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Influence of enhanced efficiency fertilisation techniques on nitrous oxide emissions and productivity response from urea in a temperate Australian ryegrass pasture

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Abstract. The effect of a nitrification inhibitor on nitrous oxide (N_2O) emissions across seasons, the effect of a urease inhibitor and a fine particle spray (both targeting ammonia (NH_3) loss) on N_2O emissions, and the potential for productivity benefits and efficiencies by using these enhanced efficiency fertilisers (EEFs) were investigated in temperate pastures. The study compared three treatments over an eight month period (April to December 2010): (1) urea (U), (2) urea with a nitrification inhibitor (3,4-dimethylpyrazole phosphate) (DMPP), and (3) urea with a urease inhibitor (N-(*n*-butyl) thiophosphoric triamide (NBTPT)) (GU). In autumn, when NH_3 loss was predicted to be high, the effect of urea applied as a fine particle spray (containing urea, NBTPT and gibberellic acid (10 g ha^{-1})) (FPA) on N_2O emissions and productivity was determined.

N_2O emissions from urea applied to pastures were low, and were larger in spring than autumn due to soil moisture and temperature. DMPP was an effective tool for mitigating N_2O emissions, decreasing fertiliser-induced N_2O emissions relative to urea by 76% over eight months. However, the urease inhibitor (NBTPT) (GU) increased N_2O emissions from urea by 153% over eight months. FPA had no impact on N_2O, but was only examined during periods of low emission (autumn). No significant biomass productivity, agronomic efficiency benefits, or improvements in apparent fertiliser recovery were observed with the DMPP and GU treatments. A significant biomass productivity benefit was observed with the FPA treatment 55 days after fertiliser was applied, most likely because of the gibberellic acid. The outcomes highlight that although DMPP effectively decreased N_2O emissions it had no impact on biomass productivity compared with urea. The use of the GU increased N_2O emissions by preserving NH_3 in the soil. To avoid this a lower rate of N should be applied with the urease inhibitor.

Additional keywords: 3,4-dimethylpyrazolephosphate, fine particle spray, nitrification inhibitor, N-(*n*-butyl) thiophosphoric triamide, urease inhibitor.

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Introduction

High nitrogen (N) fertiliser inputs, commonly as surface-applied granular urea, are typical in pasture-based dairy systems in Australia, resulting in low N use efficiency (NUE). Application of N fertilisers, such as urea, can lead to emissions of the greenhouse gas nitrous oxide (N_2O). Fertiliser-induced N_2O emissions from crops and grasslands total 0.9 million tonnes of N per year globally (IFA 2001). In Australia, agriculture represents 76% of the national total N_2O emissions, with around 50% of this from mineral fertiliser application (DCCEE 2011). Enhanced efficiency fertilisers (EEFs), including urease inhibitors, nitrification inhibitors, fine particle sprays, and controlled-release fertilisers, have the potential to decrease N losses from agriculture by either altering the rate of N transformations or slowing the release of N from fertiliser granules (Chen *et al*. 2008).

Various EEFs have been developed to target particular N loss pathways (Chen *et al*. 2008). Urease inhibitors, such as N-(*n*-butyl) thiophosphoric triamide (NBTPT), slow urea hydrolysis and are designed to decrease ammonia (NH_3) volatilisation from surface-applied granular urea. Nitrification inhibitors, such as 3,4-dimethylpyrazole phosphate (DMPP) and dicyandiamide (DCD), slow nitrification and are designed to decrease N loss as nitrate (NO_3^-), N_2O, oxides of nitrogen (NO_x) and dinitrogen (N_2). Liquid or fine particle spray fertilisers are designed to decrease NH_3 volatilisation and increase plant utilisation through enhanced foliar uptake (Dawar *et al*. 2012). The greatest benefit of the EEFs is expected in spring and autumn (urease inhibitor) and late winter–spring (nitrification inhibitor) when climatic conditions favour NH_3 volatilisation and denitrification losses, respectively. However, when a loss pathway is decreased as a consequence of EEF use

and the rate of applied N remains the same, there is a risk of increased loss from an alternate pathway unless the additional N is used by the plant.

The nitrification inhibitors DMPP and DCD have been reported to decrease N_2O emissions in laboratory studies on Australian soils (DMPP) (Chen *et al*. 2010) and in field studies in pasture systems (DCD) (Di *et al*. 2007; Kelly *et al*. 2008; Di *et al*. 2010; Gilsanz *et al*. 2016). A recent review found them effective in temperate grassland-based agriculture (Li *et al*. 2013*a*). Rowlings *et al*. (2016) studied the impact of DMPP on NUE in sub-tropical pastures, but no studies have investigated its impact on N_2O emissions and NUE in Australian temperate pasture systems to date. Although urease inhibitors are effective at decreasing NH_3 volatilisation by slowing the rate of urea hydrolysis and reducing the risk of elevated pH that drives NH_3 formation, their impact can be variable due to the influence of climatic conditions, soil type, and land use on NH_3 volatilisation. A review of the literature shows that across a range of crops and pastures, use of a urease inhibitor led to a 20% to 88% decrease in NH_3 volatilisation compared with urea (Watson *et al*. 1990; Rawluk *et al*. 2001; Turner *et al*. 2010; Suter *et al*. 2013). Due to the impacts on NH_3 volatilisation, it is expected that N_2O emissions may increase relative to urea because of greater N in the system, but some studies have reported decreases in N_2O with the urease inhibitor (Zaman *et al*. 2009; Singh *et al*. 2013; Ding *et al*. 2014). Use of fine particle sprays and suspensions have been shown to decrease NH_3 and N_2O loss and increase productivity relative to urea when applied with and without urease or nitrification inhibitors (Di and Cameron 2006; Dawar *et al*. 2011). To develop effective strategies to mitigate N_2O emissions and provide productivity benefits to facilitate adoption of these strategies in Australian pasture systems, greater knowledge of the impact of EEFs under field conditions is required.

This paper reports on a field experiment using EEFs in a ryegrass seed crop. Surface applications of granular urea (40 kg N ha^{-1}) with and without EEFs (urease inhibitor NBTPT, nitrification inhibitor DMPP) were made regularly over an eight month period (autumn to summer) in 2010. A fine particle spray containing NBTPT and gibberellic acid was also used during autumn when expected NH_3 loss is high. The impact of these amendments on soil mineral N, N_2O emissions, and biomass production and N utilisation was determined.

Materials and method

Site details

The experiment was conducted at a ryegrass (*Lolium perenne* L.) seed crop site at Murroon in south-western Victoria, Australia (38°26′10.18″S, 143°47′34.57″E). Details of the site characteristics are described in Suter *et al*. (2013). Briefly, the Chromosol soil (Isbell 1996) has a topsoil (0–10 cm) with a silty loam texture (22% clay, 38% silt, 40% sand, from 0–25 cm depth), a pH_{CaCl_2} of 4.6, 0.2% total N, 2.7% total C, a bulk density of 1.23 g cm^{-3}, and a cation exchange capacity (CEC) of 4.98 cmol (+) kg $soil^{-1}$. Initial mineral N content (0–10 cm) was 13.4 kg N ha^{-1} as ammonium (NH_4^+) and 0.84 kg N ha^{-1} as nitrate (NO_3^-). The soil porosity at 0–10 cm depth was 0.54. The site was used for ryegrass seed production and fenced off from sheep that grazed the site intermittently before (>3 weeks) establishing the experiment. The experiment was a replicated split block design of five blocks, separated by a 0.5 m buffer zone, with each treatment randomly assigned within each block to account for site spatial heterogeneity. Each treatment plot was 1 m × 2 m. The experiment commenced 12 April 2010 and finished 23 December 2010.

Local rainfall measured on site in 2010 was 803 mm. Climatic variables were measured on site with a weather station (model WXT510; Vaisala, Helsinki, Finland) until 3 October 2010 and afterwards from the two closest Bureau of Meteorology stations (Colac, 38.23°S, 143.79°E and Cape Otway, 38.86°S, 143.51°E) due to issues with the on-site weather station. Soil moisture and temperature were measured using capacitance probes (EnviroPro®) inserted vertically into the ground and logging at 10 cm intervals.

Treatments

Fertiliser (40 kg N ha^{-1}) was applied six times from April to October 2010 on 12 April, 7 June, 16 July, 3 September, 27 September and 21 October. Treatments were as follows:

(1) control (C) (no fertiliser)
(2) granular urea (U) (46% urea-N)
(3) granular urea with the urease inhibitor, NBTPT (GU) (Green Urea 14™ (45.8% urea-N with 'Agrotain®' @ 5 L t^{-1} urea))
(4) granular urea with the nitrification inhibitor, DMPP (DMPP) (Urea with ENTEC (46% urea-N with 0.4% of DMPP per unit of ammonium-N))
(5) urea applied as a fine particle spray containing NBTPT and gibberellic acid (FPA) (46% urea-N with 'Agrotain' @ 1 L t^{-1} of urea and gibberellic acid (10 g ha^{-1})). Treatment 5 was applied once on 12 April 2010 to target NH_3 loss, which was expected to be high in autumn.

Soil mineral N

Three composite soil samples (0–10 cm depth) were collected from each plot using a corer (2.5 cm internal diameter) at regular intervals (~fortnightly), immediately dried (40°C) and sieved (<2 mm). Subsamples (20 g of 105°C dried soil equivalent) were extracted with 2 M KCl (1 : 5 soil solution), by shaking for 1 h, filtering though Whatman No. 42 filter papers and were analysed for NH_4^+-N, and NO_3^--N using a SAN++ segmented flow analyser (Skalar Analytical B.V. 2005).

N_2O emissions

Nitrous oxide (N_2O) emissions were measured using manual chambers (23 cm diameter × 25 cm high) similar to those reported in Saggar *et al*. (2004). Open-topped chambers were inserted into the ground (5 cm) for the entire course of the experiment and were capped for 1 h during sample collection times. Gas flux measurements were collected at regular intervals (every second day for one week after fertilisation, and then weekly) from the capped chambers commencing between 1000 and 1200 hours. Three samples were collected at 0, 30 and 60 min after the chamber was capped. Collected samples (20 mL, injected into a 12 mL evacuated Exetainer®, (Labco Ltd, United Kingdom)) were analysed by gas chromatograph (Agilent 6890) using an electron capture

detector (ECD) with a lower detection limit of 0.2 ± 0.02 µL L^{-1}. Temperature was logged within the closed chamber (Tinytag Transit 2 TG-4080, Gemini Data Loggers). Soil moisture (ML2x theta probes (Delta-T Devices Ltd, Cambridge, UK)) and temperature (small portable temperature probe) were recorded at each sampling time.

From 3 August to 30 August 2010 free water was observed on the soil surface and gas samples were not collected during this time due to problems accessing the area and collecting samples without disturbing the soil and chambers. We hypothesised that any denitrification would be largely N_2 during this time and N_2O emissions would be minimal (Ciarlo *et al*. 2008). Recent work by Friedl *et al.* (2016) found that the $N_2/(N_2+N_2O)$ ratio increased with increasing soil moisture (to 100% water filled pore space, WFPS) in sub-tropical pastures, and Harris *et al*. (2013) hypothesised that this was the reason for low N_2O emissions from cropping sites during periods when soil WFPS exceeded 90%.

Soil N_2O flux (kg N ha^{-1} h^{-1}) was calculated using the linear regression (LR) model recommended by Venterea *et al*. (2012) and extrapolated to a daily N_2O emission. To compare treatment effects, cumulative emission was calculated by integration of the area under the daily flux curve for the period of measurement.

Biomass production

Biomass was measured on samples (2 m length × 0.4 m width; 0.8 m^2 cut per plot) collected using a lawn mower to simulate grazing rotations typical of the area at 24–28 days after fertiliser (DAF) (autumn and spring) and 42–46 DAF (winter). Biomass samples were collected on 10 May (28 DAF), 7 June (55 DAF), 19 July (42 DAF), 3 September (46 DAF), 27 September (24 DAF), 21 October (24 DAF), and 23 December (63 DAF) in 2010. The harvest on 7 June 2010 was included to assess the longevity of the impact of the EEFs following a time of expected high ammonia loss. The harvest on 23 December 2010 was 63 DAF, as the pasture was grown to seed production stage, and included both the stem and grain. All other harvests were vegetative only. Biomass was removed from the entire experimental area when each harvest was collected. Pasture N content was determined by the Kjeldahl digestion method with colourimetric analysis using a Lachat 8500 Flow Injection Analyser after samples were dried at 70°C for 72 h (Rayment and Lyons 2011).

Statistics

Statistical analysis of treatment effects at each harvest was performed using the Fisher l.s.d. analysis of variance (ANOVA, $P < 0.05$) with Minitab17. In addition, significance of soil properties (mineral N, temperature and moisture), sample time and treatment, and the interaction between these factors, on daily N_2O flux (log_{10} transformed) and dry matter production was assessed using the program RStudio, Version 0.97.248.

Results and discussion

Climatic conditions

Rainfall and temperature data (Fig. 1) showed an initially dry autumn followed by a wet, cool winter (August) and a warm, moist spring (October–November). Air temperature ranged from a low of 0°C to a maximum of 31°C. Soil moisture increased from 12 April 2010 through to late August when the site became saturated, before drying off towards the end of the year (Fig. 2). Rainfall during spring (Fig. 1) caused soil moisture contents to fluctuate between mid-September through to December. The measured volumetric soil moisture (Θ_v) ranged from 10% (19% WFPS) to 53% (98% WFPS) at 10 cm depth, 13% to 58% at 20 cm depth and 13% to 56% at 30 cm depth during the study. The upper reported Θ_v for each depth represents saturation.

Soil temperatures ranged from 4.7 to 28°C at 10 cm depth, 6.7 to 24°C at 20 cm depth and 8 to 22°C at 30 cm depth (Fig. 2), following a similar pattern to the ambient temperature (Fig. 1). There was a strong diurnal pattern of soil temperature with minimum temperature recorded between 0630 and 0900 hours and maximum between 1700 and 1800 hours.

Soil mineral N transformations

Soil ammonium (NH_4^+) levels fluctuated in response to fertiliser additions (Fig. 3), plant uptake and mineralisation. In spring,

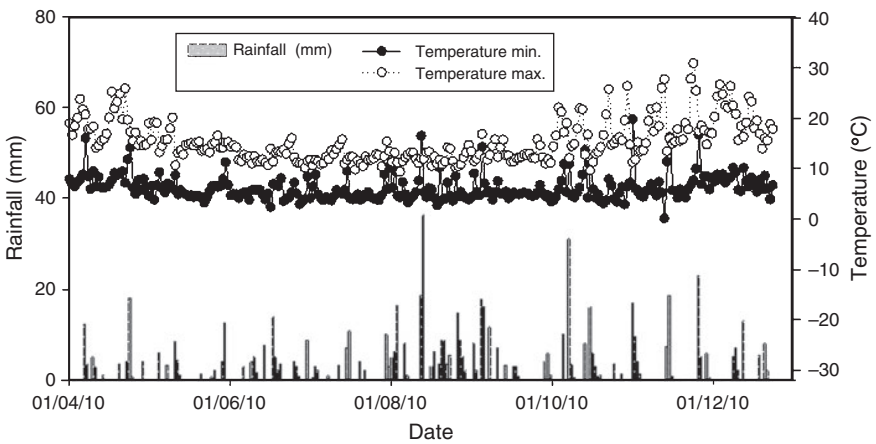

Fig. 1. Rainfall and temperature at the Murroon field site (38°26′10.18″S, 143°47′34.57″E) from 1 April 2010 to 31 December 2010.

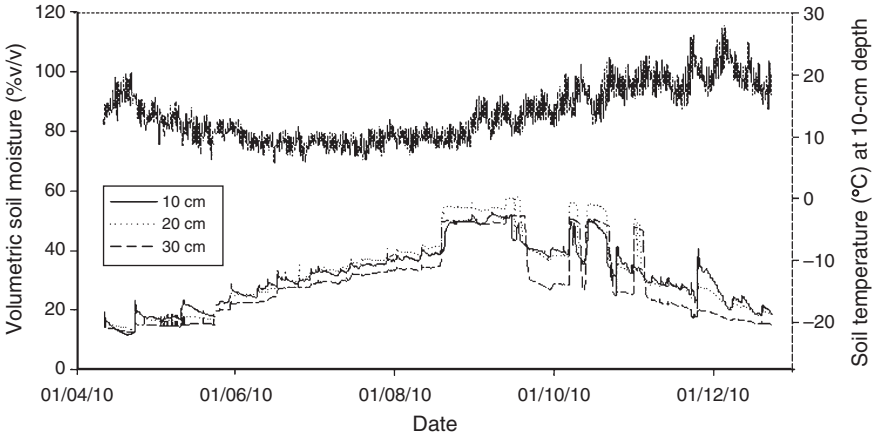

Fig. 2. Soil volumetric water content (10, 20, 30 cm depth) and temperature (10 cm depth) at the Murroon field site (38°26′10.18″S, 143°47′34.57″E) from 12 April 2010 to 23 December 2010. Note: Soil porosity (0–10 cm) is 0.54, a volumetric water content of 54% is saturation.

increased NH_4^+-N was observed in all treatments, including the control, indicating that the increased soil temperature and moist conditions (Fig. 2) stimulated mineralisation from the abundant organic matter pool (total N and C contents of 0.2 and 2.7% respectively) (Hu *et al*. 2014). There was no overall significant difference in NH_4^+-N between treatments. Slightly greater NH_4^+-N was measured in the DMPP treatment compared with U many times before 27 September 2010, most noticeably on 19 July 2010 (23.2 ± 10.0 kg N ha^{-1} for DMPP and 4.1 ± 0.2 kg N ha^{-1} for U) and 11 August 2010 (49.0 ± 20.6 kg N ha^{-1} for DMPP and 8.2 ± 1.1 kg N ha^{-1} for U) ($P < 0.1$) (Fig. 3*a*). This is expected as the inhibitor slows ammonification, and Fangueiro *et al*. (2009) found this inhibitory effect can occur for extended periods (e.g. 100 days for DMPP). In this study, the suppression of nitrification over the wetter months (July to September) when there is an increased risk of NO_3^- leaching and denitrification losses, indicates that the inhibitor could provide real benefits in decreasing gaseous emissions (N_2O and N_2) and increasing biomass production. This is reflected in the lower NO_3^--N levels in the DMPP treatment during July and August (see below). Lower NH_4^+-N in the DMPP treatment after fertilisation on 27 September 2010 compared with U and GU was not expected but the reason for this is not clear.

Addition of NBTPT to urea (GU) increased the quantity of NH_4^+-N in the soil compared with U in autumn (19 April), early winter (5 July) and for most of spring (8 October, 21 October and 19 November). This results from decreased NH_3 loss, leading to greater N remaining in the soil. A concurrent study of NH_3 loss at the site found that using NBTPT decreased N loss as NH_3 in autumn to 3.7 kg N from 12 kg N with urea (Suter *et al*. 2013). However, in July and September 2010 the higher rainfall and soil moisture (Figs 1 and 2) would lower the potential for NH_3 loss from urea so no additional N would be 'saved' in the urease inhibitor treatment, indicating no benefit from using the urease inhibitor at this time.

Applying FPA once on 12 April 2010 did not alter soil NH_4^+-N levels compared with U, and the soil NH_4^+-N returned to baseline levels by 24 May 2010 (5.9 ± 0.6 kg N ha^{-1} compared with 5.3 ± 0.5 kg N ha^{-1} for C). Samples collected on 27 September 2010 showed all treatments had similar background levels of NH_4^+-N (1.9 ± 0.2 kg N ha^{-1} for C; between 2.2 ± 0.4 and 3.4 ± 0.2 kg N ha^{-1} for the fertiliser plots (Fig. 3*a*)).

Soil NO_3^--N levels also fluctuated in response to applied fertiliser N (Fig. 3*b*) and were lower than NH_4^+-N. This indicates that NO_3^--N was either: (1) being removed by the plant material, with NO_3^--N the favoured form of N for plant uptake (Li *et al*. 2013*b*); or (2) was denitrified. Denitrification losses are expected to occur mostly over winter under anaerobic soil conditions (Butterbach-Bahl *et al*. 2013). In the early stages of the study, conditions were dry so biomass production was low (see Biomass section below), and plant uptake of NO_3^--N would also be low. At this time the DMPP and FPA treatments decreased the amount of NO_3^--N produced relative to U and GU thereby retaining N in the soil for subsequent plant uptake. In the FPA treatment, NO_3^--N levels decreased to baseline levels one month after application, indicating plant uptake or immobilisation. The urease inhibitor (GU) increased NO_3^--N levels compared with U throughout the study due to decreased NH_3 loss, and this was significantly more than that observed in the C and DMPP treatments ($P < 0.01$). During spring when high biomass production occurred, NO_3^--N did not appear to respond to fertiliser application due to plant uptake, which is supported by the lack of difference between the NH_4^+-N levels in the treatments at this time (Fig. 3).

N_2O emissions

There was a significant relationship between daily N_2O flux, day of sample collection, soil temperature, soil moisture, and the interaction between temperature and soil moisture, and temperature and day of sample collection, and GU ($F(16, 251) = 36.65$, $P < 0.005$). Daily N_2O emissions were comparatively low throughout autumn and early winter (April to early August) and higher in spring (Fig. 4*a*), reflecting soil moisture and temperature conditions (Fig. 2). This trend (Figs 2 and 4) is expected based on our understanding of the drivers of

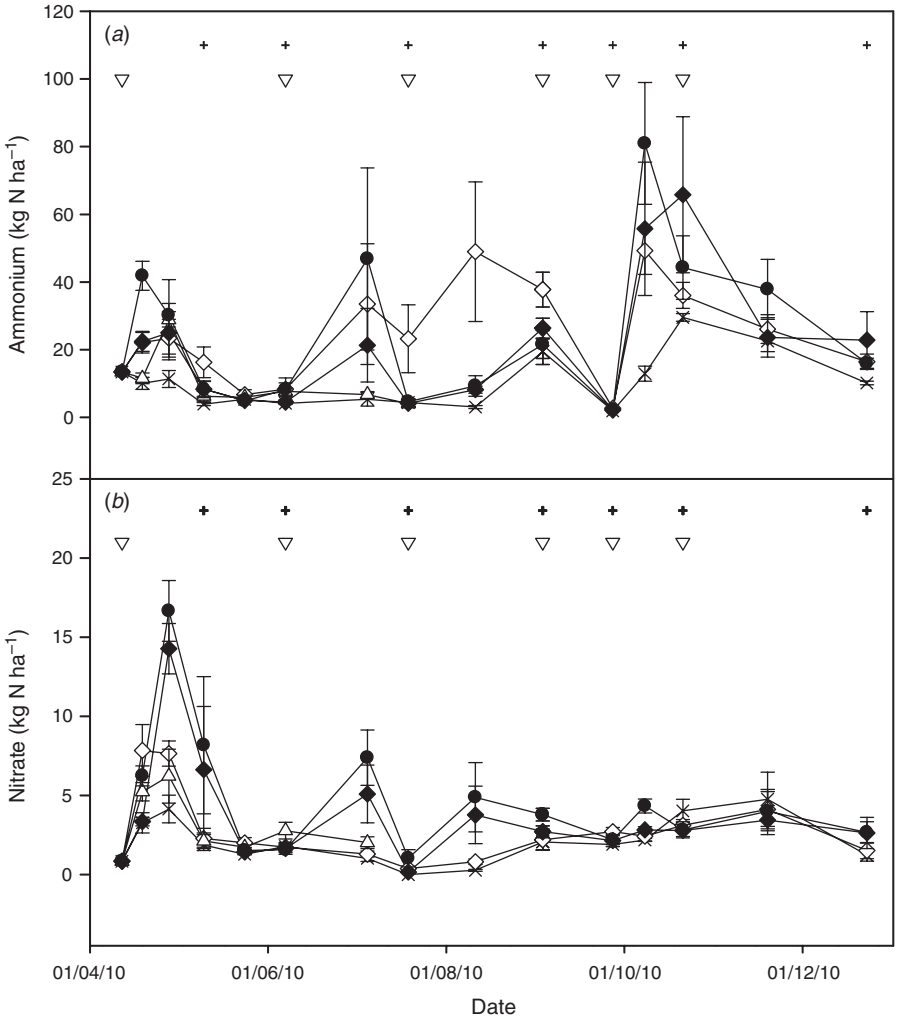

Fig. 3. Soil ammonium (*a*) and nitrate (*b*) for C (X), U (♦), DMPP (◇), GU (●) and FPA (△) showing timing of each fertilisation event (▽) and harvest (+) with standard error of the mean of five replicates.

N_2O emissions (Rafique *et al*. 2012; Butterbach-Bahl *et al*. 2013; Huang *et al*. 2013; Bell *et al*. 2015). Nitrate, the product of nitrification and the substrate for denitrification, had a significant ($P < 0.001$) influence on daily N_2O flux. The lower N_2O emissions in autumn, despite NO_3^- being higher in all treatments (Fig. 3*b*) compared with the rest of the year, is due to lower soil moisture (Fig. 2), which limits denitrification.

Minimum and maximum daily N_2O flux for each treatment was –0.28 to 24.56 g N_2O-N ha^{-1} day^{-1} for C; –0.6 to 50.93 g N_2O-N ha^{-1} day^{-1} for U; 0.07 to 94.51 g N_2O-N ha^{-1} day^{-1} for GU; –1.89 to 23.56 g N_2O-N ha^{-1} day^{-1} for DMPP; –0.28 to 15.95 g N_2O-N ha^{-1} day^{-1} for FPA. The lowest N_2O emission recorded occurred on 16 April 2010, four days after the first fertiliser application when soil moisture was low. For all but the FPA treatment (which was only used in autumn) the maximum N_2O flux occurred during spring (September to November), seven or more days after fertiliser was applied. This was due to the time required for urea hydrolysis, nitrification and denitrification and the warm, moist conditions. Similar time delays in N_2O production have been observed when cattle urine is added to soil (Bell *et al*. 2015).

The cumulative N_2O data showed that over eight months the emission of N_2O from C and U were 0.6 and 1 kg N_2O-N ha^{-1}. This represents one tenth of the denitrification loss reported by Eckard *et al*. (2003) in temperate Australian pastures (6 and 13 kg N ha^{-1} year^{-1} (N_2 and N_2O combined) from control and urea (200 kg N ha^{-1} year^{-1}) treatments). Although the $N_2O : N_2$ ratio can vary widely depending on soil type and environmental conditions (Saggar *et al*. 2013), our reported results provide a reasonable estimate of N_2O emissions from temperate Australian pastures when compared with those of Eckard *et al*. (2003). The cumulative N_2O data show that although the fertiliser treatments caused differences in N_2O emissions relative to the control during the earlier part of the experiment (12 April to 7 June 2010) (Table 1), the absolute difference was small because of the low level of emissions occurring at that time (Fig. 4*b*) compared with the remainder of the year. At that time there was no significant difference in N_2O emissions between fertiliser treatments (Table 1).

DMPP decreased fertiliser-induced N_2O emissions by 76% during the study relative to U by decreasing NO_3^--N production, the substrate for denitrification (Fig. 3). The impact of DMPP on

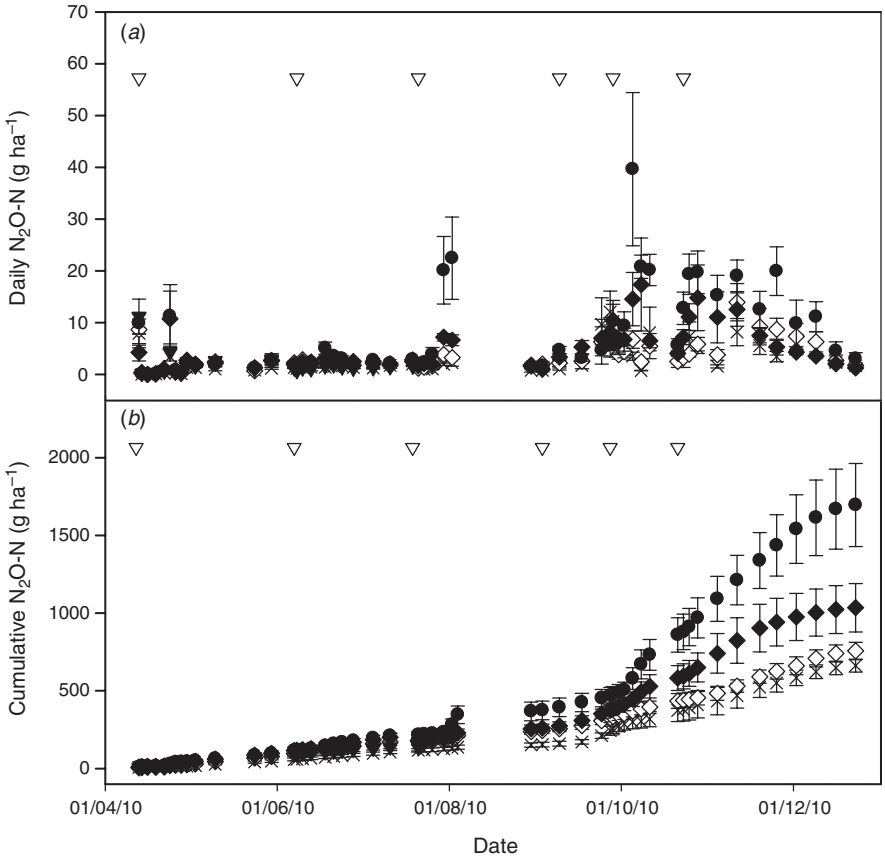

Fig. 4. Daily (*a*) and cumulative (*b*) N_2O flux for C (X), U (♦), DMPP (◇), GU (●) and FPA (▼) showing timing of each fertilisation event (▽) with standard error of the mean of five replicates.

Table 1. Average cumulative N_2O emissions ± standard error from: (1) 12 April to 7 June 2010; (2) 12 April to 23 December 2010; (3) 16 July to 23 December 2010; and (4) 27 September to 23 December 2010 calculated from integration of the daily emissions data (Fig. 4)

Within columns, different letters indicate a significant difference ($P < 0.05$); NA, not applicable (treatment only applied on 12 April 2010)

Treatment	N_2O-N (g ha^{-1})			
	12 April to 7 June	12 April to 23 December	16 July to 23 December	27 September to 23 December
C	58 ± 9a	667 ± 42a	545 ± 40a	419 ± 15a
U	116 ± 22b	1079 ± 149a	859 ± 150a	661 ± 146a
DMPP	97 ± 9ab	764 ± 55a	575 ± 58a	431 ± 63a
GU	115 ± 6b	1711 ± 265b	1480 ± 250b	1228 ± 230b
FPA	94 ± 15ab	NA	NA	NA

N_2O emissions was most noticeable during spring (September to November) when fertiliser-induced emissions with DMPP decreased by 95% (to 9 ± 63 g N_2O-N ha^{-1}) compared with U (240 ± 146 g N_2O-N ha^{-1}), with N_2O essentially reaching background (control) levels (419 ± 146 g N_2O-N ha^{-1} for C, 431 ± 64 g N_2O-N ha^{-1} for DMPP) (Table 1, Fig. 4*a*). This occurred despite low soil NO_3^- levels for all treatments during spring (Fig. 3*b*), indicating increased plant uptake due to the spring growing conditions. The calculated reductions in N_2O emissions with DMPP were similar to those reported elsewhere (Menéndez *et al*. 2009; Di and Cameron 2012; Misselbrook *et al*. 2014). The observed results indicate that DMPP is an effective N_2O mitigation tool for temperate Australian pasture systems.

Addition of NBTPT (GU) resulted in a 153% increase in fertiliser-induced N_2O emissions (1044 ± 265 g N_2O-N ha^{-1}) relative to U (412 ± 149 g N_2O-N ha^{-1}), with the greatest impact occurring from 16 July to 23 December 2010 (GU; 935 ± 250 g N_2O-N ha^{-1}, U; 314 ± 150 g N_2O-N ha^{-1}) (Table 1). Other studies have reported decreased N_2O emissions with urease inhibitors (Dawar *et al*. 2011; Sanz-Cobena *et al*. 2012; Singh *et al*. 2013). From previous work, urease inhibitors can stimulate or decrease denitrification depending on the inhibitor type and concentration used (Yeomans and Bremner 1986; Zhengping *et al*. 1991). Zhengping *et al*. (1991) observed no inhibitory effect on denitrification with NBTPT. If the urease inhibitor prevents NH_3 loss and the rate of N applied is the same as for urea, as is the case here (40 kg N ha^{-1}), then there is more NH_4^+-N retained in the soil that can undergo nitrification and denitrification to produce N_2O. The greatest impact of elevated emissions from the urease inhibitor occurred after the 16 July 2010 application. This was unexpected because in July NH_3 loss was assumed to be low, based on winter climatic conditions, and in September the measured NH_3 loss was low (Suter *et al*. (2013). So, the applied N remaining in the soil for the U and GU treatments should have been similar, but for GU the urea

would have been released more slowly making the substrate for nitrification and N_2O production available for longer compared with urea. The impact of GU on N_2O, therefore, depends on the temporal synchronisation of the substrates for N_2O production (NH_4^+ for nitrification and NO_3^- for denitrification) and conditions conducive for denitrification. This explains the differences reported here and in the literature (Ding *et al*. 2014). Whereas the mineral N data (Fig. 3) shows similar levels of NH_4^+-N for U and GU, there is a trend for greater NO_3^- with GU, supporting our hypothesis.

Biomass

Average biomass production responded to N application but, excluding spring, the response was slightly lower than that reported for long-term N studies in the same region (McKenzie *et al*. 2003) (Table 2). There was a significant relationship between dry matter production, date of sample collection, treatment, and the interaction between sample date and soil NH_4^+ levels and pasture dry matter ($F(33, 116) = 45.35$, $P < 0.005$). Total plant production increased and was significantly greater ($P < 0.001$) for the final harvest on 23 December 2010 (Table 2) because this contained both vegetative biomass and seed. Biomass production was significantly lower ($P < 0.001$) at the 7 June 2010 harvest as a result of the period between fertilisation and harvest (55 DAF) and the removal of part of the applied N in the first pasture harvest (10 May 2010, 28 DAF). Applying urea did not significantly increase biomass relative to the control at this harvest indicating insufficient N remaining in the soil (Fig. 3*a*, *b*). DMPP, GU and FPA significantly ($P < 0.05$) increased biomass production compared with C by an average of 81 ± 15 kg dry matter (DM) at this harvest. FPA significantly increased biomass relative to U at this harvest, which may be due to the presence of gibberillic acid (Biddiscombe *et al*. 1962). The greater response to the inhibitors in autumn, when conditions were drier, is similar to the results reported by Rowlings *et al*. (2016). The lack of a consistent productivity benefit with the nitrification inhibitor, despite the decrease in N_2O emissions, was due to little N being lost via denitrification (measured as N_2O), limited leaching in these texture contrast soils (Chromosols) (Isbell 1996), and the presence of sufficient N from fertilisation in all treatments.

The agronomic efficiency (kg pasture increase per kg N applied) and apparent recovery of N (net kg N taken up per kg N applied) were significantly ($P < 0.05$) greater in spring (September to October) and December than in autumn and winter, reflecting the seasonal pattern of pasture production for the region as reported by Suter *et al*. (2013) (Table 2). The lowest response to N occurred in July (average 7.7 ± 0.3 kg DM per kg of N for fertiliser treatments) where pasture was cut at 42 DAF (Table 2) due to low mineral N available for pasture growth (Fig. 3). There was a trend for increased agronomic efficiency and recovery of applied N with DMPP and GU relative to U for the May, June, July and 3 September harvests (Table 2), as well as for FPA in May and June. At other harvests this was not observed. The lack of significant

Table 2. Mean biomass production (kg DM ha^{-1}) for each biomass cut, agronomic efficiency of applied N (kg pasture increase per kg N applied), and apparent recovery of applied N (net kg N taken up per kg N applied) ± standard error

Significant differences ($P < 0.05$ (Fisher test)) between treatment for each harvest date and section are indicated by different letters. DAF, days after fertiliser application; DoG, days of growth

Treatment	Date							
	10 May	7 June[A]	May and June[B]	19 July	3 Sep.	27 Sep.	21 Oct.	23 Dec.[C]
DAF	28	55	55	42	46	24	24	63
DoG	28	27	55	42	46	24	24	63
				Biomass production (kg DM ha^{-1})				
C	450 ± 46a	132 ± 8a	582 ± 45a	266 ± 34a	224 ± 26a	239 ± 23a	419 ± 28a	2271 ± 270a
Urea	699 ± 70ab	159 ± 9ab	858 ± 77ab	569 ± 11b	876 ± 82b	818 ± 33b	1027 ± 4b	4339 ± 156b
DMPP	748 ± 97b	207 ± 36bc	955 ± 131b	618 ± 71b	949 ± 74b	754 ± 60b	1030 ± 78b	4284 ± 373b
GU	726 ± 72ab	210 ± 20bc	936 ± 76b	647 ± 42b	953 ± 53b	741 ± 47b	944 ± 48b	4196 ± 412b
FPA	823 ± 159b	225 ± 21c	1048 ± 166b	NA	NA	NA	NA	NA
				Agronomic efficiency of applied N (kg pasture increase per kg N applied)				
Urea	6.2 ± 1.8a	0.7 ± 0.2a	6.9 ± 1.9a	7.7 ± 0.3a	16.1 ± 2.0a	14.6 ± 0.8a	13.2 ± 1.0a	52.0 ± 3.9a
DMPP	7.5 ± 2.4a	1.9 ± 0.9a	9.3 ± 3.3a	8.9 ± 1.8a	18.0 ± 1.8a	13.0 ± 1.5a	13.3 ± 1.9a	50.6 ± 9.3a
GU	6.9 ± 1.8a	1.9 ± 0.5a	8.8 ± 1.9a	9.6 ± 1.0a	18.1 ± 1.3a	12.6 ± 1.2a	11.2 ± 1.2a	48.4 ± 10.3a
FPA	9.3 ± 4.0a	2.3 ± 0.5a	11.6 ± 4.2a	NA	NA	NA	NA	NA
				Apparent recovery of applied N (%) (net kg N taken up per kg N applied)				
Urea	27.8 ± 5.3a	2.8 ± 0.9a	30.6 ± 6.1.a	37.1 ± 1.1a	55.2 ± 6.5a	48.8 ± 2.3a	54.4 ± 5.2a	62.5 ± 6.7a
DMPP	32.1 ± 8.6a	6.8 ± 2.8a	38.9 ± 11.3a	41.3 ± 7.1a	69.0 ± 7.9a	46.5 ± 6.3a	52.6 ± 7.5a	61.5 ± 12.7a
GU	32.0 ± 5.4a	7.5 ± 2.0a	39.5 ± 6.4a	45.0 ± 5.7a	71.6 ± 5.0a	45.9 ± 4.2a	51.3 ± 6.5a	66.1 ± 10.4a
FPA	31.5 ± 11.0a	7.8 ± 1.8a	39.3 ± 11.2a	NA	NA	NA	NA	NA

[A]7 June data relates to fertiliser applied on 12 April 2010. Therefore, biomass, agronomic efficiency and N recovery are in addition to that achieved in data reported for 10 May 2010 for the application of 40 kg N ha^{-1} on 12 April 2010.

[B]Data is the sum of the responses collected on 10 May 2010 and 7 June 2010 cut which represents the efficiency outcome from the application of fertiliser on 12 April 2010.

[C]23 December 2010 biomass includes vegetative and seed biomass. NA: Not applicable (treatment only applied on 12 April 2010).

difference in the agronomic efficiency and apparent recovery of N with the EEFs corresponds with the response seen in other studies (O'Connor *et al*. 2012; Misselbrook *et al*. 2014; Bell *et al*. 2015). This is most likely a result of a combination of sufficient N being available for pasture growth across all treatments and other factors (water, temperature, environment) limiting pasture production. This, plus the impact of management, was concluded as the main reason for variable responses in productivity and agronomic efficiency trials using the inhibitors in a meta-analysis by Abalos *et al*. (2014). Previous research has found productivity benefits from the use of urease and nitrification inhibitors, with the greatest response from the urease inhibitor and greater efficiency observed where lower N rates were used (30 kg N ha^{-1} compared with 60 kg N ha^{-1}) (Zaman *et al*. 2013). For example, Watson *et al*. (1990) found that NBTPT increased both yield and N efficiency by 11%. Dawar *et al*. (2011) concluded that using a fine particle spray instead of granular urea and addition of NBTPT, both increased biomass production (by 27% and 38%, respectively) and N response efficiency (by 9 and 13 kg DM kg N^{-1}, respectively) compared with granular urea. This is a much greater response than observed here, which may indicate the importance of season, with Dawar *et al*. (2011) applying the fertiliser in spring. The June harvest showed that GU, DMPP and FPA all gave a production benefit when the time since fertilisation was extended, by either providing greater mineral N due to decreased NH_3 loss (GU and FPA) or retaining N in a form less prone to loss (DMPP) (Fig. 3). This reflects the ability of the inhibitors to work for extended periods (Fangueiro *et al*. 2009). However, the results reported are for a single event and due to the importance of environment and management on the performance of the EEFs (Abalos *et al*. 2014) extrapolation to different seasons and conditions is problematic.

Urease inhibitors target NH_3 loss and greatest benefits from these products are expected under conditions where NH_3 loss is high, i.e. moist, warm, windy conditions (Suter *et al*. 2013). No benefit from these products is expected during high rainfall periods, particularly when rain falls soon after fertiliser application, as NH_3 loss is low. Conversely, for the nitrification inhibitors we would expect to see the greatest benefit at times when there is high leaching and denitrification losses, i.e. during the wetter months. Studies on corn (Zea *mays* L.) found variable yield responses with a nitrification inhibitor concluding that profitable yield benefits were only likely to occur under conditions where spring and summer rainfalls were greater (40%) than the long-term average (Kyveryga and Blackmer 2014). Another study reported that one inhibitor (urease) was effective in increasing yield but not the other (nitrification) in the studied cropping system (Kawakami *et al*. 2012). This indicates that the dominant loss pathways will differ between systems, years and seasons. A synopsis of pasture trials conducted on 132 paddocks in 37 farms across New Zealand found overall biomass benefits from the use of a nitrification inhibitor (DCD) (19% increase), with variation around this dependent upon the region (Carey *et al*. 2012). The authors of the New Zealand study point out that this benefit is greater than reported on previous individual small plot studies (Carey *et al*. 2012).

Conclusions

This study found that urea boosted pasture productivity in southern Australian rainfed pasture systems by between 0.7 and 18 kg DM (vegetative) per kg of N applied, with soil moisture and climate dictating the seasonal response to N. Overall, N_2O emissions from applied fertiliser were low, but greater when conditions were conducive for denitrification (spring). Use of the nitrification inhibitor, DMPP, was consistently effective as a tool for mitigating N_2O emissions from urea across all seasons (autumn, winter and spring) with a 76% reduction recorded over eight months. However, DMPP did not significantly boost pasture productivity, with any observed biomass response influenced by seasonal conditions. The urease inhibitor, NBTPT (GU), is not an effective tool for mitigating N_2O emissions from urea, particularly during periods of high emissions (spring), with 53% more fertiliser-induced N_2O emissions produced relative to granular urea over the eight months. The importance of climate conditions for ammonia loss caused inconsistent productivity benefits from the use of GU. The potential for FPA to mitigate N_2O and increase pasture production requires investigation during periods of high emissions. These results highlight the need to use EEFs at times when their targeted loss pathway is important. This can be achieved by identifying the temporal changes in loss pathways (volatilisation, leaching and denitrification) based on climatic conditions and soil background N and C levels. To gain maximum productivity benefits from the use of EEFs other factors that restrict pasture growth, such as soil moisture and temperature, need to be effectively managed and N inputs should be lowered also.

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