

# Improving pasture growth and urea efficiency using N inhibitor, molybdenum and elemental sulphur

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## Abstract

A one year field experiment was conducted to assess the efficiency of urea fertilizer applied to pasture near Lincoln University, New Zealand. Urea with or without ( $\pm$ ) molybdenum (Mo) were applied to field plot, with a urease inhibitor [N-(n-butyl) thiophosphoric triamide (nBTPT), nBTPT + elemental sulphur (S), and nitrification inhibitor dicyandiamide (DCD) + nBTPT defined as a double inhibitor (DI) in spring 2005. Mo was sprayed at the rate of 50 g Mo/ha/yr once. Urea  $\pm$  various inhibitor and S treatments were broadcast at a rate of 30 kg N/ha 5 times over one year. The Mo alone treatment increased pasture dry matter (PDM) yield by 8.9%. Molybdenum, when applied together with urea+nBTPT+S, urea+nBTPT and urea alone, caused an initial depression in PDM yield by 14.2, 13 and 5.6% respectively. However, these depressions in yield disappeared from the second pasture cut. Over a one year period, Mo applied with urea+nBTPT, urea+DI and urea+nBTPT+S produced 18179, 16716 and 18253 kg DM ha<sup>-1</sup> respectively, compared to 16171 kg ha<sup>-1</sup> for urea+Mo. Pastures which received no Mo, but were treated with urea+nBTPT+S, or urea+nBTPT produced 18982 and 18276 kg DM ha<sup>-1</sup> respectively, compared to 16363 kg ha<sup>-1</sup> for urea alone, giving an increase of 16% and 12% over urea alone. Pastures receiving Mo, together with urea+nBTPT or urea+nBTPT+S, also showed improvement in N uptake and N recovery, compared to urea alone. Applying urea with nBTPT and S have the most potential to improve urea efficiency.

**Keywords:** Urea, nBTPT, DCD, sulphur, pasture, molybdenum

## 1. Introduction

For managed grasslands in New Zealand the supply of nitrogen (N) after every rotation of grazing is critical factor for pasture growth. The largest source of N input to these grazed pastures come from excreta (urine + dung) of grazing animals. However that N input

generally covers only 25% of a grazing paddock on an annual basis and it occurs in a non-uniform distribution known as urine and dung patches (Zaman *et al.* 2012). Further, the N released from mineralization of soil organic N, or N from organic wastes (e.g. farm or dairy effluent)

cannot meet the pasture N demand, especially during early spring when there is a growing need for animal feed after lambing or calving (Zaman *et al.* 1998, 2013a). Hence, input of additional N from chemical fertilizers is a necessity. In addition to N, the supply of other plant nutrients, such as phosphorus, sulphur (S), potassium, magnesium and a wide range of micro-nutrients including molybdenum (Mo), as well as calcium, via lime, are essential for sustainability of these managed grazed pastures.

Urea is the predominant form of N fertilizer used, both in New Zealand and worldwide (IFA, 2011), principally because of its lower cost per unit N compared to other N fertilizers such as ammonium-nitrate, calcium-ammonium-nitrate, ammonium-sulphate, and di-ammonium-phosphate (Zaman *et al.*, 2008a). However, urea has been reported to have lower N response efficiency (NRE), where NRE is defined as kg of additional dry matter produced per kg of applied N, relative to the NRE's of ammonium- and nitrate-based fertilizers. This lower NRE also appears to be prevalent if urea is applied under sub-optimal soil moisture and at too low or high temperature (Zaman *et al.* 2008a, 2013a). Several management practices and technologies have been developed to improve the NRE of urea. One such approach is to treat granular urea with the urease inhibitor N-(n-butyl) thiophosphoric triamide (nBTPT - trade-name Agrotain®). The nBTPT is either added during the manufacture of urea, or granular urea is coated with nBTPT at a rate of 250 to 500 mg per kg of urea. The nBTPT coating delays the hydrolysis of urea by 7 to 10 days after soil application (Zaman *et al.* 2008a). This in turn increases the time taken for all the urea to be converted to ammonium-N and then nitrate-N ( $\text{NO}_3^-$ -N), which is more effectively taken by plants than ammonium-N with a consequent decrease in N leaching losses as  $\text{NO}_3^-$  and conversion to gaseous nitrous oxide ( $\text{N}_2\text{O}$ ) and di-nitrogen ( $\text{N}_2$ ) (Zaman *et al.* 2008b; Saggár *et al.* 2013). Urea can be treated with a nitrification inhibitor, e.g. dicyandiamide

(DCD) as well to improve its efficiency by retaining N in the  $\text{NH}_4^+$  form for a longer period, potentially further reducing N losses via  $\text{N}_2\text{O}$  emission and  $\text{NO}_3^-$  leaching (Zaman *et al.* 2008a; Soares, *et al.* 2012; Sanz-Cobena *et al.* 2012; Zaman and Nguyen, 2012). The NRE of urea (or other  $\text{NH}_4^+$ -based fertilizers) can also be improved by S application, which acidifies the soil due to its subsequent oxidation (Zaman *et al.* 2008a, 2010). Most New Zealand soils are S deficient; therefore pasture growth generally shows a positive response to S application.

Molybdenum is an important trace element and can exist in several oxidation states ranging from 0 to 6, with oxidation state 6 being the most common form found in agricultural soils. Molybdenum is not thought to be biologically active as the metal ion, but rather is an integral part of an organic pterin complex [called the Mo-co-factor (Moco)], which binds to Mo-requiring enzymes (Williams & Frausto da Silva 2002). Moco plays a key role in carrying out redox reactions in the metabolism of carbohydrates, and also of N and S (Vistosio *et al.* 2005). Moco is also involved in abscisic acid biosynthesis and purine degradation (Schwarz & Mendel 2006). Molybdenum as a micro-nutrient fertilizer is generally applied in small amounts to managed pastures worldwide every 4 to 5 years to enhance biological N fixation by clover (Sherrell & Metherell 1986). In New Zealand's temperate grass/clover pastures, which are grazed year-round, by far the major benefit of applying Mo in areas subject to deficiency is considered to be in increasing N fixation by clover, and clover growth as a result, and in time the productivity of the entire sward due to increased soil organic N content (During 1984).

Most trials carried out on pasture N response in New Zealand have used urea fertilizer alone, without the addition of other mineral element or micronutrients. The effects of urease and nitrification enzyme inhibitors on urea efficiency and hence on pasture productivity in the presence and absence of applied

Mo have not previously been investigated. This was the primary objective of this year-long study. Also, the effect of straight nBTPT was compared to a combination nBTPT plus DCD, and the response to the application of fine elemental S was measured.

## 2. Materials and Methods

### 2.1. Site description

The experiment was established on a poorly drained silt loam (Fluvaquentic Endoaquept; Soil Survey Staff 1998) near Lincoln University, Canterbury, New Zealand and implemented over 12 months, August 2005 to August 2006. The pastures were predominantly (80%) perennial ryegrass (*Lolium Perenne* L.) with some (8 to 10%) white clover (*Trifolium repens* L.) and were managed for grazing by dairy cows (3 cows/ha). Spray irrigation at the rate of 20 to 25 mm per every 2 to 3 weeks during summer (October to March in Southern Hemisphere), is a common farming practice in the Canterbury region. The study site received a total of 250 mm during this time.

### 2.2. Application of fertilizer treatments and pasture cuts

The experimental area was fenced off six months prior to initiation of treatments so as to avoid recent excreta deposition from the dairy cows. Three rows separated by a 1 m buffer zone were established and 10 plots, each 1x1 m in area (with a 1 m buffer zone), were established in each row, giving a total of 30 plots. Prior to treatment application, three composite soil samples from 0–7.5 cm (five soil cores from each row) were collected and analysed for key soil properties (Table 1). Also, just prior to each treatment application the pasture was mowed to 4 cm above ground level. Among the 10 treatments were: control (no N), control+Mo, urea, urea+Mo, urea+nBTPT, urea+nBTPT+Mo, urea+nBTPT+S,

urea+nBTPT+S+Mo, urea+nBTPT+DCD (double inhibitor, DI), and urea+DI+Mo. Each treatment was replicated 3 times. Urea was treated with DI at 250 mg nBTPT per kg of urea. The DI contained 350 mg nBTPT and 3.5 g DCD/kg urea. Elemental S in urea+nBTPT+S was 4%.

Prior to fertilizer application, sodium-molybdate was dissolved in water and then applied onto appropriate plots at 50 g Mo/ha in late August, 2005. Urea alone, urea+nBTPT or urea+DI was broadcast by hand five times during the year in split applications, each at 30 kg N/ha, on Aug 22, Oct 30, Jan 12, Mar 20 and June 7, thus giving total N application rate of 150 kg N/ha (Table 2). In the weeks following each treatment the pasture forage from each plot was cut using a lawn mower to a base level of 5 to 6 cm (i.e. leaving a live plant shoot biomass of ca. 1200 to 1400 kg DM/ha) once average pasture cover reached grazing height. For each cut bulk pasture fresh weight was recorded. These experimental cuts occurred twice after each fertilizer application. Then urea with or without nBTPT, DI and nBTPT+S were re-applied, and the cutting procedure was repeated. Prior to these experimental pasture cuts, five randomly chosen pasture samples (100 g fresh weight) were cut from each plot with a pair of scissors, bulked and transferred into pre-weighed paper bags. The fresh weight was then recorded and the pasture samples were dried at 65 °C for 5 days and dry weights recorded. This allowed the calculation of pasture moisture percentage and pasture dry matter (PDM) yield. The dry pasture samples were then ground to less than 0.2 mm particle size and analyzed for total N on a LECO CNS-2000 elemental analyser (LECO Australia Pty. Ltd., Castle Hill NSW Australia). The NRE was calculated by subtracting the PDM of the control from that of the control from that of the fertilizer treatments, then dividing that value by the amount of N applied, as noted below:

$$\text{NRE} = \frac{\text{kg PDM of fertilizer treatment minus kg PDM of the control treatment}}{\text{kg of N applied}}$$

The apparent % N recovery was calculated by subtracting the N uptake of the control from that of the fertilizer treatments, then dividing that value by the amount of N applied, as noted below:

$$\text{N uptake} = \frac{\text{Pasture N uptake of fertilizer treatment minus pasture N uptake of control treatment}}{\text{kg of N applied}}$$

**Table 1.** Pre-treatment soil properties. Values are means (n=3).

Analyses type	Values	Optimum range*
pH	5.9	5.8–6.0
Total N (%)	0.70	0.3–0.6
Organic C (%)	14.2	7–17
Olsen P (mg/l)	42	20–30
Potassium (me/100g)	0.31	0.4–0.6
Calcium (me/100g)	7.5	5–10
Magnesium (me/100g)	0.97	1–2
Sulphate-S (mg/kg)	15	7–15
Sodium (me/100g)	0.31	0.2–0.4
CEC (me/100g)	13	12–25
Base Saturation (%)	70	40–60
Volume weight (g/ml)	1	0.60–1
Total Mo in soil (mg/kg)	0.3	0.5–2
Total Mo in ryegrass (mg/kg)	0.25	0.3–0.4

\*Cornforth and Sinclair (1982).

**Table 2.** Details of the fertilizer application and pasture cut dates of the trial.

Fertilizer application	Pasture cuts
Application-1: 22-August-2005	Cut 1: 22-September-2005 Cut 2: 29-October-2005
Application 2: 30-October-2005	Cut 3: 29-November-2005 Cut 4: 10-January-2006
Application 3: 12-January-2006	Cut 5: 17-February-2006 Cut 6: 20-March-2006
Application 4: 2-April-2006	Cut 7: 4-May-2006 Cut 8: 7-Jun-2006
Application 5: 9-June-2006	Only one cut 26-August 2006

### 2.3. Statistical analyses

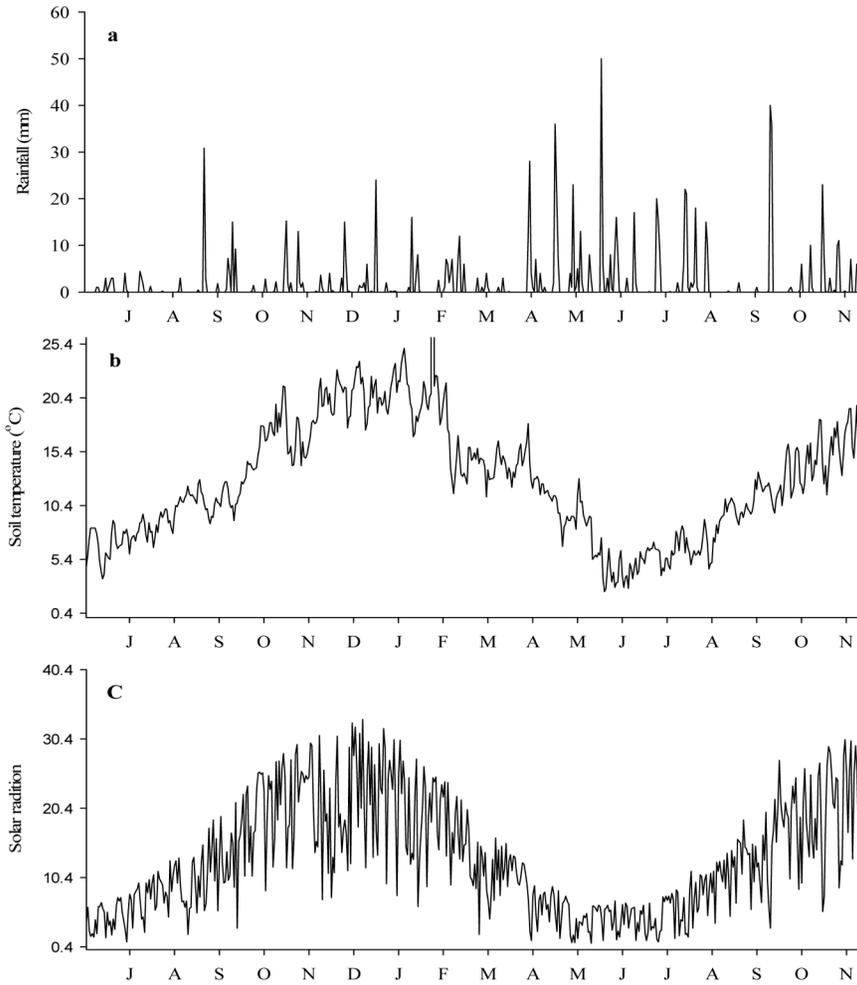
A repeated measure analysis of variance (ANOVA) was carried out in order to determine whether time (across the year) had any effect on the different measured parameters using Minitab (Version 12, Minitab Inc. USA). General linear model (GLM) was carried out at individual times when specific time x treatment interactions was statistically significant ( $p < 0.05$ ). When significant effects of treatment were observed, these were further explored using Tukey adjusted LSD value to make specific comparison among the different treatments. All the analyses were performed using Minitab (version 12).

## 3. Results

### 3.1. Soil chemical properties, soil temperature and rainfall

The soil fertility of the study site was high, e.g. soil pH, organic C, total N, available Olsen phosphorus, calcium, magnesium and sulphate-S levels in the

soil prior to treatment application were all considered adequate for normal pasture growth (Table 1). The soil potassium level was 0.31 me/100g, just below the optimum range. Therefore potassium was applied at 50 kg K<sup>+</sup>/ha using potassium chloride to ensure a near optimum level. The application of maintenance fertilizers (phosphorus and sulphur) in the spring is a common practice in pastoral agricultural systems. Therefore, these two elements were also applied at 40 kg P using triple super phosphate or 40 kg S/ha as elemental sulphur. Prior to our treatment application, Mo in the soil was assessed (0.3 mg/kg) as there was Mo in the ryegrass shoot tissue (0.25 mg/kg). Both were below the level required for optimal pasture growth; therefore we expected to get a positive response from the applied Mo. Total water inputs over the year at this site were 852 mm of rainfall and 280 mm of spray irrigation (Figure 1). Spray irrigation at the rate of 25 mm per irrigation event was applied only during summer (October to March, Southern Hemisphere), which is a common farming practice in the Canterbury region. Daily average soil temperatures in 0–10 cm soil depth were <6°C in June and July and were >19°C during December to February (see also Figure 1).



**Figure 1.** Amount of rainfall (mm) (a), soil temperature (0-10 cm) (b) and solar radiation (c) from July 2005 –Nov 2006.

**Table 3.** Cumulative pasture herbage yield (kg DM ha<sup>-1</sup>) after individual fertilizer applications as influenced by urea, urea with nBTPT, a combination of both nBTPT and DCD (DI, Double Inhibitor Treatments), urea + nBTPT + S to pasture soils. Four of the treatments are also paired with +Mo Values are means (n=3).

Treatments	Fertilizer applications				
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>
Control (no N)	3402	3152	3871	1996	909
Mo only	4010	3327	4096	2037	1052
Urea only	4215	4104	4665	2306	1073
Urea + Mo	3981	4190	4690	2198	1113
Urea + nBTPT	4778	4780	5099	2475	1144
Urea + nBTPT + Mo	4157	4801	5443	2594	1183
Urea + DI*	4745	4201	4463	2411	1115
Urea+ DI + Mo	4732	4306	4401	2113	1163
Urea + nBTPT + S	5055	4877	5278	2634	1137
Urea + nBTPT + S + Mo	4339	4742	5543	2470	1159
LSD ( $\alpha=0.05$ )	827	560	409	160	NS
<u>Percent change over treatment with Mo</u>					
Control (no N)	17.9	5.5	5.8	2.1	15.7
Urea alone	-5.6	2.1	0.5	-4.7	3.7
Urea + nBTPT	-13.0	0.4	6.7	4.8	3.4
Urea+ DI	-0.3	2.5	-1.4	-12.4	4.3
Urea + nBTPT + S	-14.2	-2.8	5.0	-6.2	1.9

### 3.2. Molybdenum effects

During the first two pasture cuts, compared to the control (no +N or +Mo), +Mo alone increased PDM yield by 17.9% (Table 3). This initial PDM yield for the +Mo alone (late August, 2005) treatment decreased in subsequent pasture cuts. As mentioned earlier, the +Mo was applied once only, at the commencement of the trial. Molybdenum on its own gave a significant increase in pasture dry matter, e.g. +8.9% over the control at the end of the year (Table 4). The analysis of three ryegrass samples after the first pasture cut showed Mo level > 1 mg/kg (data not shown). However, Mo levels in ryegrass samples in subsequent cuts (after urea applications 2 and 3) were below 0.4 mg/kg, possibly due to earlier Mo uptake by the pasture sward.

### 3.3. Individual and cumulative pasture production and N response efficiency

Pasture growth after each fertilizer application showed temporal variations and was also influenced by urea  $\pm$  inhibitors of urease and nitrification activity (nBTPT and DCD, respectively) (Table 3). Molybdenum applied with urea  $\pm$  the inhibitors caused an initial depressions in yield. After the first fertilizer application in the spring (August, 2005), only the urea plus nBTPT+S treatment produced significantly ( $p<0.01$ ) more PDM yield than urea alone. The PDM yield also increased in plots receiving urea plus either nBTPT or the DI treatments, relative to urea alone, though those increases were not statistically significant at  $p<0.05$ . There was an initial depression in PDM yield by 14.2, 13 and 5.6% when Mo was co-

applied with urea plus nBTPT+S, urea plus nBTPT and urea alone treatments respectively (Table 3). However, these depressions in yield by +Mo, when applied with urea or with urea plus the inhibitors either disappeared or became minimal with time in subsequent pasture cuts. When no +Mo treatment was applied, the PDM yield after applications 2, 3 and 4, were significantly ( $p<0.01$ ) higher for urea plus nBTPT+S and urea + nBTPT treatments, relative to urea alone. Urea plus the DI, however, did not show a significant PDM yield increase, relative to urea alone. Also, when no +Mo treatment was applied, urea  $\pm$  the inhibitors applied in late spring (2<sup>nd</sup> application) and early summer (3<sup>rd</sup> application), produced more PDM yield than when applied in the autumn. The lowest PDM yield occurred during the winter (after the 5<sup>th</sup> application). Here, with no +Mo treatment, urea  $\pm$  the inhibitors had no significant effect on PDM yield compared to control (no N).

When cumulative pasture dry matter yield of the five fertilizer applications (over the full year) were considered (Table 4), the +Mo alone treatment increased pasture dry matter by 8.9% compared to the control. However, a slight depression in PDM yield for the year was seen for the urea only treatment when applied with Mo. Over the year, pastures receiving +Mo and each of urea plus nBTPT, urea + DI and urea with nBTPT+S, produced 18,179, 16,716 and 18,253 kg DM ha<sup>-1</sup> respectively, compared to 16,171 kg ha<sup>-1</sup> for pastures receiving urea alone with +Mo. Similarly, pastures receiving urea plus nBTPT+S and urea plus nBTPT, all without Mo, produced 18,982 and 18,276 kg DM/ha respectively, compared to 16,363 kg DM/ha for urea alone (without +Mo), representing increases of 16% and 12% relative to urea alone (Table 4). The average annual NRE was also significantly ( $p<0.01$ ) higher for urea plus nBTPT, urea plus nBTPT+S treatments (all without +Mo), than for urea alone (Table 4).

**Table 4.** Annual pasture yield (kg DM ha<sup>-1</sup>) and N response efficiency (kg DM kg<sup>-1</sup> of applied N) as influenced by soil applications of urea with nBTPT, a combination of both nBTPT and DCD (DI, Double Inhibitor treatment), both without and with +S and +Mo. Values are means (n=3).

Treatment	PDM	NRE	% change over urea alone
			PDM
Control (no N)	13330		
Mo alone	14522		
Urea alone	16363	20.2	*
Urea + Mo	16171	18.9	*
Urea + nBTPT	18276	33.0	12*
Urea+ nBTPT + Mo	18179	32.3	11*
Urea + DI	16935	24.0	3
Urea + DI + Mo	16716	22.6	2
Urea+ nBTPT + S	18982	37.7	16*
Urea + nBTPT + S + Mo	18253	32.8	12*
LSD ( $\alpha=0.05$ )	1277	9.5	

**Table 5.** Annual pasture N uptake ( $\text{kg N ha}^{-1}$ ), % change relative to urea alone and pasture N recovery as influenced by urea with nBTPT, a combination of both nBTPT and DCD (DI), S and Mo to pasture soils. Values are means ( $n=3$ ).

Treatment	Pasture N uptake ( $\text{kg N ha}^{-1}$ )	% change over urea alone treatment	Pasture N recovery (%)
Control (no N)	360		
Mo alone	396		
Urea alone	417		38
Urea + Mo	408		32
Urea + nBTPT	429	3	46
Urea + nBTPT + Mo	466	14*	71*
Urea + DI	437	5	51
Urea + DI + Mo	382	-6	15
Urea + nBTPT + S	476	14*	77*
Urea + nBTPT + S + Mo	461	13*	67*
LSD ( $\alpha=0.05$ )	47.6		

Average annual NRE for urea alone was also significantly increased when the initial +Mo treatment was present (Table 4). Examples from Table 4 show that nBTPT-coated urea with +S gave a NRE of 37.7 kg of pasture DM/kg of applied N. The nBTPT-coated urea with no S showed a NRE of 33.0 kg kg of pasture DM/kg of applied N, while urea alone had a NRE of only 20.2 kg of pasture DM/kg. When urea  $\pm$  nBTPT (both with +S) were applied to pastures receiving the initial +Mo treatment, the NRE dropped slightly, e.g. to 32.8 DM/kg of applied N. Urea applied with the DI treatment also showed a 19% improvement in the annual NRE, though that was a non-significant increase.

#### 3.4. Cumulative pasture N uptake and apparent % N recovery

Pastures receiving urea with nBTPT+Mo, nBTPT+S and nBTPT+S+Mo showed a significant ( $P<0.01$ ) improvement in N uptake of 14, 14 and 13% respectively, compared to urea alone (Table 5). Pastures receiving urea with nBTPT, or with the DI (both without the initial +Mo treatment), showed a

trend of higher cumulative N uptake relative to urea alone, but it was non-significant. A calculation of N apparent recovery [where N apparent recovery is defined as additional amount of N taken up by pasture as a percentage of applied N] from applied N fertilizers by pasture herbage over the year (Table 5), suggests a apparent recovery of only 38% for urea alone, compared to a significant ( $p<0.01$ ) 77% for urea plus nBTPT+S, and 71% for urea+nBTPT+Mo. Urea applied with nBTPT alone or urea applied with DI alone showed non-significant trends for improvement in N apparent recovery.

#### 4. Discussion

There were indications of initial (first and sometimes second cut) depressions in PDM yield when Mo was applied with urea, and more so when it was applied with urea plus nBTPT and urea plus nBTPT+S, compared to the same treatments in the absence of Mo (Table 3). Thereafter, these treatments performed as well as their no-Mo partners.

The highest total PDMs and NREs for the full length of the trial were obtained for the 4 treatments containing nBTPT, all of which had high NREs of over 30, compared to the NREs of 19-20 for straight urea, although the latter PDM responses were still statistically significant ( $p < 0.01$ ) (Table 4). Interestingly however, PDM and NREs achieved with the double inhibitor (nBTPT plus DCD) were not significantly better than those with urea alone.

Moraes *et al.* (2009) applied urea + Mo to glasshouse-grown rice plants. Dry matter yield, chlorophyll concentration, net photosynthetic rate, and urease activity were all increased, although nitrate reductase activity was reduced. Application of Mo alone also has been reported to increase dry matter yield, chlorophyll concentration and net photosynthetic rate for canola plants (Liu *et al.* 2010).

We attribute the high N response efficiencies (NREs) to treatments containing nBTPT in our research to the interaction of four chemical and biochemical reactions which appear to be facilitated by nBTPT in the presence of urea. These reactions include (i) delayed urea hydrolysis which reduces ammonia losses (Zaman *et al.* 2008a; 2013b; Sanz-Cobena *et al.* 2012; Zaman & Nguyen 2012), (ii) additional time for increased lateral and downward movement of urea in the soil (Dawar *et al.* 2011), (iii) reduced nitrification (Sanz-Cobena *et al.* 2012), and (iv) more efficient N uptake and conversion into plant protein (Castle *et al.* 2006; Dawar *et al.* 2012, Zaman *et al.* 2013a).

Indications (not statistically significant) of better performance of urea plus nBTPT and S, compared to urea plus nBTPT without S, can be attributed to either the benefit of S for pasture growth, and/or to the acidifying effect of the oxidation of S further reducing  $\text{NH}_3$  losses (Zaman *et al.* 2008a). However extreme care should be taken while adding nBTPT to urea in the presence of elemental S, as we found decomposition of nBTPT at low pH (Zaman *et al.* 2013a). Techniques to minimize nBTPT

decomposition include coating or treating urea with nBTPT in the presence of lime to neutralise the acidity of S.

The surprisingly lower effectiveness of urea with the DI *viz.* only a 3% (non-significant) improvement in pasture productivity compared to urea alone (Table 4) is possibly a result of the ratio of nBTPT to DCD [within the DI mixture], which has been shown to be a critical factor in minimizing N losses and improving pasture productivity (Zaman & Blennerhassett 2010; Zaman *et al.* 2013a). The addition of DCD with nBTPT can retard conversion of ammonium-N to nitrate-N to the point where short-term N deficiency is induced. Where Mo is applied to Mo-deficient pasture, increased N fixation and growth by clover can cause an initial suppression of ryegrass growth.

The largest increases in N uptake were seen in treatments receiving urea plus nBTPT particularly in the presence of S and/or Mo (Table 5). Our results are in agreement with those of Watson (2000) and Zaman *et al.* (2008a, 2013b), who also reported that nBTPT-treated urea increased pasture N apparent recovery better than urea alone. Mo is a limiting factor in N fixation in legumes as their symbiotic bacteria requires an optimal level of Mo for efficient N assimilation (Hewitt 1983). However, recent literature indicates that Mo is also a limiting factor in N fixation for asymbiotic bacteria, especially in highly acidic soils (Barron *et al.* 2009). The high pasture N uptake for treatments with +Mo could thus be related to higher N fixation rates by ryegrass-associated bacteria. Certain N-fixing bacteria have been previously isolated from perennial ryegrass plants (Shoebitz *et al.* 2009).

The efficiency of mineral assimilation by plant tissues is dependent on environmental conditions. For example, in a study with radish plants treated with urea or urea + Mo, the yield was significantly greater during seasons when rainfall, air temperature and solar radiation were highest (Smolen & Sady 2010).

For N in particular, the efficiency of the assimilation process increases at higher levels of photosynthetic productivity (Masclaux-Daubresse *et al.* 2002). However, Mo assimilation can also vary significantly based on the amount of solar radiation and air temperature, as was demonstrated for Chinese cabbage (Moreno *et al.* 2002).

Lower than optimum soil temperature and solar radiation can significantly limit pasture yield in cooler areas of New Zealand, especially during the period from late autumn to spring. Minor changes in solar radiation may be more important than other climatic factors. As this was a one-year study only, we were not able to study the effects of climatic changes year-to-year on the relative efficiency of the treatments, but the climatic data (Figure 1) were not atypical for this site.

## 5. Conclusions

Pasture growth responded positively when Mo was applied at 50 g Mo/ha; however, caution is required with regard to Mo applications in order to avoid molybdenosis in the grazing livestock. When Mo was applied with urea alone, or urea ± N inhibitors, an initial depression in pasture yield was observed, though that effect disappeared in subsequent pasture cuts. Pasture growth showed seasonal variability, as expected, due to changing soil temperature, moisture and solar radiation. Our results suggest that applying urea with nBTPT alone or with nBTPT+S, and optimising Mo application, has the potential to improve the efficiency of urea fertilizer use. While the DI treatments may have environmental benefits, such as possible reduction in N<sub>2</sub>O emission and reduced NO<sub>3</sub><sup>-</sup> leaching, the agronomic benefits in this study were considerably poorer than those achieved by using urea with nBTPT alone and warrants further investigation the ratio of nBTPT to DCD.

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