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Nitrogen fertilisers with urease inhibitors reduce nitrous oxide and ammonia losses, while retaining yield in temperate grassland.

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## Abstract

Nitrogen fertilisation, although a cornerstone of modern agricultural production, negatively impacts the environment through gaseous losses of nitrous oxide (N<sub>2</sub>O), a potent greenhouse gas (GHG), and ammonia (NH<sub>3</sub>), a known air pollutant. The aim of this work was to assess the feasibility of urea treated with urease inhibitors to reduce gaseous N losses in temperate grassland, while maintaining or improving productivity compared to conventional fertiliser formulations. Urease inhibitors were N-(n-butyl)-thiophosphoric triamide (NBPT) (urea + NBPT) and N-(n-propyl)-thiophosphoric triamide (NPPT) (urea + NBPT + NPPT), while conventional fertilisers were urea and calcium ammonium nitrate (CAN). N<sub>2</sub>O emission factors were 0.06%, 0.07%, 0.09% and 0.58% from urea + NBPT, urea, urea + NBPT + NPPT and CAN, respectively, with CAN significantly higher than all the urea formulations, which were not significantly different from each other. Ammonia loss measured over one fertiliser application was significantly larger from urea, at 43%, compared with other formulations at 13.9%, 13.8% and 5.2% from urea + NBPT, urea + NBPT + NPPT and CAN, respectively. Changing fertiliser formulation had no significant

impact on grass yield or N uptake in four out of five harvests. In the last harvest urea + NBPT significantly out-yielded urea, but not CAN or urea + NBPT + NPPT. Overall, urea treated with either one or both urease inhibitors significantly reduced emissions of  $N_2O$  and  $NH_3$ , while preserving yield quantity and quality. Therefore, changing fertiliser formulation to these products should be encouraged as a strategy to reduce GHG and air pollution from agricultural practices in temperate climate.

Key words: nitrogen, nitrous oxide, ammonia, urease inhibitor, NBPT, NPPT

## 1. Introduction

Nitrogen (N) fertilisation is a cornerstone of production in intensive agricultural systems, with an estimated 48% of the global population in 2008 fed by N fertilisers (Erisman et al., 2008). Use of N fertilisers has seen an approximately 8 fold increase since 1961 with growing demand for agricultural products (Lu & Tian, 2017). The increased fertilisation however leads to large reactive N losses to the environment (Galloway et al., 2004). The United Nations Economic Commission for Europe (UNECE) Task Force on Reactive Nitrogen estimates that approximately 80% of N is lost from agriculture through leaching and run-off of nitrate or organic nitrogen and gaseous emissions to air (UNECE, 2018). Gaseous losses of reactive N following fertilisation are predominantly of nitrous oxide ( $N_2O$ ) and ammonia ( $NH_3$ ). Nitrous oxide is a potent greenhouse gas (GHG) and ozone-destroying substance (Ravishankara et al., 2009), while  $NH_3$  is an air pollutant contributing to eutrophication and acidification of terrestrial and aquatic ecosystems, and an indirect source of  $N_2O$  (Sutton et al., 1992). Global  $N_2O$  and  $NH_3$  emissions from fertiliser N are estimated to be approximately 4 and 10-12 Tg N yr<sup>-1</sup>, respectively (Beusen et al., 2008; Reay et al, 2012). European

countries committed to reduce emissions of  $N_2O$  and  $NH_3$  under the European Union's Effort Sharing Regulation (European Commissions, 2018) and the National Emissions Ceiling Directive (European Commission, 2016), respectively. These gaseous N losses from fertilisation of agricultural soils not only negatively impact ecosystems but also represent loss of agricultural productivity (Forrestal et al., 2017), therefore their mitigation is of paramount importance.

Intensively managed grasslands in temperate climates are hotspots of  $N_2O$  emissions due to high fertilisation rates combined with high rainfall (Sutton et al., 2011). Nitrous oxide is produced and emitted by agricultural soils primarily through the processes of nitrification and denitrification (Meixner and Yang, 2006). In particular, nitrate-based fertilisers are associated with high  $N_2O$  production rates in temperate grasslands, with  $N_2O$  emissions factors of up to 3.81% reported by Bell et al. (2016) and Harty et al. (2016). These high emissions are due to the fact that nitrate is a direct substrate for denitrification (Baggs, 2011). Changing from nitrate-based fertiliser to urea can offer reductions in  $N_2O$  emissions by avoiding rapid increases in soil nitrate concentrations post fertilisation, which have been shown to increase  $N_2O$  emissions (Dobbie and Smith, 2003). However, urea is vulnerable to  $NH_3$  volatilization, potentially leading to significant N losses through this alternative N loss pathway. Chambers & Dampney (2009) reported up to 30 % of applied N lost as  $NH_3$ , while Forrestal et al. (2017) recorded up to 53% N loss via  $NH_3$  in temperate grassland in spring, when environmental conditions were favourable to volatilisation. .

Enhanced-efficiency fertilisers frequently contain urease and/or nitrification inhibitors and have been developed to increase N use efficiency by crops (Timilsena et al., 2014). Urease inhibitors are compounds that delay the hydrolysis of urea, whereas nitrification inhibitors

are compounds that delay bacterial oxidation of  $\text{NH}_4^+$  by depressing the activities of nitrifiers in soil (Akiyama et al., 2010). Example inhibitors are N-(n-butyl) thiophosphoric triamide (NBPT), urease inhibitor which is a structural analogue of urea (Engel et al., 2015), and dicyandiamide (DCD), a nitrification inhibitor. The urease inhibitor NBPT has been shown to reduce  $\text{NH}_3$  losses from surface-applied urea (Watson et al., 2008, Forrestal et al., 2016) and increase yield and N uptake (Watson et al., 2009, Harty et al., 2017) in temperate grasslands. Dicyandiamide has also been reported to reduce fertiliser  $\text{N}_2\text{O}$  emissions (Bell et al., 2015; Harty et al., 2016; Roche et al., 2016), with contrasting reports in regards to its effectiveness in increasing yield and plant N use efficiency (Dennis et al 2012; Di & Cameron, 2012; Harty et al., 2017).

While there is a substantial body of research on the effects of enhanced efficiency fertilisers on elements of the agricultural system such as emissions of  $\text{N}_2\text{O}$  or  $\text{NH}_3$  and on crop agronomy (Watson et al., 1990, Harty et al., 2016, Burchill et al., 2017, Forrestal et al., 2017), a more integrated approach quantifying these responses simultaneously is desirable. Additionally, most research studies to date have focused on inhibitors such as NBPT and DCD, while other compounds received little attention. One such compound is a urease inhibitor similar to NBPT, N-(n-propyl) thiophosphoric triamide (NPPT), available in a commercial fertiliser developed by BASF SE, containing 75% NBPT and 25 % NPPT (Limus<sup>®</sup>) at a total rate of 0.03%. To the best of our knowledge, there have been no field experiments with urea + NBPT + NPPT in grasslands to date, with the only available data obtained in arable crop field trials (Li et al., 2015) or in laboratory incubations (Sunderlage & Cook, 2018; Kira et al., 2019). This study addresses the above knowledge gaps by (1) simultaneously quantifying major gaseous N loss pathways- $\text{N}_2\text{O}$  and  $\text{NH}_3$ , and crop yield

quantity and quality, and (2) investigating the effectiveness of a new enhanced efficiency fertiliser, which incorporates two urease inhibitors in its formulation: NBPT and NPPT.

Therefore the objectives of this work are: (1) to assess performance of temperate grassland system fertilised with a selection of N fertiliser formulations, (2) to evaluate effectiveness of using a combination of two urease inhibitors NBPT + NPPT compared to NBPT alone, and (3) to report  $N_2O$  and  $NH_3$  emission factors from enhanced efficiency fertilisers compared to traditional urea and nitrate-based fertilisers.

## 2. Materials and Methods

### 2.1 Experimental design and treatments

The trial commenced on 24<sup>th</sup> January 2017 and lasted for 12 months at the Teagasc Johnstown Castle Research Centre, Co. Wexford. The field site was a moderately drained soil with a sandy loam texture in the top 10 cm (51.7% sand, 33.9% silt, 14.4% clay; pH 5.7; 0.3% N; 2.8% C) classified as Eutric Cambisol (FAO-Unesco, 1988). The pasture sward consisted predominantly of perennial ryegrass (*Lolium perenne* L.) and no clover. The site was last reseeded in 2013. This trial consisted of agronomy plots (10 m x 2 m),  $N_2O$  measurement plots (0.4 m x 0.4 m) and ammonia measurement plots using two methods (Integrated horizontal flux-IHF, and wind tunnel methods). The agronomy and  $N_2O$  trial plots were set up in a randomized block design, with five blocks and four fertiliser treatments: control, calcium ammonium nitrate (CAN), urea (UREA), urea with urease inhibitor N-(n-butyl) thiophosphoric triamide, NBPT, where source of NBPT was Agrotain® (Koch Agronomic Services, Wichita, KS, USA) (urea + NBPT) and urea with Limus® (BASF, Ludwigshafen, Germany), which combines two urease inhibitors NBPT and N-(n-propyl) thiophosphoric triamide (NPPT) (urea + NBPT + NPPT). Treatments were applied to agronomy and  $N_2O$  plots

on the same days in six splits, with 20 kg N ha<sup>-1</sup> applied at first application, 40 kg N ha<sup>-1</sup> at applications second, third and fourth, and 30 kg N ha<sup>-1</sup> at applications fifth and sixth (Table 1). Split fertilisation was used to reflect a common practice to match plant demand and reduce N losses to the environment. Ammonia measurement plots using wind tunnel method (Lockyer, 1984) were situated beside the agronomy and N<sub>2</sub>O plots. These plots for ammonia measurements (2.5 m x 2 m) were arranged in a fully randomized block design with three blocks and the same four treatments as above. These plots received one fertiliser application on 4<sup>th</sup> July at a rate of 40 kg N ha<sup>-1</sup>. Along with the wind tunnel measurements, ammonia was also measured using integrated horizontal flux method. Plots using this method were situated within 500 m from the main trial. These plots were two 25 m diameter circles, which were fertilised with urea on the same day and at the same rate as the wind tunnel method plots. Fertilisation of ammonia and agronomy and N<sub>2</sub>O plots was offset by six days to facilitate intensive N<sub>2</sub>O and ammonia measurements. One fertiliser application date was selected for ammonia measurements to coincide with high ammonia loss conditions from urea representing a 'worst-case scenario'.

Table 1. Details of agronomic management practices including dates of six N fertilisation dates and associated N application rates, and harvest dates following each fertilisation cycle.

N application date	N application rate kg N ha <sup>-1</sup>	Grass harvest date
23/01/2017	20	01/03/2017
06/03/2017	40	13/04/2017
18/04/2017	40	25/05/2017
29/05/2017	40	06/07/2017
10/07/2017	30	07/09/2017
11/09/2017	30	17/10/2017

## 2.2 Environmental and soil conditions

Rainfall and air temperature were recorded at the meteorological station 200 m from the experimental site. Soil volumetric water content was measured on each N<sub>2</sub>O sampling occasion using Theta probe (type ML2, Delta-T Devices). One measurement per block was taken next to a randomly selected N<sub>2</sub>O measurement plots within any given block. Based on the soil volumetric water content and soil dry bulk density, water-filled pore space (WFPS) was calculated according to Maljanen et al. (2007).

### 2.3 Soil sampling and analysis

Measurements of soil mineral N concentration were performed following third fertilisation split in mid-April 2017 to represent early to mid-growing season. Soil samples were collected in triplicate from all treatments on five separate occasions between 12/04/2017 and 17/05/2017. Five soil cores were taken from across the agronomic plot on each sampling occasion (0-10 cm depth). The cores were bulked, placed in a labelled sample bag and kept in cool conditions until analysis. Samples were sieved using a 4 mm sieve and extracted in 2 M KCl (5:1 ratio, shaken for 1 h) within 24 hours of collection (Minet et al., 2018). The extracts were analysed for NH<sub>4</sub><sup>+</sup>-N (Standing Committee of Analysts, 1981) and NO<sub>3</sub><sup>-</sup>-N (Askew, 2012) by colorimetric analysis using an Aquakem 600 discrete analyser (Thermo Electron OY, Finland). Gravimetric moisture content was determined on sub-samples by drying for 24 hours at 105°C to enable the expression of soil mineral N concentrations in dry soil. Mineral N measurements were confined to one fertilisation split due to limited resources personnel available and the labour-intensive nature of the procedure.

### 2.4 Nitrous oxide sampling and analysis



Nitrous oxide fluxes were measured using the closed static chamber technique (de Klein and Harvey, 2012). Following treatment application, N<sub>2</sub>O was sampled daily for the first two weeks, twice a week for the next two weeks, and then once a week for the rest of the fertilisation cycle. Square stainless steel collars inserted to a depth of minimum 5 cm into the soil and 10 cm high lids, both with dimensions of 40 cm × 40 cm, were used for sampling. Collars were covered with a neoprene strip and a 10 kg weight was placed on top of cover compressing the neoprene in order to ensure airtight seal of the headspace at sampling. Chambers were sampled at 0 min, 15 min, 30 min and 45 min after closure. At sampling, 10 mL air sample was removed through a rubber septum (Becton Dickinson, UK) using a 10 mL polypropylene syringe (BD Plastipak, Becton Dickinson, UK) fitted with a hypodermic needle (BD Microlance 3, Becton Dickinson, UK). Air samples were injected into a pre-evacuated (to -1000mbar) 7 mL screw-cap septum glass vials (Labco, UK). Samples were returned to ambient pressure immediately before analysis and introduced into the analyser by a Combi-Pal automatic sampler (CTC Analysis, Switzerland). Nitrous oxide concentrations were analysed using a gas chromatograph (GC) (Varian CP 3800 GC, Varian, USA) fitted with a <sup>63</sup>Ni electron capture detector (ECD) with high purity helium as a carrier gas. Areas under N<sub>2</sub>O peaks were integrated using Star Chromatography Workstation (Varian, USA). Hourly N<sub>2</sub>O emissions were calculated based on the rate of change in N<sub>2</sub>O concentration inside the chamber during the measurement period. Gas sampling was performed between 10 am and 1 pm as this time is considered to be representative of the average hourly flux of the day (Blackmer et al., 1982). Hourly values were used to calculate daily emissions. Cumulative emissions were obtained by integration of daily fluxes and linear interpolation between measurement points (de Klein and Harvey, 2012). Nitrous oxide emission factors were calculated by subtracting cumulative control N<sub>2</sub>O from treatment

$\text{N}_2\text{O}$ , and division by the N fertilisation rate, to lead to a  $\text{N}_2\text{O}$  emission associated with fertilisation unit.

## 2.5 Ammonia sampling and analysis

Ammonia measurements were carried out using two methods: integrated horizontal flux and wind tunnel method. Integrated horizontal flux method was used to obtain reliable values for urea fertiliser that can be integrated into the Irish  $\text{NH}_3$  inventory. This method requires a sizeable land resources and restriction to any farm operations potentially producing  $\text{NH}_3$  in the vicinity of the trial, and therefore it was limited to this particular fertiliser treatment. Wind tunnel method requires less land and allows for testing of more treatments in a replicated manner, therefore this method was employed for investigation of potential  $\text{NH}_3$  loss reductions with changing fertiliser formulation. Including urea treatment in both methods also allowed for comparison between these methodologies.

Measurements employing both methods were conducted simultaneously, in order to produce comparable results. Differences between these two methods are primarily attributed to effects of rainfall and wind conditions (Ryden & Lockyer, 1985). Since rainfall practically did not occur during this experiment, and the wind speed inside and outside the wind tunnels did not vary by over 20%, both methods remained in good agreement.

### 2.5.1 Integrated horizontal flux method

Integrated horizontal flux method using passive air samplers 'shuttles' (Leuning et al., 1985) was used to measure  $\text{NH}_3$  emissions from grassland fertilised with urea. Two replicates of 25 m diameter fertilised circles were established in a line perpendicular to the prevailing wind direction, with a measurement tower placed in the centre of each plot. Samplers were

placed on each tower at 0.2 m, 0.4 m, 0.8 m, 1.2 m, 2.2 m, 3.3 m height. Two background towers were positioned in the field with samplers at 0.2 m, 0.8 m, 2.2 m. Only one background mast was used at any given time depending on the wind direction. Emissions were measured continuously for 14 days following fertilisation. Samplers were changed daily in the first week, and every second day in the second week of measurements.

Samplers were charged with a solution of 3% oxalic acid in acetone and extracted with deionized water according to Leuning et al. (1985). Samples were analysed for  $\text{NH}_4^+$ -N using the same method as for soil  $\text{NH}_4^+$ -N content.

#### 2.5.2 Wind tunnel method

Ammonia volatilisation was also measured using wind tunnels with three replicate tunnels per treatment. The wind tunnel method utilised units which are described by Lockyer (1984), while the tunnel construction and validation is described by Meisinger et al. (2001). In brief the units consisted of (a) a canopy (0.5 m x 2 m) made of polycarbonate sheeting, into which an inlet air sample line was integrated, (b) a galvanized steel duct housing an axial fan, anemometer and an outlet air sample line and (c) a control box housing a diaphragm pump for the air sample lines, a flow meter and a critical orifice for both air sample lines. The air pumped through the inlet and outlet air sample lines passed through two individual conical absorption flasks, which contained 100 mL of 0.02 M orthophosphoric acid ( $\text{H}_3\text{PO}_4$ , 85%; Merck, Darmstadt, Germany), to capture  $\text{NH}_3$ -N in the air (i.e. acid traps). The wind tunnel canopy was placed over the fertilised plot immediately after treatment application. Emissions were measured continuously for a period of 15 days. Absorption flasks were changed daily in the first week, and every second day in the second week of measurements. Samples were analysed for  $\text{NH}_4^+$ -N using the same method as for soil  $\text{NH}_4^+$ -N

content. The rain shielding effect of the wind tunnel canopy in periods of rain was minimized by moving the canopy back and forth within the fertilised plot each time the acid traps were changed.

## 2.6 Yield and N uptake sampling and analysis

Grass was harvested five to six weeks after each fertilisation (Table 1). The length of the period varied to reflect the change in grass growth over the growing period. At each harvest, a 1.5-m-wide strip was cut through the centre of each grass plot to a height of approximately 5 cm using a Haldrup harvester (Haldrup, Løgstør, Denmark). The grass was removed from the plot, fresh weight recorded, and a 200-g subsample collected for dry matter (DM) and for N content analysis. Grass sample was oven dried at 70°C until constant weight to determine moisture content and dry matter of the sample. Grass dry matter yield was calculated using fresh weight from a known sampled area, dry matter content of the sample, and converted to a yield expressed in kg or t ha<sup>-1</sup>. Grass sample was ground and subsampled into tin capsules and N content analysed using a C and N elemental analyzer (Leco Corp., St. Joseph, Michigan, USA). Grass yield and its N content were used to calculate N uptake. Apparent fertiliser recovery was calculated by subtracting N uptake of control from N uptake of a treatment and divided by N application rate.

## 2.7 Statistical analysis

The effect of fertiliser N treatment on the dependent variables cumulative N<sub>2</sub>O emissions, N<sub>2</sub>O emission factors, cumulative NH<sub>3</sub> emissions, dry matter yield, N uptake and apparent fertiliser recovery was tested using the general linear mixed model using the PROC GLIMMIX procedure of SAS 9.4 (2002–2010) (SAS Institute Inc.). Fertiliser formulation was a fixed

effect and block as a random effect. The effect of fertiliser N treatment on yield and N uptake during the growing season was tested using a repeated-measures analysis of variance (ANOVA) using the MIXED procedure of SAS. The factors in the model were fertiliser formulation as fixed effect, harvest as the repeated measure and block as a random effect. The F-protected Least Significant Difference (L.S.D.) test with Tukey-Kramer adjustment for multiple comparisons was used to determine statistically significant differences between treatments at 5 % level of significance. Least significant differences are presented along with the pooled SEM. All data was checked for normality of distribution and log-transformed prior to analysis, if needed.

### 3. Results

#### 3.1 Environmental and soil conditions

The experimental site received 912 mm of rainfall during the 365 days of the experiment (154 days with rain), which is approximately 14% less than the 30 year average (Fig. 1). There were 19 events with the level of rainfall exceeding 10 mm, four of them reaching and even exceeding 30 mm. Air temperature followed a typical pattern for this location throughout the experimental period, with daily averages recorded between 1.0°C and 18.0°C. Mean air temperature throughout the experiment was 10.0°C, similar to the 30 year average. In the first three and the last four months of the experiment soil water-filled pore space (WFPS) was at over 80% continuously. WFPS averaged 74% for the experimental period and ranged between 28% and 111% (Fig.1), with 58 out of total 86 N<sub>2</sub>O measurement days with WFPS above 65% suggesting that conditions were favourable to denitrification (Dobbie & Smith, 2001).

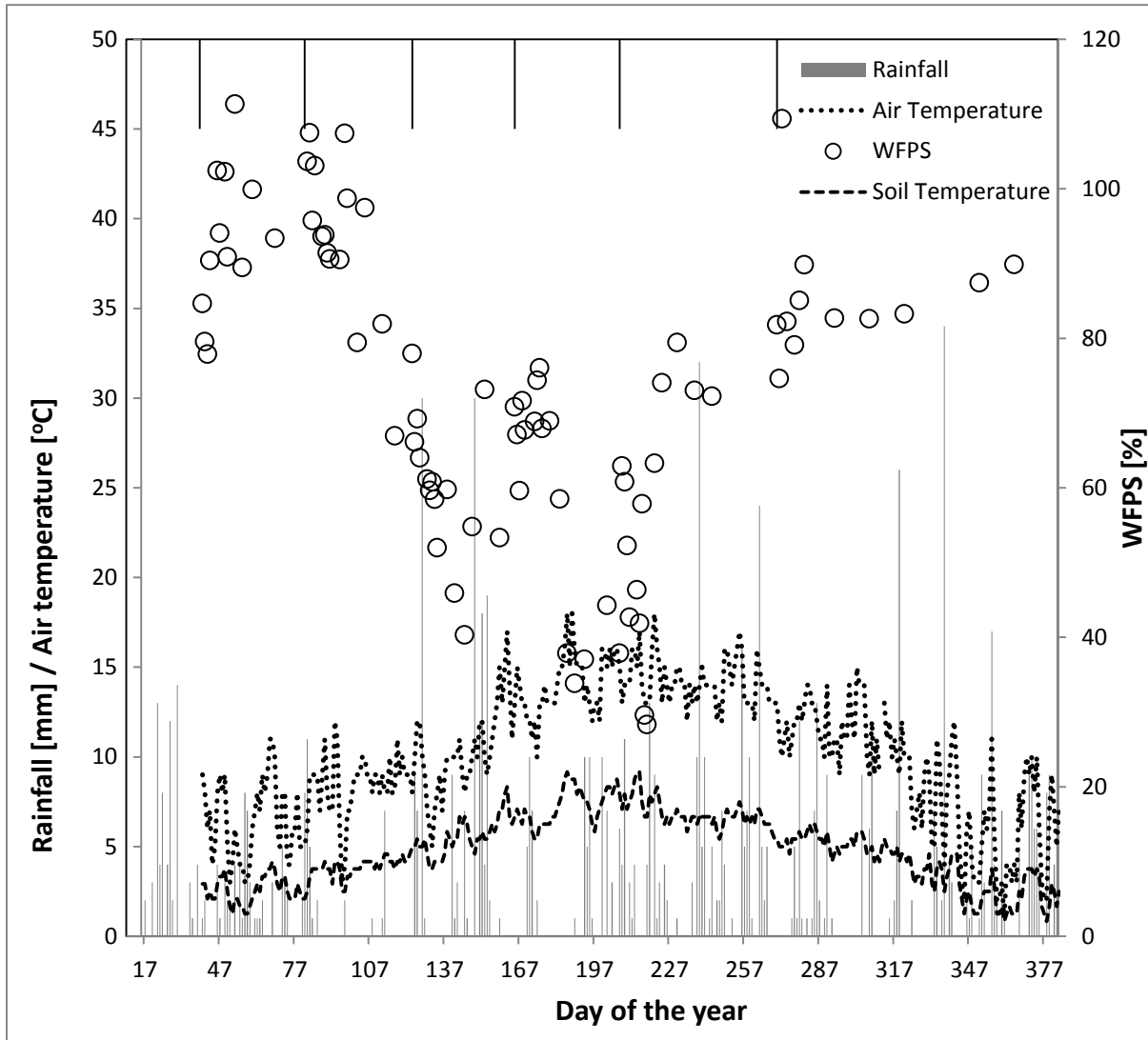


Fig. 1 Air and soil temperature at 5 cm depth, rainfall and water filled pore space (WFPS) over the experimental period. Black vertical lines indicate fertilisation events.

### 3.2 Soil mineral N content

Temporal patterns of soil mineral N: ammonium ( $\text{NH}_4^+\text{-N}$ ) and nitrate ( $\text{NO}_3^-\text{-N}$ )

concentrations in the 0-10 cm soil depth during fertilisation cycle three (12/04/2017 –

17/05/2017) are presented in Fig. 2 a & b. Fertilisation increased  $\text{NH}_4^+\text{-N}$  in all cases, leading

to peak concentration in the urea treatment reaching  $28.8 \text{ kg N ha}^{-1}$  the day following

fertiliser application (Fig. 2 a), however this increase was not seen in the soil  $\text{NO}_3^-\text{-N}$  (Fig. 2

b). The largest increase in soil  $\text{NO}_3^-$ -N was seen in the CAN treatment reaching  $12.1 \text{ kg N ha}^{-1}$ . Mean soil  $\text{NH}_4^+$ -N concentrations throughout the fertiliser cycle varied from  $5.6$  to  $14.1 \text{ kg N ha}^{-1}$  from control and urea treatments respectively, while mean soil  $\text{NO}_3^-$ -N concentrations were between  $1.3$  and  $5.1 \text{ kg N ha}^{-1}$  from control and CAN treatments. In all treatments, mineral N concentrations returned to background levels within two weeks of fertilisation. The urea based fertilisers had soil  $\text{NO}_3^-$ -N below  $12.1 \text{ kg N}$  over the duration of this fertiliser cycle.

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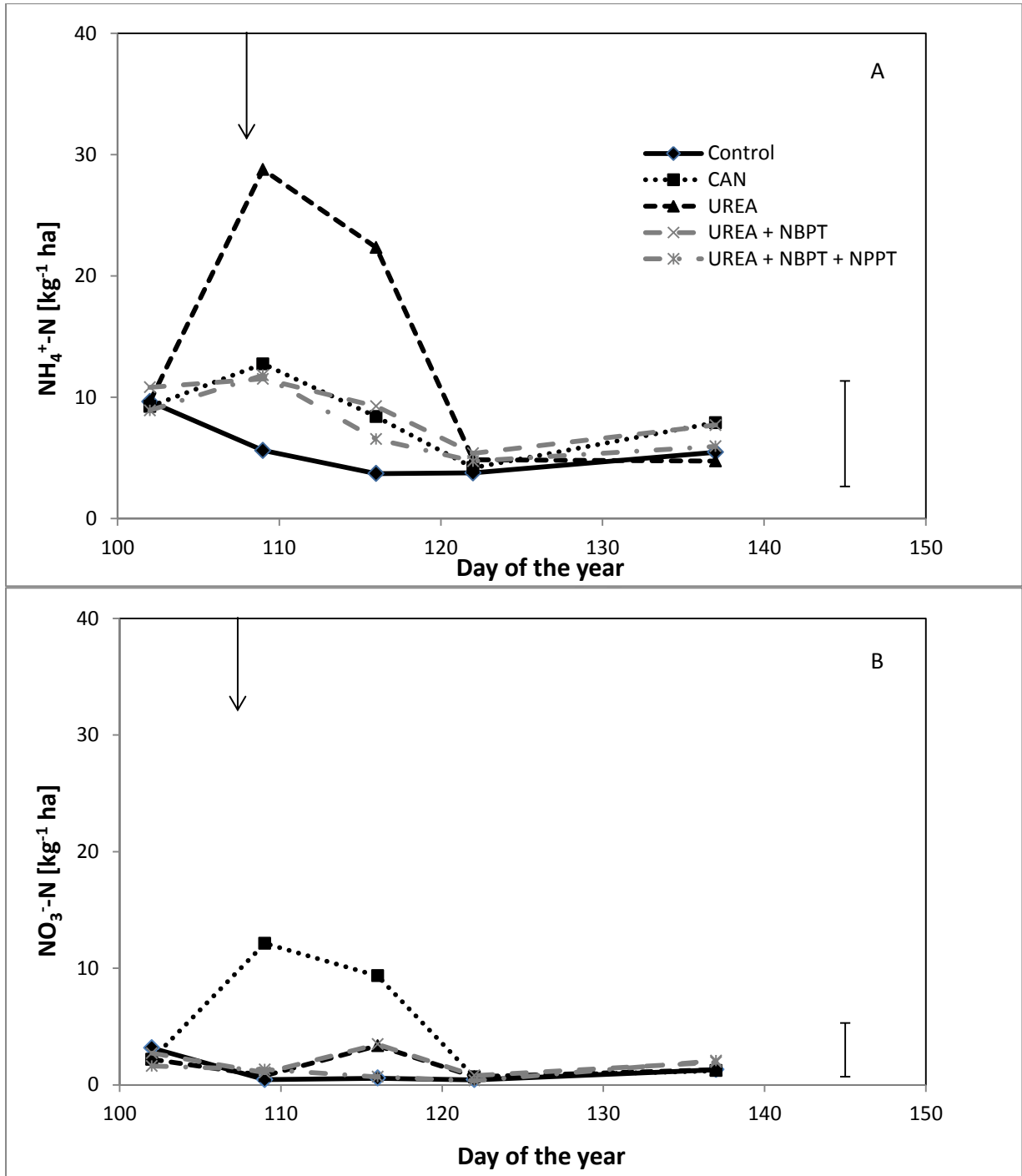


Fig 2 a & b. Mean temporal trace of soil  $\text{NH}_4^+\text{-N}$  (a) and  $\text{NO}_3^-\text{-N}$  (b) in the top 0-10 cm from control and fertilisation treatments during fertilisation cycle three. Vertical arrows indicate fertilisation event. Vertical lines to the right indicate pooled standard error of the mean.

### 3.3 Nitrous oxide emissions



Temporal fluxes of N<sub>2</sub>O during the experimental period are shown in Fig. 3 and N<sub>2</sub>O emission factors (cumulative N<sub>2</sub>O fluxes and N<sub>2</sub>O emission factors- emissions scaled to N fertilisation rate) are shown in Table 2. While N<sub>2</sub>O from control plots ranged between -3.7 and 9.6 g N<sub>2</sub>O-N ha<sup>-1</sup> d<sup>-1</sup>, fertilisation led to large increases in emissions. Small negative fluxes were recorded in unfertilised control plots and were retained in the dataset as suggested in literature (Kim et al. 2013). Increased N<sub>2</sub>O emissions were seen particularly after the first, second, and fourth fertiliser applications, with the largest mean daily N<sub>2</sub>O at 227 g N<sub>2</sub>O-N ha<sup>-1</sup> d<sup>-1</sup> observed from CAN three days post-fertilisation. The majority of N<sub>2</sub>O was emitted within a week after fertilisation. The highest cumulative N<sub>2</sub>O emission was observed from the CAN treatment (1.5 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>), as opposed to approximately 0.5 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup> from all the urea-based fertilisers and 0.3 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup> from the control. Cumulative N<sub>2</sub>O emissions from urea-based fertilisers were not different from the control, whereas CAN emission was significantly higher (P<0.05) than the control and all other fertiliser formulations. Similarly, the highest annual N<sub>2</sub>O EF was found for CAN of 0.58% ± 0.15% was significantly higher than the urea products which were 0.06% ± 0.11%, 0.07% ± 0.07% and 0.09% ± 0.03% from urea + NBPT, straight urea and urea + NBPT + NPPT, respectively (± denoted SE, standard error of the mean). There was no significant difference between the urea fertiliser products (P>0.05).

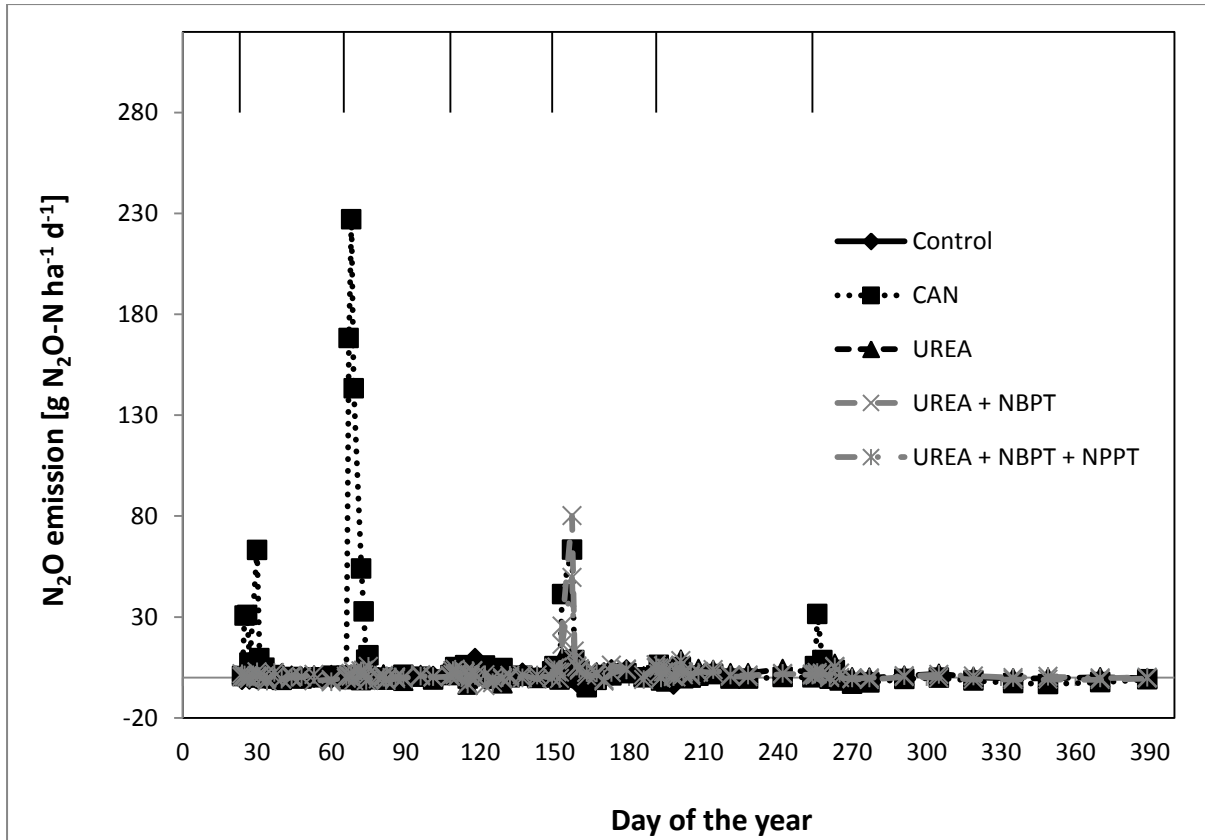


Fig. 3 Mean temporal trace of soil  $\text{N}_2\text{O}$  emission from control and various N fertilisation treatments. Black vertical lines indicate fertilisation events. Following each fertiliser application,  $\text{N}_2\text{O}$  was sampled daily for the first two weeks, twice a week for the next two weeks, and then once a week for the rest of the fertilisation cycle.

Table 2. Cumulative annual  $\text{N}_2\text{O}$  emissions and direct  $\text{N}_2\text{O}$  emission factors.

Fertiliser†	Cumulative $\text{N}_2\text{O}$		$\text{N}_2\text{O}$ emission factor	
	kg $\text{N}_2\text{O-N ha}^{-1} \text{yr}^{-1}$		%	
Control	0.34	b §	-	
CAN	1.50	a	0.58	a
UREA	0.47	b	0.07	b
UREA + NBPT	0.46	b	0.06	b
UREA + NBPT + NPPT	0.51	b	0.09	b

† CAN, calcium ammonium nitrate; NBPT, N-(n-butyl) thiophosphoric triamide; NPPT, N-(n-propyl) thiophosphoric triamide

§ Within columns values with different lowercase letters are significantly different. Mean comparison by F-protected LSD test ( $P < 0.05$ ). Pooled SEM was  $0.21 \text{ kg N}_2\text{O-N ha}^{-1} \text{yr}^{-1}$  and  $0.12\%$  for cumulative  $\text{N}_2\text{O}$  emissions and  $\text{N}_2\text{O}$  emission factors, respectively.

### 3.4 Ammonia emissions from urea fertiliser using integrated horizontal flux method

The overall temporal trend of the mean total  $\text{NH}_3\text{-N}$  loss from the urea fertilisation of grassland is shown in Fig. 4. The maximum rate of  $\text{NH}_3\text{-N}$  loss from urea occurred on the second day after urea application. The mean total  $\text{NH}_3\text{-N}$  loss over the 14 day measurement period ranged from 34 to 59% with a mean of 47%. Approximately half of this loss occurred in the first three days following fertilisation. Weather conditions during two weeks of ammonia measurements were warm and dry. Mean daily temperatures fluctuated between 13 and 16°C and there was only 1mm of rainfall recorded 12 days post-application.

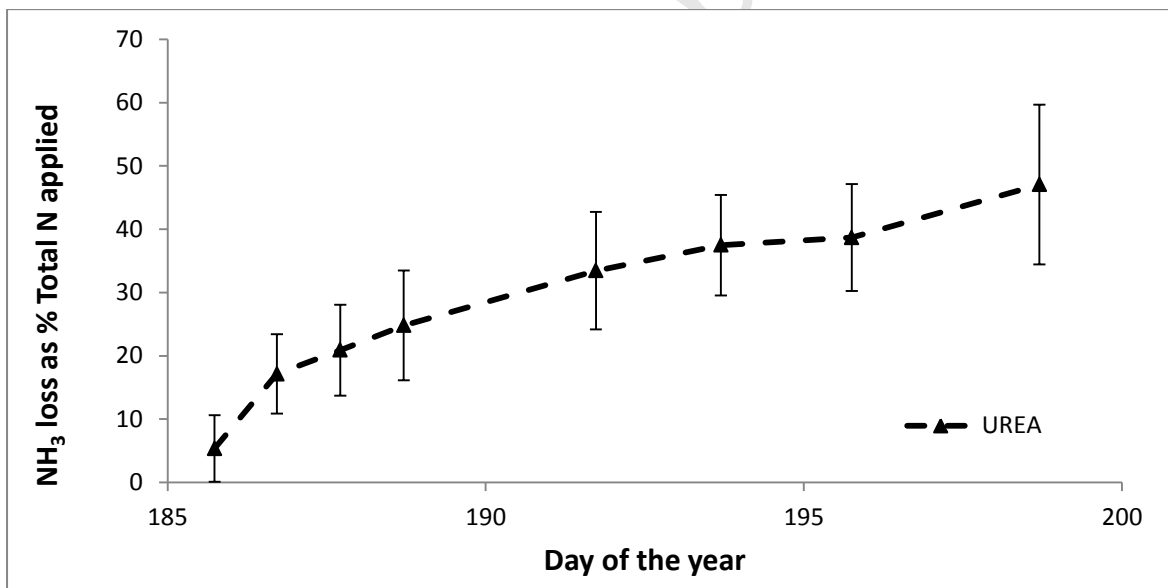


Fig. 4. The effect of fertilisation on cumulative  $\text{NH}_3$  emissions (as a % of total N applied), from urea fertiliser treatment applied to grassland and measured using the IHF method. The vertical bars represent standard error of the mean.

### 3.5 Ammonia emissions from N fertiliser formulations using wind tunnel method

The temporal  $\text{NH}_3\text{-N}$  loss trend, following fertiliser application, is shown in Fig. 5. Similarly to the IHF measurements, the maximum rate of  $\text{NH}_3\text{-N}$  loss from urea occurred within two days of urea application. This two-day period contributed over half of the ammonia loss from urea. The mean total ammonia loss over the same 14 day measurement period was

largest from urea, at 43%, followed by 13.9%, 13.8% and 5.2% from urea + NBPT, urea + NBPT + NPPT and CAN, respectively. Ammonia loss from urea was significantly higher ( $P < 0.05$ ) in comparison with all other fertiliser formulations.

Ammonia measured with the two methods did not differ significantly over the measurement period, with approximately 43 and 47% of N in urea fertiliser lost as  $\text{NH}_3$  using wind tunnel (Fig. 5) and IHF (Fig. 4) methods, respectively. Temporal emission patterns were also comparable between the two methods (repeated measures ANOVA) (Fig. 4 and 5).

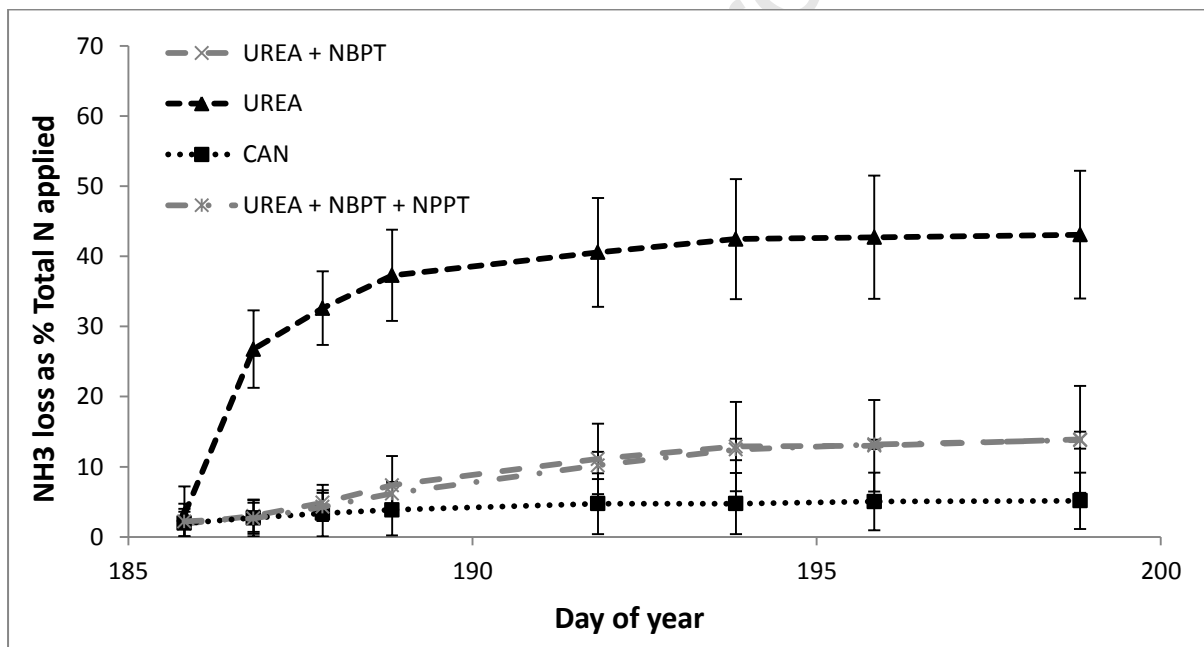


Fig. 5. The effect of fertiliser formulation on cumulative  $\text{NH}_3$  emissions (as a % of total N applied) over a 14 day period post fertiliser application to grassland, measured with the wind tunnel technique. The vertical bars represent standard error of the mean.

### 3.6 Grass dry matter yield

A significant effect of fertilisation treatment on annual grass dry matter yield and N uptake was observed (Table 3). Annual yield and N uptake for the zero N control was significantly lower than for all fertiliser treatments, where yield increased from  $4536 \text{ kg ha}^{-1}$  to  $>10233 \text{ kg ha}^{-1}$  and N uptake increased from  $85 \text{ kg ha}^{-1}$  to  $> 184 \text{ kg ha}^{-1}$  with application of  $200 \text{ kg N}$

ha<sup>-1</sup>. Urea had the lowest yield and N uptake of the fertiliser treatments, significantly lower than urea + NBPT (Table 4) which was reflected in relatively low apparent fertiliser recovery for urea.

The repeated measures ANOVA showed a significant yield and N uptake response to fertiliser N application for all N applications except for the first application, in January, which was 20 kg N ha<sup>-1</sup>. Amongst fertiliser N treatments there was no significant yield or N uptake difference for the first 4 harvests (Table 5). In harvest 5 urea + NBPT significantly out-yielded urea and CAN but not urea + NBPT + NPPT.

Table 3. Analysis of variance to evaluate the effect of fixed effects harvest and fertiliser and their interactions on annual DM yield and N uptake and repeated measures analysis of individual harvests of DM yield and N uptake.

Effect	Annual DM yield, Pr > F	Annual N uptake, Pr > F	Effect	Individual harvest DM yield, Pr > F	Individual harvest N uptake, Pr > F
Fertiliser	***	***	Fertiliser	***	***
			Harvest	***	***
			Fertiliser x Harvest	***	***

\*, \*\*, \*\*\* indicates significant differences at the 0.05, 0.01 and 0.001 probability levels

† No significant difference.

Table 4. The effect of fertiliser type on annual dry matter yield, N uptake and apparent fertiliser recovery.

Fertiliser†	Annual DM yield	Annual N uptake	Apparent fertiliser recovery
	kg ha <sup>-1</sup>		%
Control	4,536 c §	85 c	

CAN	11,066 ab	197 ab	56
UREA	10,233 b	184 b	49
UREA + NBPT	11,733 a	215 a	65
UREA + NBPT + NPPT	11,214 ab	200 ab	57

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† CAN, calcium ammonium nitrate; NBPT, N-(n-butyl) thiophosphoric triamide; NPPT, N-(n-propyl) thiophosphoric triamide

§ Within columns yields with different lowercase letters are significantly different. Mean comparison by F-protected LSD test ( $P < 0.05$ ). Pooled SEM was 324 and 6.45 kg N ha<sup>-1</sup> for annual DM yield and annual N uptake, respectively.

Table 5. The effect of fertiliser type on dry matter yield and N uptake at six individual harvests.

Fertiliser	Harvest 1		Harvest 2		Harvest 3		Harvest 4		Harvest 5		Harvest 6													
	DM yield	N uptake	DM yield	N uptake	DM yield	N uptake	DM yield	N uptake	DM yield	N uptake	DM yield	N uptake												
	kg ha <sup>-1</sup> harvest <sup>-1</sup>																							
Control	330	a	10	a	242	b	4	b	720	b	11	b	1,366	b	21	b	1,620	c	32	b	257	a	6	b
CAN	419	a	14	a	1,017	a	22	a	3,447	a	52	a	2,856	a	39	a	2,606	b	53	a	721	a	17	a
UREA	462	a	15	a	1,012	a	23	a	3,089	a	45	a	2,547	a	34	a	2,502	b	51	a	622	a	16	a
UREA + NBPT	464	a	16	a	1,239	a	27	a	3,243	a	51	a	3,022	a	42	a	3,012	a	61	a	754	a	18	a
UREA + NBPT + NPPT	414	a	14	a	1,160	a	26	a	3,298	a	46	a	2,848	a	39	a	2,736	ab	56	a	758	a	19	a

Within column values with different lowercase letters are significantly different. Mean comparison by F-protected LSD test ( $P < 0.05$ ).

Pooled SEM was 130 and 2.85 kg N ha<sup>-1</sup> for DM yield and N uptake, respectively.

#### 4. Discussion

##### 4.1 The effect of N formulation on direct N<sub>2</sub>O emissions and associated N<sub>2</sub>O emission factors

Nitrous oxide emissions exhibited substantial differences between fertiliser formulations. Four out of six fertilisation events produced N<sub>2</sub>O emissions from CAN fertiliser which were elevated relative to the other fertilisers, while only one fertilisation led to substantially enhanced N<sub>2</sub>O from urea-based fertilisers (Fig. 3). Moreover, nitrate-based formulation had significantly cumulative higher N<sub>2</sub>O emissions and emission factor and exhibited more variable N<sub>2</sub>O emission pattern compared to urea-based formulations. The cumulative emissions were 69 and 66-69 % lower in the urea and urea with urease inhibitor treatments compared to CAN. The emission factor was 88 and 85-90 % lower in the urea and urea + urease compared to CAN. Similar to Bell et al. (2016), high losses from the nitrate-based fertiliser were associated with environmental conditions favourable to denitrification, such as high WFPS resulting from rainfall and mild to warm temperature. In fact, over two-thirds of N<sub>2</sub>O measurements carried out in this study were collected in soil conditions with WFPS above 65%. The N<sub>2</sub>O loss pattern in this experiment was also comparable to findings of Harty et al. (2016), whose work carried out on the same experimental site found the largest N<sub>2</sub>O losses from CAN compared to urea-based N fertiliser formulations with or without urease and / or nitrification inhibitors. Harty et al. (2016) also reported that CAN led to most variable N<sub>2</sub>O responses (coefficient of variation of 61% for three sites over two years of measurements), while urea-based fertilisers produced universally lower N<sub>2</sub>O at all times. The annual N<sub>2</sub>O emissions measured in the current study were lower than other experiments carried out in temperate grasslands (Burchill et al., 2014; Hyde et al., 2016; Harty et al., 2016; Bell et al., 2016). Since the experimental site was under cutting regime



with no additions of animal manure since 2014, it is possible that the levels of soil available carbon were reduced leading to decreasing microbial activity, which in turn can inhibit emissions. Carbon limitation has previously been found in temperate grasslands managed for long term cutting, where carbon offtake in yields was not supplemented by any additions of organic materials (O'Neill et al., 2020). Although the magnitude of N<sub>2</sub>O losses was lower than in Harty et al. (2016), CAN N<sub>2</sub>O EF at 0.58% was comparable to the previously reported EF of 0.65% for this soil type (Harty et al., 2016; N<sub>2</sub>O EF for CAN in site JC2014). Nitrous oxide EF reported here was slightly lower than the value of 0.76% found by Cowan et al. (2019) in temperate grassland in Scotland. The same study also found N<sub>2</sub>O to be substantially lower from urea compared to nitrate-based fertiliser, while urease inhibitor reduced ammonia emissions from urea-based formulation, reducing both gaseous N loss pathways. That fact that Cowan et al. (2019) used ammonium nitrate rather than calcium ammonium nitrate can account for some of the differences in N<sub>2</sub>O. In their review of N<sub>2</sub>O EFs from various fertiliser sources used in grasslands across Ireland and the UK, Cowan et al. (2020) reported EFs for nitrate-based fertilisers to be approximately twice as high as for urea-based formulations, with slight difference between ammonium nitrate and calcium ammonium nitrate. Study of Cowan et al. (2020) analysed 20 years of experimental data and is by far the most comprehensive review of N<sub>2</sub>O EFs in temperate grasslands of North-West Europe clearly indicating disaggregation of N<sub>2</sub>O EFs by fertiliser type.

Addition of urease inhibitors to urea had no significant effect on N<sub>2</sub>O emissions (Table 2) compared to urea, contrary to some previous studies which observed reduced N<sub>2</sub>O emissions by the addition of the NBPT to urea (Tian et al., 2015; Abalos et al., 2012). This lack of observed effect of urease inhibitors could be due to generally low emissions from all urea-based fertilisers over the experimental period, which is similar to the findings of Smith

et al. (2012). Where Smith et al. (2012) found differences in  $N_2O$  between urea and nitrate-based formulations, these were small when adjusted for ammonia volatilisation, indicating importance of this N loss pathway.

Soil pH of the experimental site was 5.7, which is below optimum for productive agricultural grassland (Wall & Plunkett, 2016) and might increased  $N_2O$  emissions through disruption of expression of  $N_2O$  reductase in denitrifying bacteria causing reduction of  $N_2$  production (Stevens et al., 1998; Russenes et al., 2016). Indeed, nitrous oxide emission factors have been found to be negatively correlated with soil pH, increasing in acidic soils (Wang et al., 2018), however this effect does not seem to be clear in this study, where emissions were relatively low.

After fertiliser N application, concentrations of soil mineral N increased, with urea producing highest  $NH_4-N$  and CAN highest  $NO_3-N$  concentrations. Increased soil  $NO_3-N$  concentration coupled with environmental conditions favourable to denitrification could explain high temporal increases in  $N_2O$  emissions from CAN shortly after fertilisation. At the same time, there were no spikes in soil mineral N concentrations in urea + NBPT and urea + NBPT + NPPT treatments, which often give rise to high  $N_2O$  emission. Therefore lower mineral N concentrations in the soil from fertilisers amended with urease inhibitors can serve as an explanation of low  $N_2O$  emissions and additionally point to slower N transformations in the soil, which can benefit plant uptake.

Limitations of this work needs to be acknowledged. With  $N_2O$  emissions varying largely between experimental years (Burchill et al., 2014), it is important to realise limitations of short to medium-length studies in drawing recommendations for changing fertiliser formulation to reduce emissions. However, this experiment adds to the existing body of evidence on the efficacy of urea-based fertilisers in temperate climates, where conditions

are conducive to N<sub>2</sub>O loss from nitrate-based fertilisers through denitrification. Static chamber methodology for gas measurements also has its drawbacks. This method creates a microenvironment and is associated with a number of uncertainties, from sample collection all the way to calculation of cumulative fluxes (Levy et al., 2011). However, static chambers are still widely applied, especially to plot-scale trials, and universally recognised. This study followed static chamber methodology guidelines to minimize uncertainty and ensure high quality of the data (de Klein and Harvey, 2012).

#### 4.2 The effect of N formulation on ammonia emissions

Ammonia is the main gaseous loss of reactive N in fertilised agricultural grasslands (Burchill et al., 2016; Hoekstra, et al. 2020), with magnitude of emissions which can have a significant impact on agronomic performance. Here, even measured at one of the fertilisation events, it also proved to be the major driver of gaseous N loss. Ammonia losses found in this study were large, with between 43 and 47% of the applied urea N lost through this pathway. However, urease inhibitors offered significant reductions in ammonia emissions of approximately 67%. Observed loss values were high compared to 12.7% reported by Burchill et al. (2017) for a nearby Irish grassland, 30% by Chambers & Dampney (2009) for UK grassland, and near the upper limit of the range previously reported by Forrestal et al. (2016) on the same grassland site (20 to 53%), all measured with wind tunnels. Weather conditions had large implications on the magnitude of emissions, with combination of temperature and moisture leading to high fluxes. Mean daily air temperature during the two week measurement period in July 2017 ranged from 13 to 16°C with an average of 15°C, while the only rainfall recorded during this period was 1mm 12 days following fertilisation. Contrary to the expectation that dry ground conditions would

impede dissolution of fertiliser granules and infiltration of fertiliser N solution into the soil, largest ammonia emissions from urea occurred on the second day after fertilisation, indicating rapid hydrolysis, similar to Watson and Miller (1996) and Forrestal et al. (2016). Since urea granules absorb moisture, dew present in the evenings and mornings was sufficient to initiate the hydrolysis process, leaving the granules exposed to large volatilisation losses.

At the initial soil pH of 5.7 and an average temperature of 15°C, approximately 20% of  $\text{NH}_4^+$  from urea hydrolysis would volatilize as  $\text{NH}_3$  (Barnabe, 1990; Smith et al., 1997). However, hydrolysis leads to a localised increase in soil pH (Selbie et al., 2015). An estimated increase to approximately pH 8.5 would shift the chemical balance between  $\text{NH}_4^+$  and  $\text{NH}_3$  towards  $\text{NH}_3$ , resulting in volatilisation at levels recorded in this study (Barnabe, 1990; Smith et al., 1997). However, inclusion of urease inhibitors significantly reduced  $\text{NH}_3$  losses from 43% to below 14% from urea + NBPT and urea + NBPT + NPPT. This amounts to a 68% reduction in  $\text{NH}_3$  compared to an average reduction of 78.5% reported by Forrestal et al. (2016), 77% by Chambers and Dampney (2009) and approximately 90% by Cowan et al. (2019), albeit at lower  $\text{NH}_3$  loss levels. The reduction in  $\text{NH}_3$  also represents savings in indirect  $\text{N}_2\text{O}$  associated with emissions of volatilised and re-deposited N. Performance of urea with one or a combination of two urease inhibitors was not significantly different, which is unlike findings of Pasda et al. (2016), where the combination of NBPT + NPPT outperformed NBPT in experiments carried out in cropland soils. At the same time, mean ammonia loss from CAN was 5.2% which was comparable with 4.2% found by Forrestal et al. (2016), but larger than 0.8% reported by Black et al. (1985). Half of N in this formulation was already in a nitrate form, which naturally limited potential volatilisation. Additionally, such moderate level of emissions could also partially be explained by the small initial rise of pH localised

around the fertiliser granule caused by the rapid dissolution of calcium carbonate in CAN (Black et al., 1985). Ammonia emissions were measured using two different methods due to practical considerations. Micrometeorological method (IHF) was used to produce absolute  $\text{NH}_3$  loss values from urea fertiliser, the goal was to test emissions under conditions conducive to high loss to assess 'worst case scenario', and provide an answer to the pertinent question of the magnitude of possible losses following urea application.

#### 4.3 The effect of N formulation on yield and N uptake

A significant yield and N uptake response to fertiliser N indicated that the site was responsive to N application with yields more than doubled by addition of fertiliser N. Similar levels of response, though with higher control yields, have been reported in previous temperate grassland N response trials (Harty et al., 2017; Forrestal et al., 2016). Addition of the urease inhibitor NBPT to urea significantly increased yields and N uptake compared with standard urea, which is in line with the findings of previous studies (Chambers and Dampney, 2009; Forrestal et al., 2016), that NBPT reduces ammonia loss from urea and therefore can boost yield through better N use efficiency. However, impact on yield is not always detectable, with Carswell et al. (2019) finding no differences in yield quantity and quality between fertiliser formulations. Here, urea + NBPT + NPPT and CAN, which were also shown to have low ammonia loss relative to urea, were not significantly different to urea + NBPT in terms of annual yield or N uptake.

In the present study the zero N control took up  $85 \text{ kg N ha}^{-1}$  of mineralised soil N, which was lower than the 107 to  $184 \text{ kg N ha}^{-1}$  reported by Harty et al. (2017), but was consistent with reports by Herlihy (1979) and McDonald et al. (2014), also highlighting that N mineralisation varies across seasons and soils. To some extent, lower mineralisation N rate could have

been caused by a possible carbon limitation of the study site, similar to that found in O'Neill et al. (2020). The finding that urea + NBPT + NPPT did not significantly increase yield compared to urea, but that NBPT+NPPT positively impacted on N recovery efficiency due to decreased ammonia loss is consistent with the report of Li et al. (2015) in wheat studies in China. Penultimate harvest which occurred in early autumn (harvest 5) was the only harvest during this study, in which a significant yield difference was detected between fertiliser types, however this did not translate into N uptake on this occasion.

## 5. Conclusions

Effects of enhanced-efficiency nitrogen fertilisers on  $N_2O$  and  $NH_3$  losses from agricultural production systems have been a focus of much research in recent years. This study takes a system-wide approach quantifying both agronomic responses and gaseous N losses to evaluate the efficacy of urea-based fertiliser formulations with urease inhibitors. Urea-based fertilisers significantly lowered  $N_2O$  emissions by 66-69% and associated EFs by 85-90% in comparison to CAN, most likely as a result of lower levels of soil nitrate following fertilisation events. In fact,  $N_2O$  from urea-based fertilisers were not significantly different to those of control. Ammonia loss from urea fertiliser was high at over 40% in conditions favourable to volatilisation, however addition of the urease inhibitors into the fertiliser formulation significantly reduced  $NH_3$  by approximately 67%. Importantly, both yield and N uptake were observed to be the same for nitrate and urea-with urease inhibitor fertiliser formulations. This is most likely due to lower  $NH_3$  losses, which due to their magnitude can affect agronomic response. Therefore, the overall impact on agricultural production in this temperate grassland study suggests inclusion of urease inhibitors is important option for optimising yield while reducing  $N_2O$  and  $NH_3$  emissions.

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## Competing Interest Statement

BASF had no decision on the publishing of this data nor did they influence the results, the analysis or the interpretation of the results. The company viewed a copy of this manuscript before submission.

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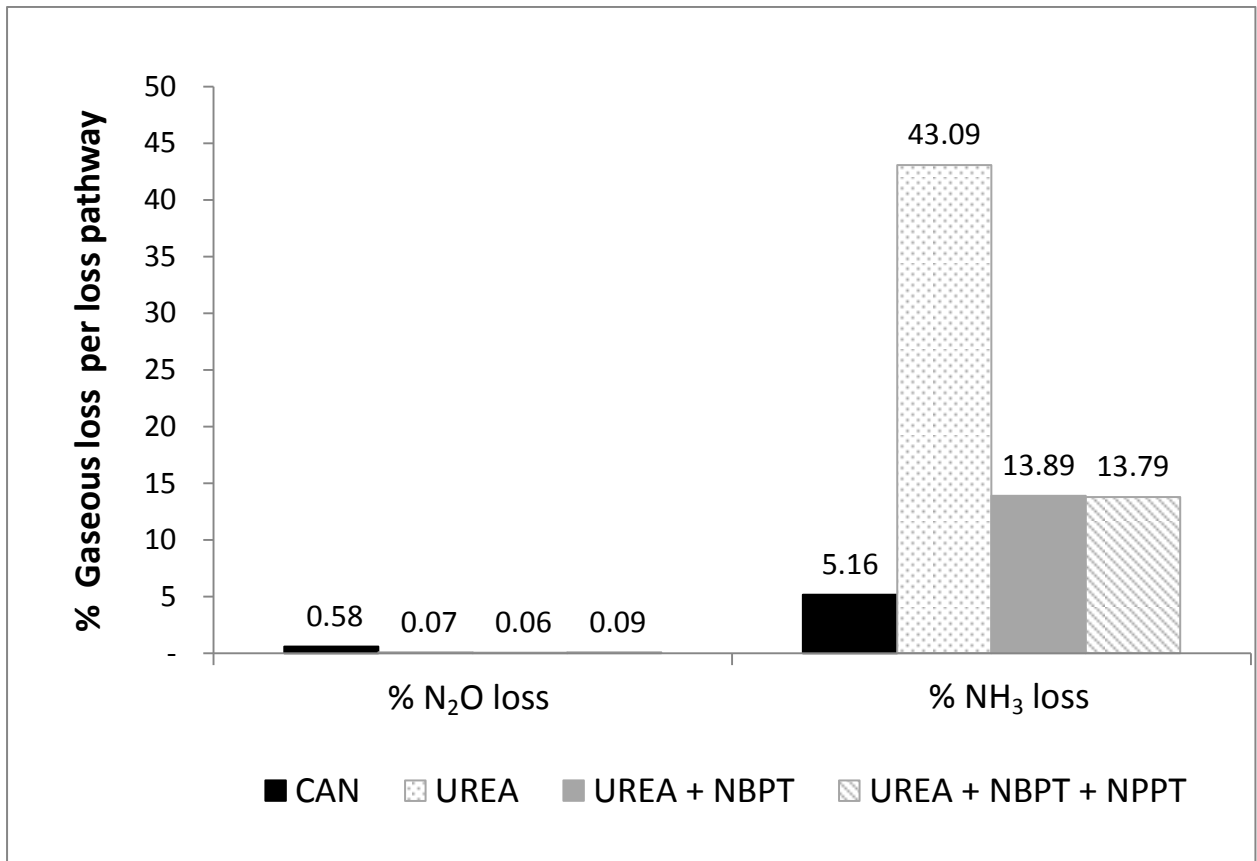
**Declaration of interests**

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.

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## Graphical abstract



## Highlights

- N fertilisation is a large source of  $\text{N}_2\text{O}$  and  $\text{NH}_3$  in agriculture
- Fertiliser formulations largely impact gaseous N losses in agricultural systems
- Urea with urease inhibitors reduced  $\text{N}_2\text{O}$  to level comparable to unfertilised control
- Addition of urease inhibitors reduced  $\text{NH}_3$  by nearly 70%
- Fertiliser formulation had no effect on yield quantity and quality

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