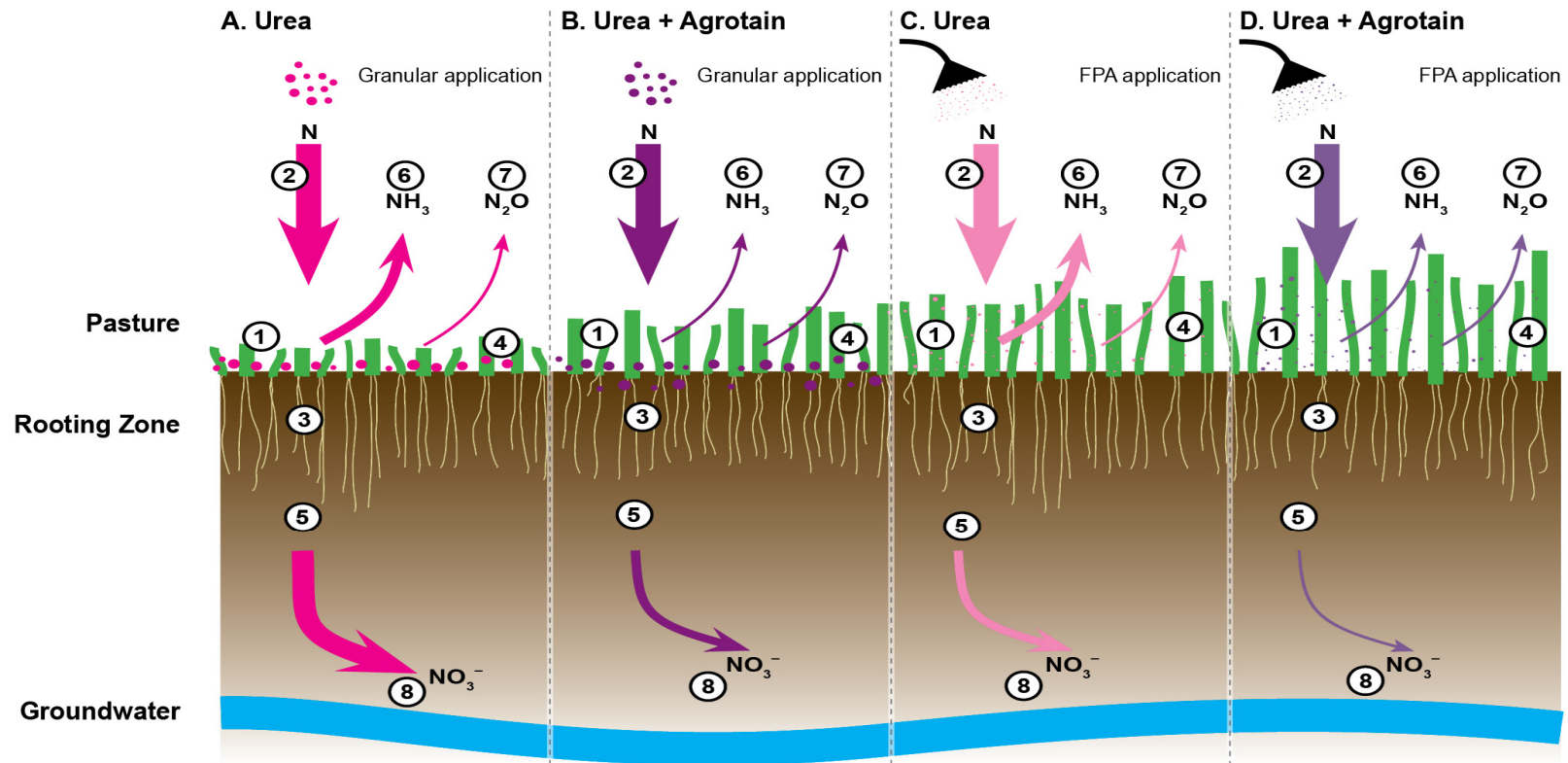
Fig. 6.1 A schematic diagram of the main results of the experimental work reported in Chapters 2, 3, 4, and 5. Details for each numbered response can be found in the text.

6.2 Discussion of principal findings

6.2.1 N use efficiency as influenced by Agrotain and FPA

Agrotain consistently increased pasture dry matter and N uptake compared with urea alone and compared with other forms of chemical fertilisers (Chapters 2 and 3) both under glasshouse and field conditions (chapter 5). A number of studies carried out in different agricultural systems (pastures versus crops) have reported increased DM yield and N uptake (Bundy and Observe 1988; Buresh et al. 1988; Joo et al. 1991; Hendrickson 1992; Watson et al. 1998; Xu et al. 2000; Blennerhassett et al. 2006; Zaman et al. 2008, 2009; Zaman and Blennerhassett 2010) which were related to the delayed urea hydrolysis and reduced NH_3 losses by urease inhibitors. However, Zaman et al. (2008) reported that the improvements in N uptake and fertiliser efficiency after applying Agrotain-treated urea were likely greater than can be explained solely by reductions in NH_3 volatilisation, N_2O emission and NO_3^- leaching. This suggests that other mechanisms could be involved in higher N responses in the presence of Agrotain (see below).

Applying urea in FPA form was more effective in producing higher herbage DM yield and taking up N more efficiently than other forms of ammonium- or nitrate-based fertilisers (Chapter 3, 5). Relatively few studies conducted under New Zealand pastures have compared urea applied with or without Agrotain in FPA and in granular forms and reported a similar increase in herbage DM yield (Quin et al. 2006; Zaman and Blennerhassett 2009). These studies attribute the response to delayed urea hydrolysis by Agrotain or a more even spread of applied urea, both of which improve plant access and reduce hot spots for N losses (NH_3). However, the even spreading of urea N may only be part of the mechanism involved in improving N use efficiency of the applied urea. Increases in herbage DM could also be related to the type of N taken up by

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plants as influenced by urease inhibitor and FPA and its effects on the biochemical processes of N assimilation in the leaves.

Many plants can take up N as urea through the roots when urea is not rapidly hydrolysed (Kirkby and Mengel 1970; van Beusichem and Neeteson 1982; Bradley et al. 1989; Watson et al. 1990). Urea can also be rapidly absorbed and assimilated by leaves following foliar application (Wittwer et al. 1963; Nicoulaud and Bloom 1996; Turley and Ching 1986). Franke (1967) has reported that foliar uptake of urea-N is more successful than other forms of N as urea improves the permeability of the cuticle and facilitates diffusion in to the leaf. After absorption, urea is quickly hydrolysed by the urease enzyme in the cytosol. The NH_3 released may be transported in to the chloroplast where it is assimilated by the chloroplastidic glutamine synthetase (GS) (Lam et al. 1996). Alternatively, NH_4^+ may be assimilated directly by the cytosolic GS, which has been reported to be limited to the phloem parenchyma cells in leaves (Edwards et al. 1990). Ammonia losses are most likely to occur when excess quatities of NH_4^+ are present in the leaves (apoplast) (Schjoerring et al. 2000). A delay in urease activity on the leaf surface by the action of Agrotain has the potential not only to minimise the risk of N losses via NH_3 volatilisation from the plant (Schjoerring et al. 2000) but also to improve the bioavailability of applied N (Zaman et al. 2008). Quin et al. (2006) have suggested that there is a potential for very substantial increases in the efficiency of use of fertiliser N, by manipulating supply, uptake and assimilation of fertiliser N in urea form, through the use of the urease inhibitor NBPT.

The ^{15}N studies conducted under glasshouse conditions improve our understanding of the mechanism by which urea applied with Agrotain in granular as well as in FPA forms improve N

uptake and N use efficiency (Chapters 2 and 3). The improved N uptake may have been in large part due to fact that Agrotain was retaining the applied N in its uncharged urea form, which provided plants an opportunity to take up an increased proportion of the applied urea-N through roots or leaves than in the case with urea alone. One potential benefit of this is that less energy is required to assimilate urea into amines, amides, amino acids and protein than NH_4^+ or NO_3^-. Plants have the ability to take up N both as NH_4^+ or NO_3^-. The abundant form of mineral N in temperate soils is reported to be NO_3^- and most plants are reported to prefer NO_3^- over NH_4^+ (Clark et al. 1979; Glass et al. 2002). However, the rate of uptake of NH_4^+ is often found to be greater than that of NO_3^-, especially at low temperatures. The uptake of urea may also be faster than that of NH_4^+ and NO_3^-. This due to the fact that urea does not affect the pH of rhizosphere as plants do not release either hydrogen (H^+) or hydroxyl (OH^-) ions after urea uptake to offset changes in charge balance. The release of these ions by plants also affects the uptake of other anions and cations. Clearly, the uptake and assimilation of N by plants is a complex cellular and biochemical process that involves a series of membrane transporters and N assimilatory enzymes (Fig 6.2) (Stewart et al. 1980; Lea 1993) – many of these issues have been beyond the scope of this study. However, the present study does provide some indicators of future directions for research (see below).
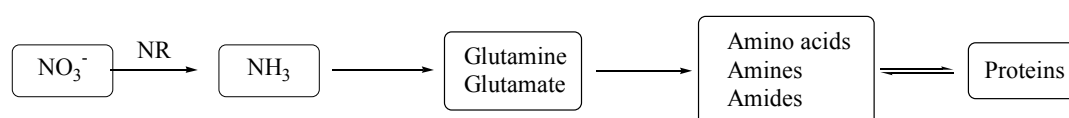
Fig. 6.2 Schematic illustration of N metabolism within plants

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A second major explanation for improved N use efficiency and N uptake could be the enhanced movement of urea both downward as well as laterally as influenced by Agrotain (Chapter 4). Being an uncharged particle, urea does not bind to negatively charged soil particles or organic matter, and thus it diffuses more easily into the rooting zone at moderate-high soil moisture levels. Delayed urea hydrolysis by Agrotain allows more time for rainfall or irrigation to move the added urea from the surface layer to sub-soil layers. The persistence of urea and then its downward and lateral movement is likely to provide plants an opportunity to take urea through a greater proportion of the root system, and thus enhance N-use efficiency. Importantly, the enhanced movement of urea also minimises N losses via ammonia volatilisation from surface-applied urea as rainfall or irrigation dilutes surface NH_4^+ concentration, reduces NH_3 partial pressure and thereby minimises NH_3 losses (Whitehead and Raistrick 1993). The distribution and movement of applied N during an irrigation event will depend on N form (urea versus NH_4^+).

6.2.2 The impact of Agrotain and FPA on N losses

The field study provided significant insights into N losses via gaseous emission of NH_3, N_2O and NO_3^- leaching as well as pastures dry matter, N uptake and N recovery as influenced by application methods and Agrotain under variable field conditions (Chapter 5). Gaseous emissions of NH_3 were not significantly influenced by FPA versus granular application of urea. It is generally believed that FPA results in a more even spread of applied urea and thus avoids creating 'hotspots' responsible for NH_3 emissions. However this was not the case in the field experiment, when both FPA and granular applications exhibited similar amounts of NH_3 emission. These results further suggest that NH_3 losses are related to increased urea hydrolysis, which can be controlled only by the use of a urease inhibitor like Agrotain. A number of studies

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have reported reduced NH_3 losses after granular application of urea with Agrotain (Sanz-Cobena et al. 2008; Watson et al. 2008; Zaman et al. 2008), but no reported study has highlighted this aspect of NH_3 losses resulting from FPA.

N losses via N_2O followed a similar trend to that of NH_3 emissions - there was no difference in N_2O emissions between the two application methods. Nitrous oxide emissions after fertiliser applications are related to the N transformation processes (urea hydrolysis, NH_4^+ and NO_3^- production) as affected by soil moisture and temperature (Dalal et al. 2003; Saggar et al. 2004; Luo et al. 2008; Menendez et al. 2009; Zaman et al. 2008, 2009). Generally more N_2O is emitted from grazed pastures in spring than that in autumn because of the wet soil conditions in the later season (Saggar et al. 2004, 2007; Zaman et al. 2009). Nitrification is considered to be the predominant source of N_2O production (Inubushi et al. 1996) in the presence of large amounts of NH_4^-N which result from urea-N hydrolysis, especially when the soil water content is at water filled pore space (WFPS) of 60% or less. However, denitrification may become the major source of N_2O production when NO_3^- accumulates after NH_4^+ oxidation coupled with higher soil water content (WFPS > 60%) after heavy rainfall or application of irrigation water (Saggar et al. 2004, 2007; Luo et al. 2008). The small reduction in N_2O emission as a result of Agrotain suggests that delaying urea hydrolysis not only lowers the nitrification rate, one of the major mechanisms contributing to N_2O production (Kyveryga et al. 2004; Zaman and Chang 2004; Sahrawat 2008), but may also have the potential to reduce N_2O emission via denitrification.

Reduced N losses via nitrate leaching are also an important potential benefit of the utilisation of urease inhibitor and FPA. The environmental effects of NO_3^- leached to groundwater and other

waterways and the potential damage to soils are a major concern worldwide. The accumulation of NO_3^- in the environment results mainly from non-point-source runoff from the over-application of N fertilisers, voided urine and dung, and from poorly or untreated effluents and sewage (Sing et al. 2008). High concentrations of NO_3^- in lakes, rivers and estuaries can result in eutrophication and algal blooms, and links have also been made between high NO_3^- and toxicity in fish eggs, amphibian eggs, and tadpoles. Nitrate toxicity can manifest itself in both humans and livestock, although the NO_3^- concentrations that produce toxicity are much higher for livestock than those for humans (Bolan et al. 2004b). The reduced NO_3^- leaching as result of Agrotain-mediated improvements in pasture N uptake (that minimises the chances of N being lost via NO_3^- leaching) has important implications for mitigation of environmental impacts of fertilisation. This will be an important issue for the future sustainability of intensive agricultural practices, but further work is needed to provide a detailed understanding of how these potential benefits can be optimized in specific land-management scenarios.

6.2.3 The economic benefits of urea with Agrotain applied in FPA form

The financial benefit to the grower depends on how well Agrotain-treated urea in FPA performs over granular urea and the returns for meat or milk from the extra dry matter produced. A cost-benefit calculation developed by using Chapter 5 yield results, fertiliser costs and milk solids payouts is presented in Table 6.1. For a given response rate and milk solid payout, the table shows how much financial benefit there is to using urea with Agrotain in eiather granular or FPA form. This clearly indicates that there will likely be significant economic benefits to the grower from using Agrotain-treated urea, especially in FPA form.

Table 6.1. Cost-benefit analysis

Cost	Urea-G		urea+Agr-G			urea+Agr-FPA	
Product cost ($/t)	$	624.00	$	726.00		$	726.00
On truck fluidising cost (per ha)	$	-	$	-		$	26.00
Bulk Cartage ($/t)	$	50.00	$	50.00		$	100.00
Spreading and tracking ($/ha)	$	12.00	$	12.00		$	14.00
Application cost ($/ha)	$	86.00	$	86.00		$	83.00
Total cost ($/t)	$	772.00	$	874.00		$	949.00
Spread rate (kg urea/ha/yr)		217		217			217
% N in product					46%		
N applied (per ha/yr)		100		100			100
Nitrogen Response Efficiency (kg DM per kg N) – see Ch 5		10		16			23
Additional herbage (kg DM/ha/yr)		1000		1600			2300
Cost of fertilser ($/ha)	**$**	**167.83**	**$**	**190.00**		**$**	**206.30**
Price premium for Agr-treated FPA			$	22.17		$	38.48
Extra herbage (kg DM/ha/yr)				600			1300
Returns							
Pasture utilisation					80%		
Kg DM/kg Milk Solids					12		
Milk payout ($/kg MS) – (Jan 2011 value)					$ 7.20		
Kg MS from additional grass produced		67		107			153
Gross return per ha	$	480.00	$	768.00		$	1,103.99
Net return	**$**	**312.17**	**$**	**578.00**		**$**	**897.69**
Cents per kg DM	$	0.17	$	0.12		$	0.09
Additional Potential Return from Using Age-treated urea over Granular urea			$	265.83		$	585.52

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6.3 Future studies

The present study has improved our understanding of the mechanism of N use efficiency of urea applied with or without Agrotain in granular as well as in FPA form under glasshouse and field conditions. Apart from agronomic benefits, these results also provide important insights into the impacts of applying urea with Agrotain on N losses, especially NH_3 and NO_3^- leaching. However, the results have raised a number of important questions, which need to be addressed in future research studies.

(1) The improved pasture N response exhibited under optimum soil moisture (80% FC) and temperature (25 ^{o}C) conditions suggest that variable soil temperatures and moisture levels should be included in future studies to further our knowledge in this area.

(2) Further field information is required to verify the relationships between high pasture dry matter and N uptake as observed under controlled conditions in the glasshouse.

(3) Estimates of N-use efficiency and N losses come from a field study conducted on a dairy farm under spray irrigation in late spring. Such measurements should be conducted under a wider range of soil management practices (irrigated versus non irrigated) and at different times of the year (spring versus, summer and autumn) to confirm the appropriateness of using Agrotain in improving N uptake and mitigating N losses. A fruitful line of investigation would be how different soil types with

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different physiochemical properties and management practices behave in terms of pasture productivity and N losses after being treating with urea with Agrotain.

(4) Pasture responses to applied N fertilisers will clearly vary with the timing of the fertiliser application. Future studies should focus on different fertiliser timing to explore this relationship.

(5) The present studies have concerned the application of N alone. Future studies should investigate the extent to which additional plant nutrients such as sulphur (S) and trace elements (molybdenum, selenium, zinc and boron) benefit N-use efficiency and under what circumstances the benefits can be optimized.

(6) The responses of plant N content and dry matter observed in Chapters 2 and 3 suggest that N consumption is greater in the early stages of the fertiliser response, but translation into dry-matter yield is, by comparison, much slower. It seems clear that for all forms of applied N, uptake of N precedes initiation of growth by some time. There would be great benefit in research investigating approaches to manipulate plant growth response (e.g. hormonal applications) so that it more closely coincides with the timing of N application.

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APPENDIX

The impact of urease inhibitor on the bioavailability of nitrogen in urea and in comparison with other nitrogen sources in ryegrass (*Lolium perenne* L.)

K. Dawar[A]*, M. Zaman*[B]*, J. S. Rowarth*[C]*, J. Blennerhassett*[B]*, and M. H. Turnbull* [A,D]

[A]School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch, New Zealand.
[B]Summit-Quinphos (NZ) Ltd, Private Bag 12508, Tauranga Mail Centre, Tauranga 3143, New Zealand.
[C]Institute of Natural Resources, Massey University, Private Bag 11222, Palmerston North, New Zealand.
[D]Corresponding author. Email: matthew.turnbull@canterbury.ac.nz

Abstract. Improving nitrogen (N)-use efficiency of applied urea is critical to maximise its uptake and decrease environmental impact. Two glasshouse-based studies were conducted to investigate the potential of incorporating urea fertiliser with urease inhibitor (N-(n-butyl) thiophosphoric triamide (NBPT) or 'Agrotain') to enhance fertiliser N uptake
efficiency. Topsoil (0–0.075 m, Typic Haplustepts silt loam) from a pasture site near Lincoln, Canterbury, New Zealand,
was collected and ryegrass (*Lolium perenne* L.) was grown from seed in standard plant trays maintained at soil moisture
contents of 75–80% field capacity. Urea, Agrotain-treated urea, ammonium nitrate, ammonium sulfate, or sodium nitrate,
were applied in granular form at rates equivalent to 25 or 50 kg N/ha with 4 replicates. Herbage was harvested 21 and 42 days after application of treatments to assess dry matter (DM) production, N uptake, leaf amino acid, ammonium (NH_4^+) and nitrate (NO_3^-) concentrations, and nitrate reductase activity (NRA). In a separate pot experiment, granular
^{15}N urea (10 atom%) with or without Agrotain was applied to ryegrass at 25 kg N/ha. At 0.5, 1, 2, 3, 5, 10, and 21 days
after treatment application, 3 pots per treatment were destructively sampled to determine urea hydrolysis, herbage DM,
and ^{15}N uptake. In both experiments, Agrotain-treated urea improved bio-availability of added N and resulted in significantly higher herbage DM yield and N uptake than urea alone or other forms of N fertilisers. Agrotain-treated urea
applied at 25 kg N/ha increased N response by 66% compared with urea alone (and by greater proportions compared with
the other fertiliser forms). Agrotain-treated urea applied at 25 kg N/ha produced significantly higher uptake efficiency (13 g DM/g of applied N) than at 50 kg N/ha (5 g DM/g of applied N). Tissue amino acids, NH_4^+ and NO_3^- contents, and
NRA were not significantly influenced by any type of fertiliser. Results from the ^{15}N experiment support the suggestion
that a delay in urea hydrolysis by Agrotain provided an opportunity for direct plant uptake of an increased proportion of
the applied urea-N than in the case of urea alone. Treating urea with Agrotain thus has the potential to increase N-use efficiency and herbage production.

Additional keywords: Agrotain, herbage dry matter, hydrolysis, N response, ^{15}N, N-(n-butyl) thiophosphoric triamide (NBPT).

The full article is available in the print copy of this thesis, or at doi:10.1071/CP09205

Urea hydrolysis and lateral and vertical movement in the soil: effects of urease inhibitor and irrigation

K. Dawar & M. Zaman & J. S. Rowarth &
J. Blennerhassett & M. H. Turnbull

Abstract A glasshouse-based study was conducted to investigate the effect of urease inhibitor N-(n-butyl) thiophosphoric triamide (‘Agrotain’) and irrigation on urea hydrolysis and its movement in a Typic Haplustept silt loam soil (in 72 repacked soil cores). Half (36) of these cores were adjusted to soil moisture contents of 80% field capacity (FC) and the remaining 36 cores to 50% FC. Granular urea with or without Agrotain was applied at a rate equivalent to 100 kg N ha^{-1}. There were three replicates to these two sets of soil cores. After 1 day of treatment application, soil cores of the 50% FC were adjusted to 80% FC by applying surface irrigation. Twelve pots were destructively sampled at each day after 1, 2, 3, 4, 7 and 10 days of treatment application to determine urea hydrolysis and its lateral and vertical movement in different soil layers. Agrotain-treated urea delayed urea hydrolysis during the first 7 days after its application. This delay in urea hydrolysis caused by Agrotain enabled added urea, which is uncharged, to move away from the surface soil layer to the sub-surface soil layer both vertically and laterally. In contrast, most urea hydrolysed to soil NH_4^+ within 2 days of its application. Irrigation after 1 day resulted in further urea movement both laterally and vertically from the surface soil layer (0−10 mm) to the sub-soil layer (30−50 mm) in Agrotain-treated urea. These results suggest that Agrotain delayed urea hydrolysis and allowed more time for rainfall or irrigation to move added urea from the surface layer to sub-soil layers where it is likely to make good contact with plant roots. This distribution of urea in the rooting zone has the potential to enhance N use efficiency and minimize N losses associated with ammonia volatilization from surfaceapplied urea.

Keywords Agrotain . Hydrolysis . Irrigation . Movement . N-(n-butyl) thiophosphoric triamide (NBPT) . Urea . Urea Inhibition