Author/s:
Belyaeva, O;Ward, G;Wijesinghe, T;Chen, D;Suter, H

Title:
Influence of soil organic matter, fertiliser formulation and season on fertiliser nitrogen use efficiency in temperate pastures

Date:
2024-08-01

Citation:
Belyaeva, O., Ward, G., Wijesinghe, T., Chen, D. & Suter, H. (2024). Influence of soil organic matter, fertiliser formulation and season on fertiliser nitrogen use efficiency in temperate pastures. Nutrient Cycling in Agroecosystems, 129 (1), pp.133-150. https://doi.org/10.1007/s10705-024-10362-9.

Nutr Cycl Agroecosyst (2024) 129:133–150
https://doi.org/10.1007/s10705-024-10362-9

ORIGINAL ARTICLE

Influence of soil organic matter, fertiliser formulation and season on fertiliser nitrogen use efficiency in temperate pastures

Oxana Belyaeva · Graeme Ward · Thushari Wijesinghe · Deli Chen · Helen Suter

Received: 26 January 2024 / Accepted: 25 May 2024 / Published online: 18 June 2024

Abstract Intensively grazed dairy systems use high inputs of fertiliser nitrogen (N), and often supplementary irrigation, to ensure adequate pasture production to support milk output and meet the growing food demand. However, the efficiency of N use in these systems can be low and potential environmental impacts high. This study aimed to test the hypothesis that (1) use of two inhibitors, the urease inhibitor N-(n-butyl) thiophosphorictriamide (NBTPT) and the nitrification inhibitor 3,4-Dimethylpyrazole phosphate (DMPP) reduced N loss and improved pasture production compared to conventional N fertiliser (urea) in irrigated temperate perennial ryegrass (*Lolium perenne* L.) dairy pasture, and (2) their efficiency was affected by soil and environmental parameters. The effect of repeated applications of urea, at different rates, and the inhibitors were studied on pasture production and agronomic apparent fertiliser N use efficiency (NUE) over 2.5 years. The fate of a single application of N was determined through recovery of ^{15}N-labeled fertiliser applied at 20 and 40 kg N ha^{-1} was studied in the field for one year. The highest yield and NUE occurred in spring–summer (from August to February) reflecting optimal growing conditions. The highest NUE occurred at low rates of urea application (20 and 40 kg N ha^{-1}). Mineralisation played a key role in supplying N to pasture with 64–82% of total plant N derived from soil organic matter (SOM). Less than 50% of the applied N was recovered in the pasture (37–43%) with a large component retained in the soil (26–43% after one year, 0–40 cm), and slowly released in small amounts (< 2%) to the pasture over time, highlighting the abundant capacity of the native soil N pool to supply pasture N. Loss of N fertiliser (14–31%) was attributed to primarily ammonia (NH_3) volatilisation and nitrate (NO_3^-) leaching. Use of the inhibitors NBTPT and DMPP did not significantly affect pasture yield or NUE, most likely because fertiliser N saved with the inhibitors only played a minor role in plant nutrition with the majority of the plant nutrition provided by the soil organic matter pool.

Keywords NUE · Inhibitor · Pasture · Temperate · ^{15}N

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10705-024-10362-9.

O. Belyaeva (✉) · G. Ward · T. Wijesinghe · D. Chen · H. Suter (✉)
School of Agriculture, Food and Ecosystem Sciences, Faculty of Science, The University of Melbourne, Melbourne, VIC 3010, Australia
e-mail: ksu999@mail.ru

H. Suter
e-mail: helencs@unimelb.edu.au

Introduction

The demand for livestock products has increased globally over recent decades, mainly in response to rapid population growth and increasing income in developing countries (FAO 2019). This offers a major opportunity for livestock producers in industrialised countries to intensify production and this situation is likely to continue well into the foreseeable future, with a predicted 70% increase in livestock production by 2050 (Thornton 2010). Agricultural systems intensification, and as consequence, the increased use of N fertiliser, has been one of the most pronounced changes to accommodate increased food supply (Jankowski et al. 2018). Demand for N fertilisers globally was 111 million tonnes in 2022, an increase from 105 million tonnes in 2016, with Oceania requiring around 2 million tonnes (2016–2022) (FAO 2019). This alarming increase in fertiliser use raises concerns because reported N use efficiency (NUE) is quite low on average in agricultural systems globally, typically ranging from 8 to 64% of applied N (Powell et al. 2010). There are numerous approaches across scientific literature in which NUE can be assessed, reflecting crop N uptake or utilisation efficiency, profitability and environment aspects. Nonetheless, it is generally assumed that the higher N output/ N input ratio of recovered applied N represents the efficient use of the N fertiliser by crop (EU Nitrogen Expert Panel 2015). In this research the agronomic NUE of applied fertilisers was used as a one of the benchmarks cropping system NUE indicators, reflecting environmental and nutritional constraints to crop production and off-site N losses (Antille and Moody 2021). Reduced NUE often develops when fertiliser rates surpass crop requirements and is of concern in intensive production systems such as intensive dairy pastures. A low NUE is associated with increasing losses of N to the environment, potentially contributing to soil acidification and eutrophication, enhancing N gaseous losses, including ammonia (NH_3) and the potent greenhouse gas nitrous oxide (N_2O) (Cameron et al. 2013) and nitrate (NO_3^-) pollution of surface and ground waters. In intensively managed dairy pastures in southern Australia, 40–50 kg N ha^{-1}as urea applied per grazing rotation (around 21–56 days) is common place where climate or irrigation availability are conducive to all year pasture production, resulting in high annual inputs(e.g. > 400 kg N ha^{-1} yr^{-1}) (Dougherty et al. 2016). However, there is concern regarding potential negative effects on the environment from this high N input. Understanding how management practices affect the efficiency of N use by pasture plants is critical to improve NUE.

Enhanced efficiency fertilisers (EEFs) are designed to reduce the risk of N losses, by slowing the transformation of urea to NH_3/NH_4^+ (urease inhibitors), or NH_3/NH_4^+ to NO_3^- (nitrification inhibitors), or by slowing the release of N (controlled or slow-release fertilisers) and therefore can better synchronise N supply with plant uptake (Azeem et al. 2014). The urease inhibitor N-(n-butyl) thiophosphorictriamide (NBTPT) and the nitrification inhibitor 3,4-Dimethylpyrazole phosphate (DMPP) are widely used commercially available inhibitors. NBTPT inhibits urea hydrolysis and can effectively reduce ammonia (NH_3) volatilization from surface-applied granular urea in pasture systems (Suter et al. 2020). DMPP reduces the activity of the ammonia mono-oxygenase (AMO) enzyme in ammonium-oxidizing bacteria (AOB) in the first step of nitrification, the oxidation of NH_3 to NO_2^-, and thereby reduces the rate of nitrate (NO_3^-) production and minimises the risk of leaching and subsequent denitrification losses (N_2O, N_2) (Zerulla et al. 2001).

Whilst previous studies have shown that application of EEFs can effectively reduce N gaseous losses (Akiyama et al. 2010; Wu et al. 2021), decrease N leaching (Yang et al. 2016) and improve crop yield (Abalos et al. 2014), in pasture systems, the findings are largely inconsistent in terms of N losses, pasture productivity and NUE (Duncan et al. 2017; Nauer et al. 2018; Rowlings et al. 2016; Suter et al. 2013). The reported inconsistencies are mainly attributed to spatial variability of climatic and edaphic conditions, and differing management techniques (Chen et al. 2008).

While pasture N nutrition using fertilisers is a major component of managing dairy systems in Australia, a substantial quantity of N taken up by plants is not directly sourced from the fertiliser (Suter et al. 2020). Previous research has found that sometimes less than half of the N taken up by crops comes from applied fertiliser, and the rest is provided by depletion of soil organic matter and decomposition of plant residues (Angus et al. 2006). Despite some reported research on strategies to improve NUE and optimise agricultural production (Harris et al. 2016a, b), few studies have addressed the effects of these practices over more than a growing season or year. Comprehensive information on the combined effects of variable fertiliser rates and EEFs on seasonal and annual variability in productivity, NUE and environment impact, from applied N in irrigated temperate dairy pastures of southern Australia is lacking.

This manuscript reports on a research trial that examined (1) the influence of N rate and use of EEFs, and season on ryegrass pasture productivity and NUE, (2) the fate of applied N and magnitude of N loss via nitrous oxide, and (3) the source of N utilised by pasture plants (fertiliser or soil), in irrigated pastures in a high rainfall zone of Victoria, Australia using data from a 32-month (November 2016–June 2019) small plot field experiment.

Materials and methods

Site details and soil

The field trial was conducted on a commercial dairy farm at Allansford, in south-west Victoria, Australia (38° 24′ 34.59″ S 142° 38′ 16.83″ E). The site is located in the 'high rainfall zone' (HRZ) of Victoria with long-term average annual precipitation of 743 mm, 70% of which falls during April–October. The mean annual monthly maximum and minimum temperatures are 17.9 °C and 9.6 °C respectively (Commonwealth Bureau of Meteorology). The trial was established on a site with a history of long-term (> 20 years) pasture production (perennial ryegrass (*Lolium perenne* L.), fertilisation, grazing and irrigation. The soil is a Melanic-Mottled, Subnatric, Brown Sodosol (Agriculture Victoria 2020) [Solonetz (WRB 2015)]. Physico-chemical properties of the soil are presented in Table S1. The $pHCaCl_2$ of the soil profile (up to 0.8 m depth) was slightly acidic throughout, ranging from 6.3 (0–0.2 m) to 6.0 (0.7–0.8 m). At commencement of the experiment, both total C and N were high, particularly in the topsoil (0–0.1 m), which contained 3.8% and 0.32% respectively. In the topsoil (0–0.1 m) mineral N was 21.1 kg ha^{-1}, and was dominated by nitrate (14.9 kg N ha^{-1}), Colwell P was115 kg ha^{-1} and Colwell K was 158 kg ha^{-1}. Across the whole profile (0–0.8 m) mineral N was 61 kg ha^{-1}, Colwell P was 199.3 kg ha^{-1} and Colwell K was 580 kg ha^{-1}. Particle size analysis revealed that the texture changed from a loamy sand (84% sand, 11% silt and 4% clay) in the topsoil to a clay loam (48% sand, 6% silt and 45% clay) in the subsoil (> 0.4 m depth). Soil bulk density increased abruptly from 1.2 Mg m^{-3} in the surface (0–0.1 m) to 1.7 Mg m^{-3} at 0.2–0.3 m depth. Further details of the soil properties and climate are provided in the Supplementary Information.

Experimental design and management

The trial was carried out over two and half years, commencing on 26th of October 2016 and finishing on 20th of July 2019. Grazing animals were excluded from the site two months prior to commencement of the trial with the pastures mown and clippings removed and discarded three times over this period. The experiment was a randomised block design small plot trial with nine treatments replicated five times. Each plot measured 3 m × 3 m, with a 1 m buffer established between each of the five blocks. The treatments were: control (no-N) (C), Urea (U) at five rates – 20, 40, 60, and 80 kg N ha^{-1} (U20, U40, U60, U80), and urea applied as an EEF program including a urease and nitrification inhibitor at two rates—20 and 40 kg N ha^{-1} (EEF20, EEF40). The urease inhibitor fertiliser used was the commercial product Green Urea NV™ containing N-(n-butyl) thiophosphorictriamide (NBTPT) and the nitrification inhibitor fertiliser was the commercial product Urea with ENTEC® containing the nitrification inhibitor 3,4-Dimethylpyrazole phosphate (DMPP). NBTP or DMPP was used depending on the season and predicted loss pathway (Table S2). Fertiliser treatments were surface broadcast following each pasture harvest, such that the total application to the pastures was 660, 1320, 1980 and 2640 for the total study period, and 240, 480, 720 and 960 annually for the 20, 40, 60 and 80 kg N ha^{-1} treatments respectively. Phosphorus (P) and potassium (K) fertilisers were applied as required at rates representative of farming practice in the region from 22 to 35 kg ha^{-1} for P and from 50 to 150 kg ha^{-1} for K depending on the season, to prevent nutrient deficiency.

Soil mineral N

Soil surface samples (0–10 cm) were collected on a monthly basis initially, immediately after harvest and before fertilisation (21st of November 2016 to 6th of June 2017), and then two weeks after each fertilisation (16th of August to 27th of May 2019), using a corer (2.5 cm internal diameter), with 5 cores per plot collected and composited per sample time. Collected samples were analysed for NH_4^+-N and NO_3^--N using the methodology outlined previously. Mineral N was also determined for the soil profile (0–80 cm) on 4th of April 2017 from 5 locations within the buffer zones (non-fertilised) of the experimental site to determine a baseline profile N content and then on 17th of April 2019 from the control, U40, U80 and EEF40 plots with 5 replicates per treatment, using deep soil cores of 3.8 cm inner diameter. Samples were analysed for 0–0.1 m, 0.1–0.2 m, 0.2–0.4 m and 0.4–0.6 m depths and the profile N calculated from the sum of all depths accounting for the soil bulk density.

Nitrous oxide emissions

Nitrous oxide (N_2O) emissions were measured over three periods; in autumn 2018 (16th of March to 11th of April), spring 2018 (12th of September to 1st of October) and summer-autumn 2019 (23rd of February to 17th of March) over three weeks in the days following fertilisation using a vented closed static chamber method, to provide an indication of N_2O loss from the control, U40 and U80 treatments at different time periods. A chamber base (0.5 m × 0.5 m) was installed permanently into the ground to a depth of 0.1 m on selected plots. At gas collection times, which occurred between 10:00 to 14:00 h to avoid the potential effect of diurnal variation, chambers (0.5 m × 0.5 m × 0.3 m high) were temporarily sealed onto the bases and samples (20 ml) were collected three times over a one-hour period (0, 30 and 60 min). Measurements were taken each day during the first week after fertilisation, each second day during the second week and each third day till the end of the sampling period. Collected gas samples were transferred to 12 ml pre-evacuated vials for analysis by gas chromatography (Agilent 7890A) using an electron capture detector (ECD) with a lower detection limit of < 0.2 ppmv. The N_2O flux (kg N ha^{-1} h^{-1}) was determined using the linear flux method (Venterea et al. 2012) and converted to a daily emission. Cumulative and net-cumulative (less control) emissions were determined by integration of the daily flux curve for each measurement time.

Harvest, dry matter and NUE determination

Pasture dry matter (DM) production was determined for each plot at the 3-leaf stage of perennial ryegrass regrowth by cutting pasture to a height of 6 cm above the ground using a rotary lawnmower (0.5 m width), from an area of 3.0 m^2 (two strips 0.5 m by 3.0 m located centrally in each of the plots). Pasture fresh weight was recorded and a subsample of approximately 0.2 kg was removed and oven-dried at 70 °C to constant weight to determine pasture DM content. After harvest, all remaining pasture was mown and clippings removed from the plots. Pasture DM and cumulative production were determined respectively for (i) each harvest and (ii) each season and year by summing individual harvests.

Pasture N removal was calculated from the DM yield and herbage N content. Agronomic apparent N utilisation efficiency of fertiliser (NUE) for each harvest was calculated as harvested product (kg) per kg of applied N, according to Suter et al. (2013);

$$NUE = \frac{pasture\ yield\ at\ N_x - pasture\ yield\ at\ N_0}{kg\ of\ N\ applied\ at\ N_x} \tag{1}$$

where N_x is the fertiliser N rate, N_0 is the control. Values for NUE were determined for individual harvests and averaged for each season.

Fertiliser ^{15}N recovery

The long-term recovery of urea-N fertiliser was studied using two sets of ^{15}N microplots (23.7 cm inner diameter × 25 cm depth) installed on the site to a depth of 0.2 m on 4th of April 2017 and 14th of September 2017 to measure the fate of urea applied in autumn and spring respectively. Granulated ^{15}N urea (10.2 atom %) was applied at 20 kg N ha^{-1} and 40 kg N ha^{-1} rates to the microplots, replicated three times. Plants were removed from the microplots regularly after ryegrass reached the 3-leaf stage, following the timeline of the main plot harvest regime. Harvested pasture was analysed for biomass (t DM ha^{-1}), total N (%) and ^{15}N enrichment (%). On 1st of May 2018 both sets of microplots were removed from the site (after the completion of the ^{15}N response trial) with the area remaining free of grazing animals until that time), representing a total time of thirteen and eight months for the autumn and spring applications respectively. Two soil cores (0.2–0.4 m length, 9.2 cm internal diameter) were collected below the base of each microplot after they were removed. The soil was removed from the microplots in two 0.1 m increments to 0.2 m depth. Plant and soil samples were dried at 60 °C and 40 °C respectively, ground (< 50 µm) and analysed for TN and ^{15}N by Isotope ratio mass spectrometry (IRMS) (Hydra 20–20, SerCon).

Recovery of ^{15}N in the plant and soils was calculated according to Malhi et al. (2004) as follows;

$$Recovery\ of\ applied\ N\ in\ the\ plant\ (\%) = \frac{(\%N\ plant) \times DM \times (atom\ \%\ ^{15}N\ excess\ of\ total\ N\ in\ plant)}{Rate\ of\ N \times atom\ \%\ ^{15}N\ excess\ of\ total\ N\ in\ fertiliser} \quad (2)$$

where *%N plant* is the grams of total N per 100 g of pasture (%), *DM* is the dry matter production (kg ha^{-1}), *atom % ^{15}N excess of total N in the plant* = (atom % ^{15}N excess of total N in the plants of the fertilised plots) – (atom % ^{15}N excess of total N in the plants of the control plots), *Rate of N* is the rate of applied N (kg N ha^{-1}), and *atom % ^{15}N excess of total N in fertiliser* = (atom % ^{15}N of fertiliser) – (atom % ^{15}N natural abundance);

$$Recovery\ of\ applied\ N\ in\ soil\ (\%) = \frac{(\%N\ soil) \times SW \times (\text{atom\%}\ ^{15}N\ \text{excess of total N in soil})}{Rate,\ of\ N \times atom\%\ ^{15}N\ excess\ of\ total\ N\ in\ fertiliser} \quad (3)$$

where *%N soil* is the grams of total soil N per 100 g of soil, SW is the weight of dry soil (kg ha^{-1}), *atom % ^{15}N excess of total N in the soil* = (atom % ^{15}N excess of total N in the soil of the fertilised plots) – (atom % ^{15}N excess of total N in the soil of the control plots), and *Rate of N* and *atom % ^{15}N excess of total N in fertiliser* are as described above.

The total plant N derived from fertilisers (%Ndff) was calculated as described by Malhi et al. (2004);

$$\%\ Ndff = \frac{Atom\ \%\ ^{15}N\ excess\ of\ total\ N\ in\ plant}{Atom\ \%\ ^{15}N\ excess\ of\ total\ N\ in\ fertiliser} \times 100 \quad (4)$$

and the total plant N derived from soil (%Ndfs) was calculated as described by Malhi et al. (2004);

$$\%Ndfs = 100 - \%Ndff \quad (5)$$

Statistical analysis

Statistical analysis was conducted using GenStat 16th edition. Analysis of variance (ANOVA) and repeated measures were used to calculate differences on each individual date and over the whole experimental time. Differences between treatments were calculated at the 5% level using Fisher's significance test. No transformations were required to improve the residual normality among N_2O treatments.

Results

Climatic variations and soil mineral N

During the trial the annual rainfall was lower than the long-term (25-year) average (735 mm), being 587 mm in 2017 and 454 mm in 2018. In the first six months of 2019 the site received 264 mm of rain which was lower than the long-term mean (330 mm) for this period. However, irrigation, applied from spring to autumn each year, substantially increased the amount of water received by the site to 1390 mm in 2017, 1185 mm in 2018 and to 640 mm in the first six months of 2019 (Fig. 1a). The volumetric soil moisture content in the 0–0.2 m layer consistently stayed above the permanent wilting point (PWP) (15%) and the readily available water point (RAW) (26%), reflecting irrigation inputs coupled with rainfall, apart from at commencement of the trial in October 2016—March 2017, when some moisture deficiency was observed especially in the top 0.1–0.2 m layer (Fig. 1b). The soil moisture at 0.2–0.3 m depth

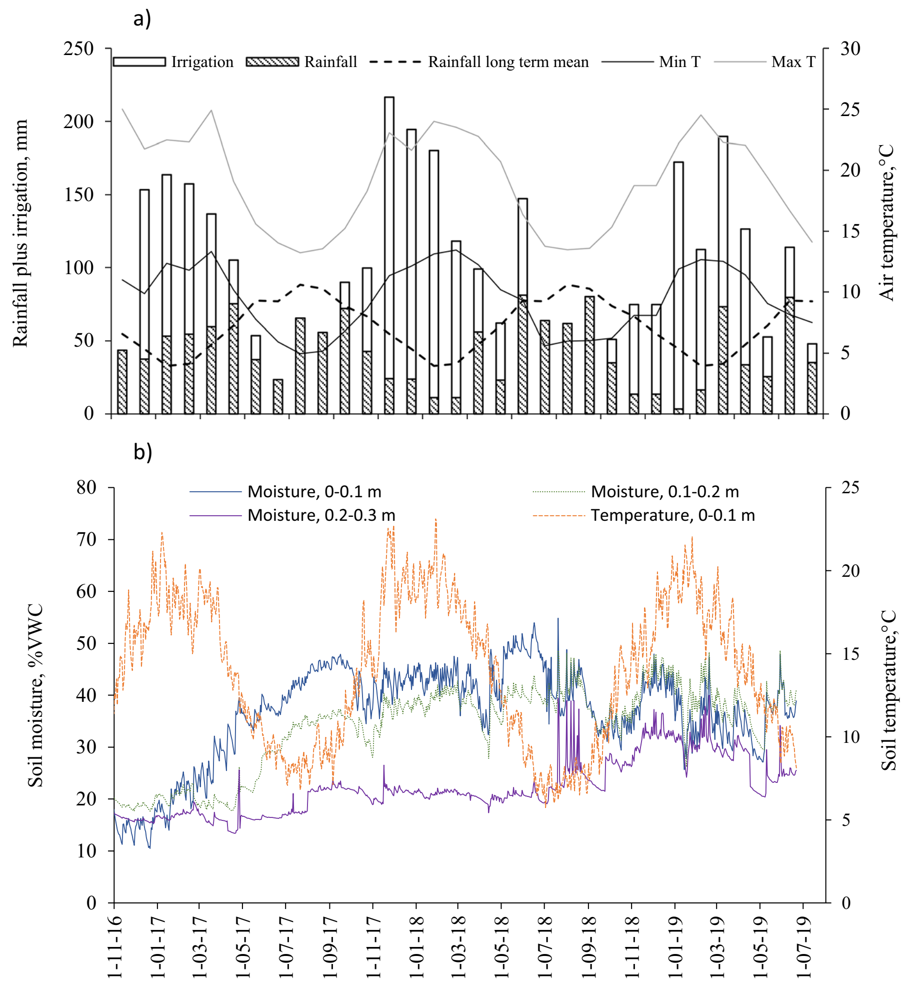


Fig. 1 Environmental parameters at the Allansford site from November 2016 to June 2019: **a** Monthly precipitation, and irrigation (mm), and maximum and minimum air temperature (°C) plus long-term (25 years) rainfall; **b** Daily soil volumetric moisture (0–0.1 m, 0.1–0.2 m and 0.2–0.3 m) and soil temperature (0–0.1 m)

followed a slightly different pattern remaining low at the start of the study (13–26%) through to July 2018 after which it increased to a level similar to that in the 0–0.2 m depths. Fluctuations of soil moisture were more pronounced in the surface (0–0.1 m) varying between 10 and 55%. Below the surface, the range of soil moisture changed from 17 to 49% in the 0.1–0.2 m layer and from 13 to 40% in the 0.2–0.3 m layer.

Air temperature fluctuations were typical of seasonal conditions in this region. The daily air temperature ranged from a low of -0.7 °C (July 2017) to a high of 42 °C (January 2019) (data not shown), and mean minimum and maximum monthly temperatures were between 4.9 and 25.0 °C (Fig. 1a). Daily temperature in the soil also followed the seasonal pattern and were less variable than the ambient temperature, ranging from 5.7 °C (June 2018) to 23.1 °C (January 2018) in the surface layer 0–10 cm (data not shown). At depths greater than 0.1 m the soil temperature fluctuated seasonally, but overall was more consistent ranging from 6.5 to 22.8 °C throughout the trial (data not shown).

Averaged NH_4^+-N concentrations in the 0–10 cm layer fluctuated between 15.1 kg N ha^{-1} to 31.2 kg N ha^{-1}, but there was little impact of fertiliser use on NH_4^+-N levels (Fig. 2). Nitrate–N concentrations in the 0–10 cm layer fluctuated between 0.5 and 65.6 kg N ha^{-1} and were highest at the highest rate of N application (U80) with an average of 36.2 kg N ha^{-1} throughout the experiment, followed by U60, U40 and U20 treatments, averaging 25.8 kg N ha^{-1}, 18.8 kg N ha^{-1} and 15.0 kg N ha^{-1} respectively (Fig. 2b).

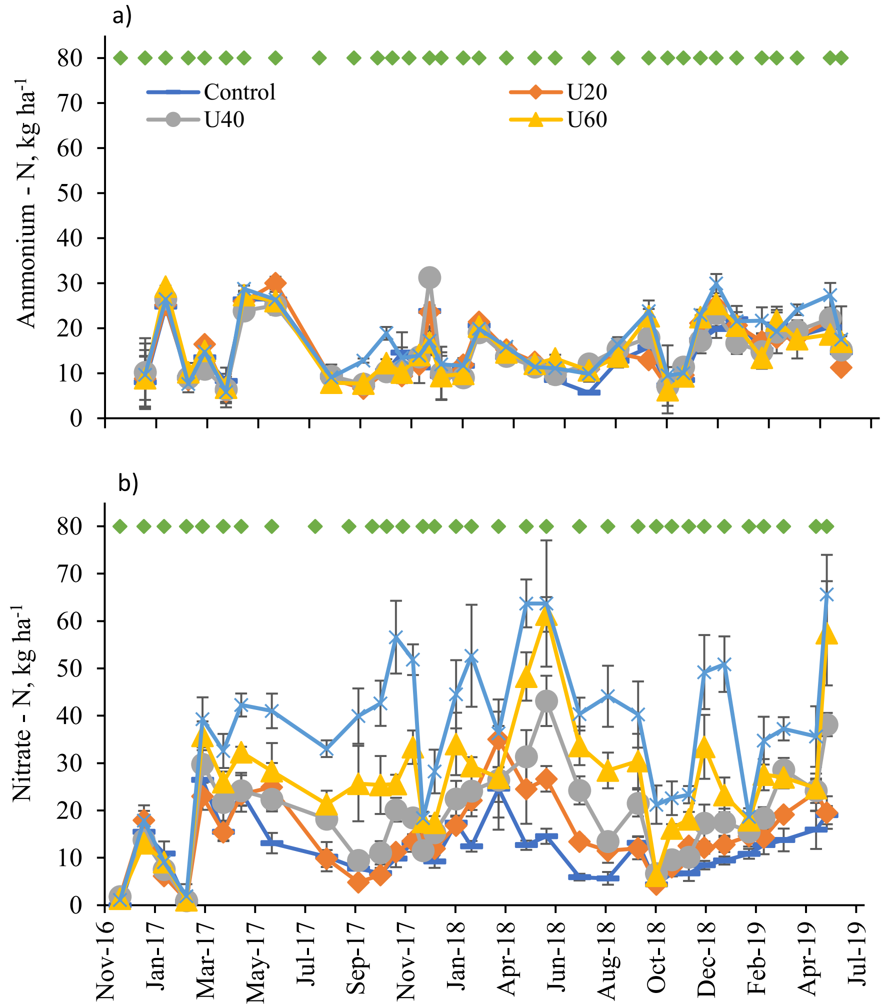


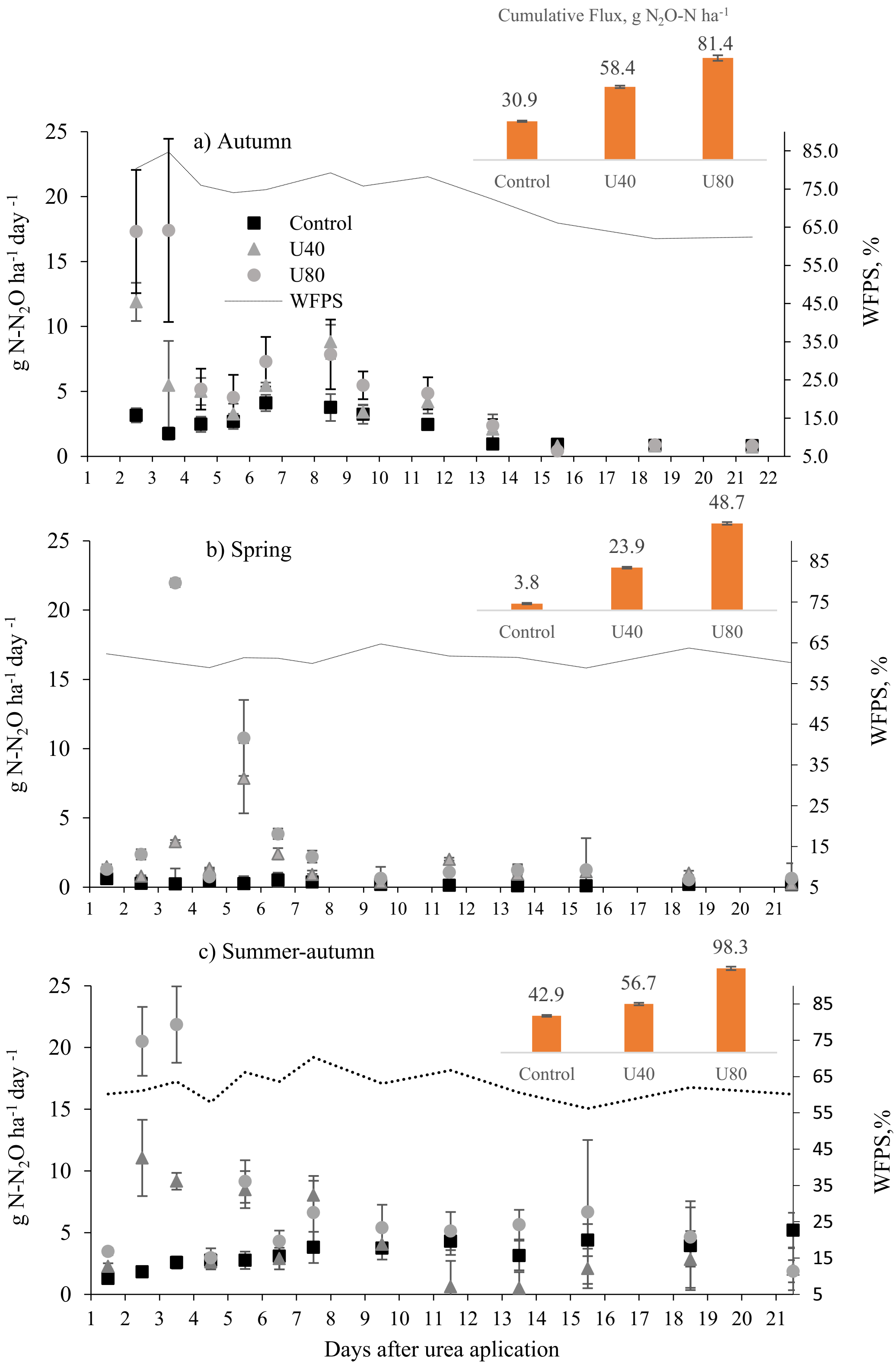
Fig. 2 Surface soil (0–0.1 m) ammonium (**a**) and nitrate (**b**) for the different N rates. Green diamonds represent fertiliser application dates. Values represent means (± standard errors) of five replicates. Note: soil sampling was done 2 weeks after fertilisation except for samples collected before August 2017

Table 1 Soil ammonium (NH_4^+-N), nitrate (NO_3^--N) and total mineral N (kg ha^{-1}) in the soil profile (0–60 cm) on the 3rd of April 2017 and on 17th of April 2019

Treatment	NH_4-N (kg ha^{-1})	NO_3-N (kg ha^{-1})	Total mineral N (kg ha^{-1})
April 2017			
Baseline level	$15.6^a \pm 1.5$	$37.2^a \pm 2.5$	$51.4^a \pm 3.3$
April 2019			
Control	$46.7^b \pm 10.4$	$23.9^a \pm 6.0$	$70.6^a \pm 19.9$
U40	$34.8^{ab} \pm 1.7$	$33.8^a \pm 7.6$	$68.6^a \pm 13.2$
EEF40	$48.6^b \pm 9.8$	$32.3^a \pm 8.7$	$80.9^a \pm 17.8$
U80	$44.3^b \pm 4.1$	$33.7^a \pm 9.7$	$76.4^a \pm 9.9$

Means followed by the same letter in a column are not significantly different at $P \leq 0.05$

Application of EEFs had no significant effect on NH_4^+ or NO_3^- in the topsoil (0–0.1 m) (Fig. S1). The soil-profile (0–0.6 m) total mineral N content increased from a baseline of 51 kg N ha^{-1}on 3rd of April 2017 to an average of 74 kg N ha^{-1} across all treatments on 17th of April 2019, although the change was not significant (Table 1). This change reflected the significant increase in soil NH_4^+-N levels on the 17th of April 2019 (average of 44 kg N ha^{-1} across all treatments) compared to 3rd April 2017 (15.6 kg N ha^{-1}). Soil NO_3^- was essentially the same at both sampling times (average 32 kg N ha^{-1}). Despite the variation in N inputs ranging from 0 kg N/ha (control) to 2640 kg N/ha (U80), and the use of EEFs, the mineral N content was not


Cumulative Flux, g N2O-N ha-1
a) Autumn
30.9
58.4
81.4
Control
U40
U80
Control
U40
U80
WFPS
g N-N2O ha-1 day -1
WFPS, %
b) Spring
3.8
23.9
48.7
Control
U40
U80
g N-N2O ha-1 day -1
WFPS, %
c) Summer-autumn
42.9
56.7
98.3
Control
U40
U80
g N-N2O ha-1 day -1
WFPS,%
Days after urea aplication

◂**Fig. 3** Daily and cumulative N_2O emissions following N fertiliser application in autumn 2018 (16th of March to 7th of April) (**a**), spring 2018 (12th of September to 1st of October) (**b**) and summer-autumn 2019 (23rd of February to 17th of March) (**c**) with soil temperature in the top 0.1 m. Values represent means (± standard errors) of five replicates

significantly different between treatments towards the end of the trial.

Nitrous oxide emissions

The application of N significantly ($P \leq 0.05$) increased daily N_2O emissions (Fig. 3) from 0.4 g N_2O-N ha^{-1} (control) to 22.0 g N_2O-N ha^{-1} (U80) across the three sampling times. Most of the fertiliser loss as N_2O (up to 89%) occurred during the first seven days after fertilisation with emissions peaking between day 2 and day 4. Cumulative emissions (over 21 days) significantly increased with the application rate N40, and with an increase of N from 40 to 80 kg N ha^{-1} in autumn 2018 only (Fig. 3). The cumulative emissions measured in summer-autumn 2019 were highset across three measurement sets, although not significantly higher that N_2O production in autumn 2018. In spring 2018, when the temperature was colder, N_2O production lowered, being 12%, 40% and 59% of the highest emissions occurred in 2019 for the equivalent treatments of U0, U40 and U80 respectively.

Pasture dry matter (DM) production

On each harvest date the lowest individual harvest DM production was from the control, ranging from 0.2 t DM ha^{-1} (August–November 2018, February 2019) to 1.41 t DM ha^{-1} (September 2017) (Table S2). Dry matter production increased with N application with the highest values, up to 3.1 t DM ha^{-1} (January 2018) obtained in the U80 treatment. There was a significant N response for the urea fertiliser treatments in all seasons, but increasing the N rate above 60 kg N ha^{-1} only increased yields significantly ($P \leq 0.05$) in spring and summer (Table 2). The highest DM production for each treatment was observed in summer, followed by spring. There was a regular, but often non-significant, increase in seasonal average pasture DM production with the EEFs compared to straight urea at the equivalent rate up to 10% (Table 2).

However, this increase tended to be more prominent during winter—early spring. Use of NBTPT with urea (GU) significantly increased DM production harvested on 6th of June 2017, compared to urea applied at the same N rate (at 20 kg N ha^{-1}) and 14th of September 2017 (at 20 kg N ha^{-1}), but not on any other dates (Table S3). Use of DMPP with urea (EU) significantly increased pasture DM production only on one occasion (out of seven harvests), the 11th of September 2018 (at 40 kg N ha^{-1}; Table S3).

Similar to the pattern of DM production, the pasture N uptake significantly increased with N fertiliser rate up to 80 kg N ha^{-1} in spring and summer, but only to the 60 kg N ha^{-1} rate in autumn and winter (Table 2). Considering the entire trial, the average N uptake by the pasture from the control treatment was 21.0 kg N ha^{-1} $harvest^{-1}$ (range for individual harvests from 3.7 to 51.4 kg ha^{-1}), whereas for the U80 treatment the average was 70.3 kg ha^{-1} $harvest^{-1}$ (range for individual harvests from 39.3 kg ha^{-1} to 108.0 kg ha^{-1}; Table S3). The effect of EEFs on the pasture N uptake was variable and significant ($P \leq 0.05$) only on a few occasions when (i) DMPP (EU) was used at rate 20 kg ha^{-1} in winter-spring 2017 (August and September harvests) and (ii) NBTPT (GU) was used at a rate of 40 kg ha^{-1} in summer 2017 (December harvest) and summer-autumn 2019 (January 31st and May harvests).

The NUE tended to be the highest under the U20 and U40 treatments, while application of 80 kg N ha^{-1} (U80) lowered NUE compared to all other N rates in each season, albeit not always significantly (Fig. 4a). In autumn NUE decreased ($P \leq 0.05$) nearly linearly with increasing N application rates from 22.2 kg DM kg N^{-1} (U20) to 13.6 kg DM kg N^{-1} (U80). In spring, summer and winter NU_AE slightly increased when N rate increased from U20 to U40 but then tended to decline when more N was applied. EEFs application did not significantly affect NUE, but occasionally increased this indicator up to 10% (Fig. 4b).

This significant ($P \leq 0.05$) increase was observed for the U20 rate in spring and winter, where an increase in NUE from 19.7 kg DM ha^{-1} kg N applied to 24.9 kg DM ha^{-1} kg N (spring), and from 16.6 kg DM ha^{-1} kg N to 24.9 kg DM ha^{-1} kg N (winter) was observed.

Table 2 Average DM yield and plant N uptake per harvest/grazing cycle within each season

	DM yield (t ha^{-1})				N uptake (kg N ha^{-1})			
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
Control	0.7^{a}	0.8^{a}	0.7^{a}	0.6^{a}	19.5^{a}	20.1^{a}	23.2^{a}	19.7^{a}
U20	1.1^{b}	1.3^{b}	1.1^{b}	1.0^{b}	29.6^{b}	32.1^{b}	38.7^{b}	34.9^{b}
U40	1.6^{c}	1.9^{c}	1.4^{c}	1.4^{d}	48.7^{c}	49.2^{c}	53.1^{c}	51.7^{d}
U60	1.8^{d}	2.3^{d}	1.7^{d}	1.8^{e}	59.4^{d}	64.2^{d}	68.6^{d}	65.0^{e}
U80	2.0^{e}	2.5^{e}	1.8^{d}	1.8^{e}	72.8^{e}	74.4^{e}	71.2^{d}	67.8^{e}
EEF20	1.2^{b}	1.4^{b}	1.2^{b}	1.2^{c}	32.5^{b}	32.6^{b}	39.8^{b}	40.5^{c}
EEF40	1.7^{c}	2.0^{c}	1.5^{c}	1.5^{d}	50.1^{c}	52.3^{c}	55.6^{c}	55.7^{d}

Means followed by the same letter in a column are not significantly different at $P \leq 0.05$

Recovery of N fertiliser (^{15}N)

The majority of the applied ^{15}Nrecovered in the plant after the 8–13 months period was recovered in the aboveground biomass (38–43%) with minimal in the roots (1.5–2.8%) (Table 3). No seasonal (autumn / spring application) effect was detected in the plant ^{15}N recovery over the 8–13-month period. ^{15}N plant uptake was rapid and the most of ^{15}N fertiliser found in the aboveground biomass, up to 82%-87%, was taken up in the first two harvest in both seasons (Table S4). The N recovery in each subsequent harvest remained approximately the same regardless of N application rate (range 0.1–2.5%). The ^{15}N recovery in the soil after 8–13 months was 26–43% with the majority of the ^{15}N recovered found in the top 10 cm (16–23%), with 5–13% recovered in the 0.1–0.2 m layer and 4–12% in the 20–40 cm layer (Table S5).

Between 69 and 86% of the applied ^{15}N was recovered in the plant-soil system with a trend of greater recovery at lower N rates (Table 3). In total, nearly twice the amount of N was unaccounted for at the U40 rate with 31.2 kg N ha^{-1} and 27.5 kg N ha^{-1} unaccounted for in autumn and spring respectively compared to 16.4 kg N ha^{-1} and 17.0 kg N ha^{-1} at the U20 rate (Fig. 5) Plant total N uptake from a single fertiliser application was found to be sourced largely (> 64%) from the soil organic matter with < 37% originating from the applied fertiliser (Ndff) (Table 3). The %Ndff was significantly ($P < 0.05$) higher with

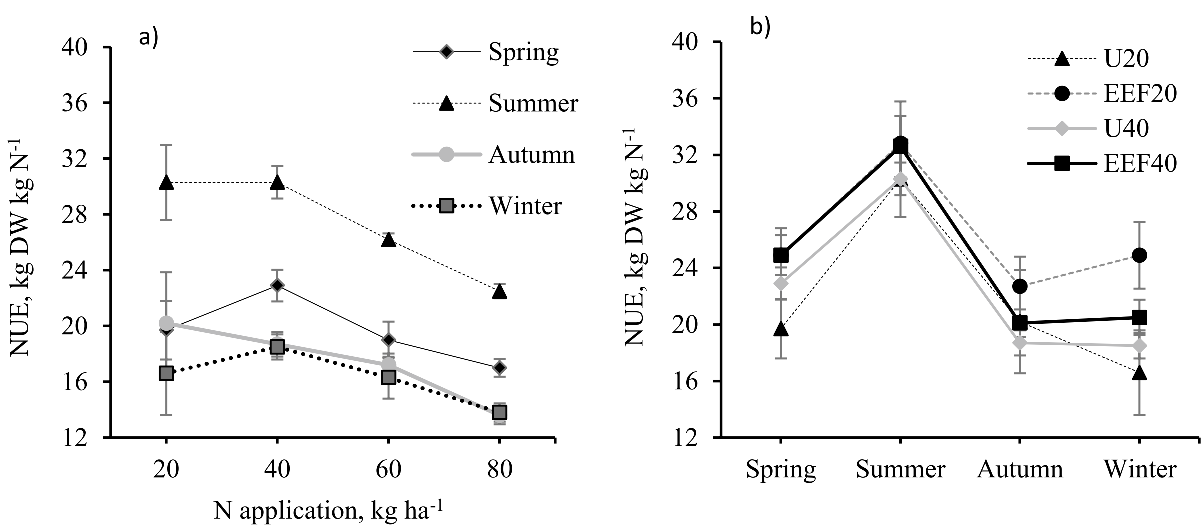


Fig. 4 Agronomic efficiency of fertiliser (NUE) as affected by season and N rate of conventional urea (**a**), and EEF urea and season (**b**). Values represent means (± standard errors) of five replicates

the higher rate of N applied (Table 3, Table S4), but there was no significant difference.

Table 3 ^{15}N recovery in plants and soil, and source of N taken up by the plant over the 13 (autumn) and 8 (spring) month measurement period at the Allansford irrigated site

N rate (kg ha^{-1})	^{15}N recovery in soil (%)		^{15}N recovery in shoots (%)*		^{15}N recovery in roots (%)		^{15}N recovery total (%)		Plant N derived from fertiliser (Ndff, %)		Plant N derived from soil (Ndfs, %)	
	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring
20	43 ± 2.3^a	39 ± 4.3^b	38 ± 5.3^a	43 ± 2.3^a	1.7 ± 0.3^a	2.7 ± 1.2^a	83 ± 5.3^a	86 ± 3.4^a	18 ± 2.5^a	21 ± 2.1^a	82 ± 2.4^b	79 ± 2.3^b
40	36 ± 1.7^a	26 ± 1.0^a	42 ± 3.5^a	40 ± 1.3^a	1.5 ± 0.4^a	2.8 ± 0.3^a	79 ± 1.6^a	69 ± 1.9^a	35 ± 1.1^b	36 ± 6.0^b	65 ± 1.3^a	64 ± 1.5^a

Means followed by the same letter in a column are not significantly different at $P \leq 0.05$

*N recovery in shoots is the cumulative data from 13 autumn and 9 spring harvests

Values represent means (±standard errors) of three replicates

Discussion

Effect of N fertilisation on seasonal pasture DM production, NUE and plant N uptake

Annual DM production averaged over the 32-months of the trial exhibited a linear response to N, significantly ($P < 0.05$) increasing from 8.9 t ha^{-1} at 0 N to 25.6 t ha^{-1} at U80 which is common for pastures (McKenzie et al. 2006). Seasonal variations in response of the perennial ryegrass to N (Table 2) reflect typical ryegrass growth patterns (Fulkerson and Donaghy 2001) as influenced by environmental parameters (e.g. soil temperature and moisture, solar radiation). Within season variability in pasture growth is mainly associated with fluctuations in soil water content (Chapman et al. 2009). Irrigation in our trial reduced the impact of soil moisture deficits (Fig. 1b) on pasture growth and N uptake, however, in autumn, when irrigation ceased, soil moisture played a critical role in pasture productivity and N responses, in particular deep soil moisture (0.2–0.3 m). This evidenced by the different N response seen in 2017, 2018 and 2019, when autumn pasture yield was lover in 2017 compared to 2018 and 2019 (Fig. S2). Perennial ryegrass pasture roots can access soil depths of 0.5 m depending on the soil type, indicating that consideration of subsoil (> 0.1 m depth) moisture is important when predicting N responses (Schultz et al. 2022).

In spring and summer, with adequate soil moisture due to irrigation, pasture was highly responsive to N, driven by the higher temperatures and high solar radiation (Fig. 1, Table 2) similar to observations of Korte (1988). The flatter DM production in response to increased fertiliser N generally seen in autumn and winter are a response to low solar radiation and temperatures coupled with drier soil conditions (autumn). NUE was higher in summer compared with other seasons due to favourable growing conditions (Belyaeva et al. 2019; Fig. 4b). The low N application rates (20 and 40 kg N ha^{-1}) tend to return higher NUE compared to rates at or above the regional standard (Fig. 4a), and consequently less is lost to the environment or immobilised into the soil organic pool (de

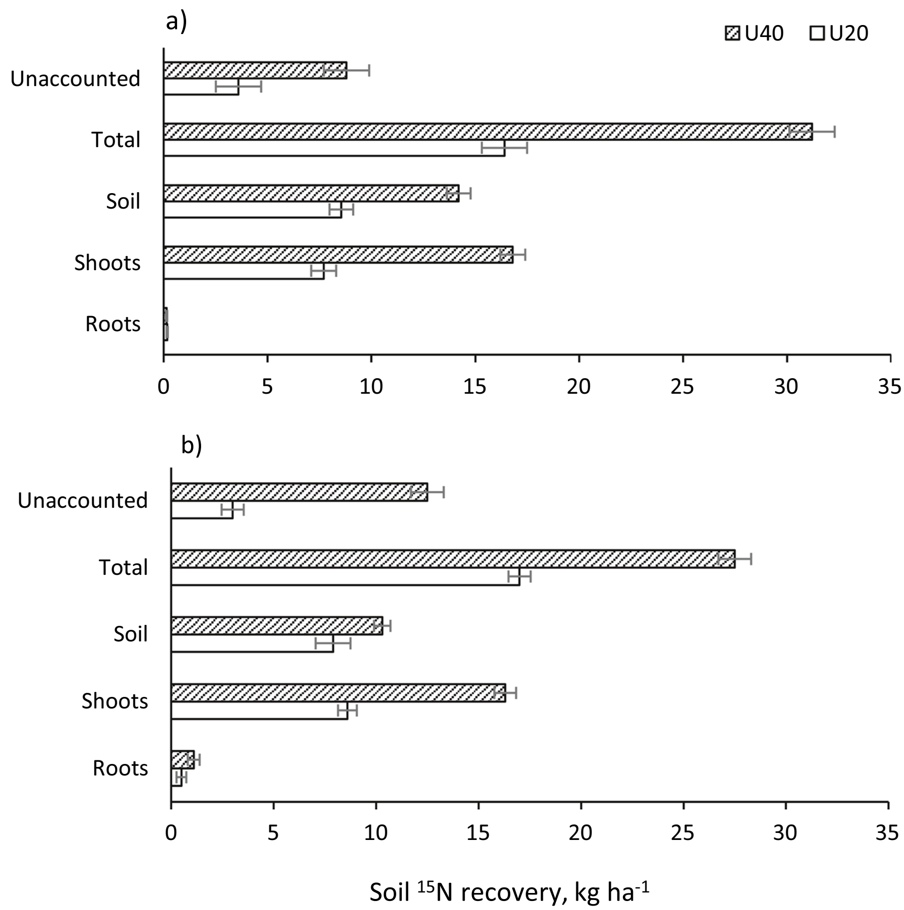


Fig. 5 The fate of ^{15}N-labeled fertiliser in plant-soil system as affected by rate and season; autumn (**a**) and spring (**b**). Values represent means (± standard errors) of three replicates

Klein et al. 2017). However, a high NUE can mean soil N is being mined (Antille and Moody 2021). The amount of N removed by pasture plants throughout the trial exceeded the amount of N applied in all treatments except the highest rate (80 kg N ha^{-1}) by 1%, 23% and 40% with U60, U40 and U20 respectively, while in the U80 treatment an excess of 340 kg ha^{-1} N was created over 2.5 years (Table S6). The apparent negative balance is often seen in cut and carry style pasture trials such as this one where there is no N return to the system from decaying plant clippings and animal wastes as in a grazed pasture.

The negative N balance indicates that mineralization of soil organic matter, with a large reserve of 4.4 t N ha^{-1} available in the experimental soil, successfully fulfilled a role in suppling N to the pasture. This is evident from the ^{15}N work, which showed > 64% of the pasture N was supplied by the soil. We expect that mineralised N would provide high levels of pasture nutrition under grazed systems as well. With continued removal of excess N, the organic N reserves will eventually diminish (N mining), leading to an associated loss of soil carbon, with implications for long-term sustainability. Therefore, replenishing N is needed, with strategic application at times when N loss is expected to be minimal.

Influence of urease and nitrification inhibitors on DM yield and NUE

The agronomic and environmental effectiveness of EEF's remains a controversial topic due to the considerable variability reported across trials, influenced by edaphic, environmental and management factors (Chen et al. 2023). A number of studies have indicated the effectiveness of EEFs in mitigating ammonia or denitrification losses, as well as enhancing plant productivity (Dawar et al. 2012; Dobbie and Smith 2003). Interestingly, some research has shown that EEFs, in particular DMPP, was notably effective, even when the application rate was halved compared to conventional urea (Rowlings et al. 2016). However, there is a growing body of recent field research, which suggests that EEFs may not significantly increase pasture production or NUE whether applied at standard or halved rates (De Antoni Migliorati et al. 2014; Nauer et al. 2018; Suter et al. 2016b) and our study aligns with them. Several factors might explain the observed lack of effect of EEFs on pasture yield and NUE, including interaction with soil and environmental site conditions including organic matter content, soil texture, soil temperature, pH and some management practices (e.g. irrigation) (Thapa et al. 2016; Yang et al. 2016).

SOM is recognised as a major factor affecting EEFs' efficiency. In soils with high organic matter, the abundance of the urease enzyme targeted by NBTPT might render this inhibitor less effective there (Hongprayoon et al. 1991). Bremner and Chai (1989) showed that NBTPT was most effective on a soil with low organic matter content. This further explains the limited impact on our carbon-rich (3.8% C) pasture soil. Similar, DMPP effectiveness may be reduced in soils with high SOM due to adsorption on soil colloids (Volpi et al. 2017). In addition, SOM is a source of energy for heterotrophic microbes that can degrade DMPP and reduce its effectiveness (Fisket al. 2015).

Soil texture plays a crucial role in the EFF's effectiveness. Due to its high-water solubility, NBPT may be less effective in soils prone to leaching, such as coarse and sandy soils (Cantarella et al. 2018). In our study, a loamy sand texture of the experimental soil, with 83% sand content up to 40 cm, could increase the risk of NBPT being washed away before exerting its full inhibitory effect. Menéndez et al. (2009) observed that the effectiveness of DMPP might be reduced at soil moisture content close to saturation. In our study, the moisture level in the upper soil was generally high (30–55 vol%) and sometime close to saturation, especially in winter, due to rainfalls and reduce evaporation. So, these environmental conditions might contribute to the reduced efficacy of the DMPP.

EEFs' efficacy is known to be actively influenced by soil pH (Liu et al. 2015; Suter et al. 2016a) with NBTP showing chemical degradation with pH decrease (Engel et al. 2015). Thus, the slightly acidic pH of the soil in our study may have shortened longevity of NBTP and hence, efficacy. Several studies, including a recent meta-analysis, have noted a positive correlation between DMPP efficacy and soil pH (Xue et al. 2012), with diminishing efficacy observed in acidic soils (Nauer et al. 2018; Liu et al. 2015; Yang et al. 2016). Alkaline soil pH accelerates DMPP hydrolysis, yielding the active compound DMP, which inhibits ammonia oxidation (McCarty 1999). Thus, the acidic pH in our study might not be conductive to the optimal performance of DMPP.

Temperature also plays a role, with NBTPT and DMPP being less effective at higher temperatures due to increased microbial activity and inhibitor degradation. Studies have shown that inhibitors are less effective at temperatures above 25 °C (NBTPT) (Clay et al. 1990) and above 20 °C (DMPP) (Chen et al. 2010). However, the relatively mild ambient and soil temperatures at our site (Figs. 1, 2) and the presence of a good pasture cover that would cool the soil, suggests that temperature effects were likely minimal.

The high inherent fertility of the soil might have contributed to the lack of the EEFs' effect on yield and NUE observed in our study. Consequently, most of the N supplied to pasture plants originated from the native N derived from mineralisation of SOM (as discussed in the next section), rather than from fertiliser. This minimal contribution of fertiliser meant that any potential savings of N with EEFs were negligible, resulting in little change in the overall N balance.

Fertiliser ^{15}N recovery and source of N used by pasture plants

The recovery of fertiliser N in the pasture biomass (38–46%) is within the range previously reported for Australian and New Zealand ryegrass and other grasses (29–49%) (Cookson 1999; Suter et al. 2020). Most of the ^{15}N uptake occurred quickly, within the first two weeks following fertilisation (Table S4), which suggests rapid immobilisation of applied N (Liu et al. 2015; Ledgard et al. 1988). This immobilised fertiliser N is a N store available for future pasture growth, however the subsequent release of this N to plants in our experiment was slow, with a large proportion (37–43%) of applied ^{15}N remaining in the soil after 12 months (Table S4) and only low amounts (i.e. 1.4–1.9%) being released at each subsequent harvest event, similar to other studies (Jamali et al. 2016; Smith and Chalk 2018). The observed fertiliser recoveries could be affected with ‘added nitrogen interaction’ (ANI) or ‘priming’ which results in enhanced mineralisation (of soil N) by N fertiliser application and consequently, increased plant uptake of soil N (Jenkinsons et al. 1985). Over time, because of the relatively large size of the organic N pool accumulated under the fertilised long-term perennial ryegrass, only a small component of the total N utilised by the plant will be from the ^{15}N fertiliser that was immobilised and re-mineralised. Our data confirmed that the contribution of native soil N to pasture nutrition (Ndfs) was large (64–82%) (Table 3). There is the evidence that NH_4^+-N causes higher ANI compared to NO_3^--N (Azam 2002), hence the presence of the EEF EU might stimulate ANI via prolonging the presence of NH_4^+-N in soil as reported for dryland pastures in a similar climate (Suter et al. 2020).

Typically, lower plant recovery of fertiliser N is expected in autumn compared to spring due to slower plant growth (Cookson et al. 2001), which we observed with the autumn fertilisation DM being nearly half that of the spring fertilisation (data not shown). This was associated with reduced solar radiation, as other climatic parameters were similar in both seasons (temperature 13.0–15.6 °C, soil volumetric water content of 48–60%). Enhanced spring plant growth resulted in more N uptake and higher recovery of ^{15}N, but this seasonal effect was noticeable after the first and second harvests only (Table S4). Nonetheless, the total N recovery in autumn-fertilised plots (30–34%) was slightly lower, than in spring-fertilised plots (35–39%).

Environmental impact of N fertiliser application

After an experimental period of 8–12 months, an average of 3.6 kg N ha^{-1} and 12.5 kg N ha^{-1} remained unaccounted for at the low (U20) and higher (U40) rates of urea, respectively, representing 14–31% of applied N. Thus, doubling the fertilisation rate had no effect on the recovery of ^{15}N in the plant, however it did cause a two to threefold increase in unaccounted for N (Fig. 5). The observed range of lost N is similar to that reported by others for temperate pastures (9–35%) (Jamali et al. 2016; Williams et al. 2000). Nitrogen loss from agricultural systems can be via major pathways including denitrification gaseous loss (N_2O and N_2), ammonia volatilisation (NH_3) and nitrification leading to nitrate (NO_3^-) loss.

N input is recognized as the major cause of N_2O emissions in pasture systems (Oenema et al. 1997); however, there can be significant differences in the magnitude of N_2O emissions following fertiliser application, depending on environmental and management factors. In our study, daily emissions measured from both fertilised and unfertilised plots (Fig. 3) were low. In the U0 treatment, this low flux was attributable to low levels of soil NO_3^- (10–20 mg kg^{-1}) under unfertilised cut and carry conditions. Similar low emissions have been observed in non-fertilised, non-grazed or extensively grazed pastures, where the daily flux is typically < 5 g N_2O-N ha^{-1} (Moiser et al. 1991). Even though fertilisation with urea increased the N_2O flux exponentially (Fig. 3), the total N lost within one week (peak loss) (under 0.13%) was still small and did not represent an economical value. This is mainly because this peak increase was short (< 1 week) as a consequence of rapid urea hydrolysis in these highly organic pasture soils (often < 7 days and can be as rapid as 24 h for complete hydrolysis) (Dougherty et al. 2016) followed by nitrification and denitrification. Additionally, low losses of N_2O observed in fertilised plots can be associated with plant uptake of N (Veldkamp et al. 1999). Well-developed roots under established perennial pasture outcompete microbial nitrifiers for NH_4^+ in the rich organic pasture soil (3.8% C in our study) (Neill et al. 1995) reducing the NH_4^+ undergoing autotrophic nitrification. Moreover, under established perennial pastures carbonaceous material is abundant in the rhizosphere, which can enhance net-immobilization of N (Wakelin et al. 2021) further reducing NH_4^+ available for nitrification. Our post fertilised N_2O emissions (0.5–22.0 g N_2O-N ha^{-1} per day) were similar to estimates from studies of temperate grasslands (Flechard et al. 2005; Randall et al. 2015; Suter et al. 2016a).

The differing magnitude of the N_2O fluxes measured in the three seasons appeared to be influenced more by soil temperature than soil moisture (Fig. 1b). We observed lower N_2O emissions (on average 50–90% less) with the cooler soil temperatures in spring 2018 (< 10.6 °C) (Fig. 1b) compared to the other sampling dates (average of 19.4 °C). These spring temperatures are below the optimal range for microbial nitrification and denitrification processes (20–40 °C) (Bouwman et al. 2002). Lower N_2O emissions during the cooler spring and autumn months, regardless of soil moisture and soil N input was observed by Philips et al. (2007), who described a two thirds reduction of N_2O fluxes when the soil temperature was reduced from 23 to 20 °C. Because of wet soil conditions [30% VWC to 46% VWC which is equivalent of 55–85% water filled pore space (WFPS)] under irrigation, we hypothesise that most of the N_2O emission in our experiment were primarily from denitrification (Saggar et al. 2013). The soil moisture at the higher end of the observed range is known to be conductive to loss of N_2 from complete denitrification (Wu et al. 2017). Under such conditions, the emission of this gas is generally expected to be greater than that of N_2O, with the ratio N_2:N_2O varying from 1 to 8:1 depending on soil and environmental conditions (Warner et al. 2019; Rolston et al 1982). However, based on this ratio, we expect that N_2 loss was a minor contributor to overall N loss in the experimental environment. Nitrate leaching is often considered to be the most important channel of N loss from the rooting zone particularly on well-drained soils and during times of high rainfall or irrigation (Di and Cameron 2002; Holz 2010). However, we do not expect this would be a major pathway of N loss at our site at spring–summer during active pasture growth, due to the competition of well-developed plant roots for NO_3^-. However, over winter, plant grow rates were low, but rainfall was high increasing the risk of N leaching. The soil at our site had reasonable internal drainage (Table 1) suggesting a possibility for N to leach, and we observed up to 12% of applied ^{15}N was found at the 20–40 cm depth after 8–12 months (Table S3). In additional, lateral flow could occur due to the texture change in the profile, so leaching and movement of N below the root zone could be substantial during the winter period.

Under drier conditions, similar to those we observed at our site in autumn, substantial NH_3 volatilization from urea applied to pastures can occur (up to ~ 30%) (Dawar et al. 2012) when the urea is not washed into the soil due to the turned off irrigation. Previous reports in similar climates shown up to 30% loss from urea applied to pasture in autumn (Suter et al. 2013).

Our findings emphasize the seasonal variability in N_2O fluxes, driven primary by soil temperature and fertilizer applications, which underscores the importance of accounting for climatic conditions in N management strategies. The potential for N leaching during periods of high rainfall, coupled with NH_3 volatilization under dry conditions, highlights the need for targeted management, which could include urease and nitrification inhibitors, especially during high N loss periods such as winter and autumn.

Conclusion

Environmental variables along with timing and rate of fertiliser application significantly influenced pasture productivity and NUE in the established perennial ryegrass pasture. Our findings indicate that the highest NUE was measured at a urea rate reflecting the standard commercial practice in the region (40 kg N ha^{-1}). NUE was higher in summer, emphasizing the importance of encompassing variability in seasonal N management strategies. Inter-year seasonal variations in pasture growth highlighted the importance of soil moisture in determining productivity, particularly in autumn when deep soil moisture appears to drive productivity as the soil dries out.

Application of EEFs with urea did not alter the pasture yield or NUE over the growing season. This is attributed to the high background levels of N in the soil contributing N from the soil organic matter pool for pasture nutrition, and the rapid immobilisation of applied N within the organic-rich soil matrix. Additionally, local soil and environmental conditions may have influenced the EFFs' efficacy. Consequently, we concluded that using EEFs was not an effective strategy in boosting pasture production, when applied at standard or lower N rates, in the investigated environment.

A significant proportion of applied N remained unaccounted for a year after application, suggestive of losses through denitrification and ammonia volatilisation. While losses of N_2O were relatively low, losses of N as volatilised ammonia or via leaching during autumn and winter respectively could be major contributors to the unaccounted for N loss. Future studies to identify all pathways of N loss, and develop measures to prevent N loss to environment would be beneficial. Effective N management in pasture system requires a comprehensive understanding of soil–plant interactions, seasonal variability and the impact of fertilisation practices on productivity and environmental sustainability. Targeted management strategies tailored to local soil and climatic conditions are essential for optimizing N use efficiency while minimizing environmental impacts.

Acknowledgements This research was supported by funding from the Australian Government Department of Agriculture, Water and the Environment as part of its Rural R&D for Profit Program, Dairy Australia and The University of Melbourne. The authors would like to thank the Croft family from Allansford for their significant contribution and cooperation which made this research possible. The authors also thank the many research assistants involved in the project: Dr. Arjun Pandey, Dr. Bhawana Bhatta, Dr. Robert Impraim, Dr. MD Shahin Rhaminur, Mr. Andrew Longmire, and Dr. Robert Edis for the soil physics assessment.

Author contributions Authors contributed to the study conception and design: Deli Chen and Helen Suter. Material preparation, samples/data collection and analysis were performed by Oxana Belyaeva, Graeme Ward, Thushari Wijesinghe. The first draft of the manuscript was written by Oxana Belyaeva and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions

Availability of data and materials No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abalos D, Sanz-Cobena A, Guardia G, Jeffery S (2014) Meta-analysis of the effect of urease and nitrification inhibitors on crop productivity and nitrogen use efficiency. Agric Ecosyst Environ 189:136–144. https://doi.org/10.1016/j.agee.2014.03.036

Agriculture Victoria (2020) SW3. In: Victorian Resources Online, State Government of Victoria. https://vro.agriculture.vic.gov.au/dpi/vro/vrosite.nsf/pages/vrohome/. Accessed 20 March 2021

Akiyama H, Yan X, Yagi K (2010) Evaluation of effectiveness of enhanced-efficiency fertilizers as mitigation options for N_2O and NO emissions from agricultural soils: meta-analysis. Glob Chang Biol 16(6):1837–1846

Angus JF, Bolger TP, Kirkegaard JA, Peoples MB (2006) Nitrogen mineralization in relation to previous crops and pastures. Aust J Soil Res 44:355–365

Antille DL, Moody PW (2021) Nitrogen use efficiency indicators for the Australian cotton, grains, sugar, dairy and horticulture industries. Ecol Indic 10:100099. https://doi.org/10.1016/j.indic.2020.100099

Azam F (2002) Added nitrogen interaction in the soil-plant system—a review. J Agron 1:54–59

Azeem B, KuShaari K, Man ZB, Basit A, Thanh TH (2014) Review on materials & methods to produce controlled release coated urea fertilizer. J Control Rel 181:11–21

Belyaeva ON, Chen D, Ward G, Suter H (2019) Field calibration of the capacitance soil moisture probes for brown sodosol. Proceedings of 19th Australian Agronomy Conference. Wagga-Wagga, Australia, 25–29 August.

Bouwman AF, Boumans LJM, Batjes NH (2002) Emissions of N_2O and NO from fertilized fields: summary of available measurement data. Global Biogeochem Cycl 16(4):6–13

Bremner JM, Chai HS (1989) Effects of phosphoroamides on ammonia volatilization and nitrite accumulation in soils treated with urea. Biol Fertil Soils 8:227–230

Cameron KC, Di HJ, Moir JL (2013) Nitrogen losses from the soil/plant system: a review. Ann Appl Biol 162:145–173

Cantarella H, Otto R, Soares JR, Gomes A, Silva B (2018) Agronomic efficiency of NBPT as a urease inhibitor: a review. J Adv Res 3:19–27. https://doi.org/10.1016/j.jare.2018.05.008

Chapman DF, Cullen BR, Johnson IR, Beca D (2009) Interannual variation in pasture growth rate in Australian and New Zealand dairy regions and its consequences for system management. Anim Prod Sci 49:1071–1079. https://doi.org/10.1071/AN09054

Chen D, Suter H, Islam A, Edis R, Freney JR, Walker CN (2008) Prospects of improving efficiency of fertiliser nitrogen in Australian agriculture: a review of enhanced efficiency fertilisers. Soil Res 46(4):289–301

Chen D, Suter HC, Islam A, Edis R (2010) Influence of nitrification inhibitors on nitrification and nitrous oxide (N_2O) emission from a clay loam soil fertilised with urea. Soil Biol Biochem 42:660–664

Chen M, Schievano A, Bosco S, Montero-Castaño A, Tamburini G, Terres J-M, Pérez-Soba M, Makowski D (2023) Evidence map of the benefits of enhanced-efficiency fertilisers for the environment, nutrient use efficiency, soil fertility, and crop production. Environ Res Lett. https://doi.org/10.1088/1748-9326/acb833

Clay DE, Maltzer CI, Anderson JL (1990) Ammonia volatilization from urea as influenced by soil-temperature, soil–water content and nitrification and hydrolysis inhibitors. Soil Sci Soc Am J 54:263–266

Commonwealth Bureau of Meteorology, http//www.bom.gov.au. Accessed 20 March 2018

Cookson WR, Rowarth JS, Cameron KC (2001) The fate of autumn-, late winter- and spring-applied nitrogen fertiliser in a perennial ryegrass (*Lolium perenne* L.) seed crop on a silt loam soil in Canterbury, New Zealand. Agric Ecosyst Environ 84:67–77

Cookson RW (1999) Nitrogen fertiliser use efficiency in ryegrass (*Lolium perenne* L.) seed crops. Ph.D. Thesis, Lincloln University, Canterbury, New Zealand.

Dawar K, Zaman M, Rowarth JS, Turnbull MH (2012) Applying urea with urease inhibitor N-n-butyl thiophosphoric triamide in fine particle application improves N uptake in ryegrass *Lolium perenne* L. Soil Sci Plant Nutr 583:309–318

De Antoni MM, Scheer C, Grace PR, Rowlings DW, Bell M, McGree J (2014) Influence of different nitrogen rates and DMPP nitrification inhibitor on annual N_2O emissions from a subtropical wheat–maize cropping system. Agric Ecosyst Environ 186:33–43

De Klein C, Monaghan RM, Alfaro M, Gourley CJP, Oenema O, Powell JM (2017) Nitrogen performance indicators for dairy production systems. Soil Res 55:479–488. https://doi.org/10.1071/SR16349

Di HJ, Cameron KC (2002) Nitrate leaching from temperateagroecosystems: sources, factors and mitigating strategies. Nutr Cycling Agroecosyst 64:237–256

Dobbie KE, Smith KA (2003) Impact of different forms of N fertilizers on N2O emission from intensive grassland. Nutr Cycl Agroecosyst 67:37–46

Dougherty WJ, Collins D, Van Zwieten L, Rowlings DW (2016) Nitrification (DMPP) and urease (NBTP) inhibitors has no effect on pasture yield, nitrous oxide emissions or nitrate leaching under irrigation in a hot-dry climate. Soil Res 54:675–683

Duncan EG, O'Sullivan CA, Simonsen AK, Roper MM, Peoples MB, Treble K, Whisson K (2017) The nitrification inhibitor 3,4,-dimethylpyrazole phosphate strongly inhibits nitrification in coarse-grained soils containing a low abundance of nitrifying microbiota. Soil Res 55:28–37

Engel RE, Towey BD, Gravens E (2015) Degradation of the Urease Inhibitor NBPT as Affected by Soil pH. Soil Sci Soc Am J 79(6):1674–1683

EU Nitrogen Expert Panel (2015) Nitrogen Use Efficiency (NUE) - an indicator for the utilization of nitrogen in agriculture and food systems. Wageningen University, Alterra, PO Box 47, NL-6700 Wageningen, Netherlands. https://www.eunep.com. Accessed 15 March 2019

FAO (2019) World fertilizer trends and outlook to 2022. FAO, Rome

Fisk LM, Maccarone LD, Barton L, Murphy DV (2015) Nitrapyrin decreased nitrification of nitrogen released from soil organic matter but not amoA gene abundance at high soil temperature. Soil Biol Biochem 88:214–223. https://doi.org/10.1016/j.soilbio.2015.05.029

Flechard CR, Neftel A, Jocher M, Ammann C, Fuhrer J (2005) Bi-directional soil/atmosphere N_2O exchange over two mown grassland systems with contrasting management practices. Glob Change Biol 11:2114–2127

Fulkerson WJ, Donaghy DJ (2001) Plant soluble carbohydrate reserve and senescence—key criteria for developing an effective grazing management system for ryegrass-based pasture: a review. Aust J Exp Agric 41:266–275

Harris RH, Armstrong RD, Wallace AJ, Belyaeva ON (2016a) Delaying nitrogen fertiliser application improves wheat ^{15}N recovery from high rainfall cropping soils in south eastern Australia. Nutr Cycl Agroecosyst 106:113–118

Harris RH, Officer SJ, Hill PA, Armstrong RD, Fogarty KM, Zolinger RP, Phelan AJ, Partington DL (2016b) Can nitrogen fertiliser and nitrification inhibitor management influence N_2O losses from high rainfall cropping systems in South Eastern Australia. Nutr Cycl Agroecosyst 95:269–285

Holz GK (2010) Sources and processes of contaminant loss from an intensively grazed catchment inferred from patterns in discharge andconcentration of thirteen analytes using high intensity sampling. Hydrol 383:194–208

Hongprayoon C, Lindau C, Patrick WJ, Bouldin D, Reddy K (1991) Urea transformations in flooded soil columns: I. Experimental results. Soil Sci Soc Am J 55:1130–1134

Jamali H, Quayle W, Scheer C, Baldock J (2016) Mitigation of N_2O emissions from surface-irrigated cropping systems using water management and the nitrification inhibitor DMPP. Soil Res 54:481–493

Jankowski K, Neill C, Davidson EA, Macedo MN, Costa JC, Galford GL, Maracahipes-Santos G, Lefebvre P, Darlisson N, Cerri CEP, McHorney R, O'Connell C, Coe MT (2018) Deep soils modify environmental consequences of increased nitrogen fertilizer use in intensifying Amazon agriculture. Sci Rep 8:13478. https://doi.org/10.1038/s41598-018-31175-1

Jenkinsons DS, Fox RH, Raumer JU (1985) Interactions between fertiliser nitrogen and soil nitrogen – the so-called "priming" effect. Soil Sci 36:425–444

Korte CJ (1988) Nitrogen fertilizer responses on Gisborne-East Coast pastures. Proc Ann Conf Agron Soc N Z 18:85–88

Ledgard SF, Steele KW, Feyter C (1988) Influence of time of application on the fate of ^{15}N-labbled urea applied to dairy pasture. N Z J Agric Res 31:87–91

Liu X, Li XG, Guo R, Kuzyakov Y, Li F (2015) The effect of plastic mulch on the fate of urea-N in rain-fed maize production in a semiarid environment as assessed by ^{15}N-labelling. Eur J Agron 70:71–77

Malhi SS, Johnston AM, Gill KS, Pennock DJ (2004) Landscape position effects on the recovery of ^{15}N-labelled urea applied to wheat on two soils in Saskatchewan, Canada. Nutr Cycl Agroecosyst 68(1):85–93

McCarty GW (1999) Modes of action of nitrification inhibitors. Biol Fertil Soils 29:1–9

McKenzie FR, Jacobs JL, Ward GN (2006) Irrigated dairy pasture yield and water use efficiency responses to summer applied nitrogen. Proc N Z Grassl Assoc 68:155–160

Menéndez S, Merino P, Pint M, González-Murua C, Estavillo JM (2009) Effect of N-(n-butyl) Thiophosphoric triamide and 3,4 dimethylpyrazole phosphate on gaseous emissions from grasslands under different soil water contents. J Environ Qual 38:27–35. https://doi.org/10.2134/jeq2008.0034

Mosier A, Schimel D, Valentine D, Bronson K, Parton W (1991) Methane and nitrous oxide fluxes in native, fertilised and cultivated grasslands. Nature 350:330–332. https://doi.org/10.1038/350330a0

Nauer PA, Fest BJ, Visser L, Arndt SK (2018) On-farm trial on the effectiveness of the nitrification inhibitor DMPP indicates no benefits under commercial Australian farming practices. Agric Ecosyst Environ 253:82–89

Neill C, Piccolo MC, Steudler PA, Melillo JM, Feigl DJ, Cerri CC (1995) Nitrogen dynamics in soils of forest and active pastures in the western Brazilian Amazon Basin. Soil Biol Biochem 27:1167–1175

Oenema O, Velthof G, Yamulki S, Jarvis S (1997) Nitrous oxide emissions from grazed grassland. Soil Use Manag 13:288–295

Powell JM, Gourley CJP, Rotz CA, Weaver DM (2010) Nitrogen use efficiency: a potential performance indicator and policy tool for dairy farms. Environ Sci Policy 13(3):217–228

Randall NP, Donnison LM, Lewis PJ, James KL (2015) How effective are on-farm mitigation measures for delivering an improved water enivronment? A systematic map. Environ Evid 4:18. https://doi.org/10.1186/s13750-015-0044-5

Rolston DE, Sharpley AN, Toy DW, Broadbent FE (1982) Field measurements of denitrification. III. Rates during irrigation cycles. SSSAJ 46:289–296

Rowlings DW, Scheer C, Liu S, Grace PR (2016) Annual nitrogen dynamics and urea fertiliser recoveries from a dairy pasture using ^{15}N; effect of nitrification inhibitor DMPP and reduced application rates. Agric Ecosyst Environ 216:216–225

Saggar S, Singh J, Giltrap DL, Zaman M, Luo J, Rollo M, Kim D-G, Rys G, van der Weerdeen TJ (2013) Quantification of reductions in ammonia emissions from fertilizer urea and animal urine in grazed pastures with urease inhibitors for agriculture inventory: New Zealand as a case study. Sci Total Environ 465:136–146

Schultz B, Mohssen M, Chau HW, Pandey VP, Prasad K, Anthony P (2022) Assessing effective pasture root depth for irrigation scheduling by water balance and soil moisture monitoring. Irrig Drain 71:971–979. https://doi.org/10.1002/ird.2708

Smith CJ, Chalk PM (2018) The residual value of fertilizer N in crop sequences: an appraisal of 60 years of research using ^{15}N tracer. Field Crops Res 217:66–74

Suter H, Sultana H, Turner D, Davies R, Walker C, Chen D (2013) Influence of urea fertiliser formulation, urease inhibitor and season on ammonia loss from ryegrass. Nutr Cycl Agroecosys 95(2):175–185

Suter H, Sultana H, Davies R, Walker C, Chen D (2016a) Influence of enhanced efficiency fertilisation techniques on nitrous oxide emission and productivity response from urea in a temperate Australian ryegrass pasture. Soil Res 54:523–532

Suter H, Lam SK, Walker C, Chen D (2020) Enhanced efficiency fertilisers reduce nitrous oxide emissions and improve fertiliser ^{15}N recovery in a Southern Australian pasture. Sci Total Environ 699:134147. https://doi.org/10.1016/j.scitotenv.2019.134147

Suter H, Walker C, Chen D (2016b) Understanding the variability in performance of the nitrification inhibitor 3, 4-Dimethylpyrazole phosphate in Australian agricultural soils. In: Proceedings of the 2016th International Nitrogen Initiative Conference.

Thapa R, Chaterjee A, Wale R, McGranahan DA, Daigh A (2016) Effect of enhanced efficiency fertilizers on nitrous oxide emissions and crop yields: a meta-analysis. Soil Sci Soc Am 80:1121–1134

Thornton PK (2010) Livestock production: recent trends, future prospects. Philos Trans R Soc Lon Ser B Biol Sci 365(1554):2853–67

Veldkamp E, Davidson E, Erickson H, Keller M, Weitz A (1999) Soil nitrogen cycling and nitrogen oxide emissions along pasture Chronosequence in the humid tropics of Costa Rica. Soil Biol Biochem 31:387–394

Venterea RT, Parkin TB, Cardenas L, Petersen SO, Pedersen AR (2012) Data analysis considerations. In: de Klein CAM, Harvey MJ (eds) Nitrous oxide chamber methodology guidelines version 1. New Zealand, pp 95–121.

Victorian resourceless online. http://www.vro.agriculture.vic.gov.au (access April 2018).

Volpi I, Laville P, Bonari E, di Nasso NN, Bosco S (2017) Improving the management of mineral fertilizers for nitrous oxide mitigation: The effect of nitrogen fertilizer type, urease and nitrification inhibitors in two different textured soils. Geoderma 307:181–188. https://doi.org/10.1016/j.geoderma.2017.08.018

Wakelin SA, Matson A, Wigley K, Waller L, Dickie IA, Whitehead D, Garrett L (2021) High maintenance of rhizosphere soil C and N equilibrium regardless of plant species or species traits. Front Soil Sci. https://doi.org/10.3389/fsoil.2021.762510

Warner DI, Scheer C, Friedl J, Rowlings DW, Brunk C, Grace PR (2019) Mobile continuous-flow isotope-ratio mass spectrometer system for automated measurements of N_2 and N_2O fluxes in fertilized cropping systems. Sci Rep 9:11097. https://doi.org/10.1038/s41598-019-47451-7

Williams PH, Rowarth JS, Tregurtha RJ (2000) Recovery of ^{15}N-labelled fertiliser by a perennial ryegrass seed crop and a subsequent wheat crop. Nutr Cycl Agroecosyst 56:117–123

Wu D, Cardenas LM, Calvet S, Brüggemann N, Loick N, Liu S, Bol R (2017) The effect of nitrification inhibitor on N_2O, NO and N_2 emissions under different soil moisture levels in a permanent grassland soil. Soil Biol Biochem 113:153–160

Wu D, Zhang Y, Dong G, Du Z, Wu W, Chadwick D, Bol R (2021) The importance of ammonia volatilization in estimating the efficacy of nitrification inhibitors to reduce N_2O emissions: a global meta-analysis. Environ Pollut 271:116365. https://doi.org/10.1016/j.envpol.2020.116365

Xue Y, Wu ZJ, Zhang LL, Gong P, Dong XX, Nie YX (2012) Inhibitory effect of DMPP on soil nitrification as affected by soil moisture content, pH and organic matter. J Appl Ecol 23(10): 2663–2669

Yang M, Fang Y, Sun D, Shi Y (2016) Efficiency of two nitrification inhibitors (dicyandiamide and 3, 4-dimethypyrazole phosphate) on soil nitrogen transformations and plant productivity: a meta-analysis. Sci Rep 6:22075. https://doi.org/10.1038/srep22075

Zerulla W, Barth T, Dressel J, Erhardt K, von Locquenghien KH, Pasda G, Rädle M, Wissemeier AH (2001) 3,4-Dimethylpyrazole phosphate (DMPP)—a new nitrification inhibitor for agriculture and horticulture. Biol Fertil Soils 34:79–84