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# Nitrogen fertiliser interactions with urine deposit affect nitrous oxide emissions from grazed grasslands

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## ABSTRACT

Cattle excreta deposited on grazed pastures are responsible for one fifth of the global anthropogenic nitrous oxide ($N_2O$) emissions. One of the key nitrogen (N) sources is urine deposited from grazing animals, which contributes to very large N loadings within small areas. The main objective of this plot study was to establish whether the application of N fertiliser and urine deposit from dairy cows synergistically interacts and thereby increases $N_2O$ emissions, and how such interaction is influenced by the timing of application. The combined application of fertiliser (calcium ammonium nitrate) and urine significantly increased the cumulative $N_2O$ emissions as well as the $N_2O$ emission factor (EF) from 0.35 to 0.74 % in spring and from 0.26 to 0.52 % in summer. By contrast, EFs were lower when only fertiliser (0.31 % in spring, 0.07 % in summer) or urine was applied (0.33 % in spring, 0.28 % in summer). In autumn, $N_2O$ emissions were larger than in other seasons and the emissions from the combined application were not statistically different to those from either the separately applied urine or N fertiliser (EF ranging from 0.72 to 0.83, p-value < 0.05). The absence of significant synergistic effect could be explained by weather conditions, particularly rainfall during the three days prior to and after application in autumn. This study

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implies that the interactive effects of N fertilisation and urine deposit, as well as the timing of the application on $N_2O$ emission need to be taken into account in greenhouse gas emission inventories.

Keys words: Calcium ammonium nitrate fertiliser, emission factors, urine, dairy cattle, yield

## 1.1 INTRODUCTION

Globally, livestock currently accounts for about 14.5 % of the world's total greenhouse gas (GHG) emissions, with bovine beef and dairy cattle production contributing about 41 % and 20 % of the sector's emissions, respectively (Rojas-Downing et al., 2017). Most of GHG emissions from bovine beef and dairy systems arise from (i) enteric fermentation in the guts of the ruminants, leading to methane ($CH_4$) emissions (Moraes et al., 2014) and (ii) nitrification and denitrification processes associated with animal excreta, manure and slurry spreading, resulting in nitrous oxide ($N_2O$) emissions (Butterbach-Bahl et al., 2013). Cattle excreta deposited on grazed pastures are estimated to be responsible for one fifth of the global anthropogenic $N_2O$ emissions (Jacobs et al., 2015). $N_2O$ is a particularly potent GHG and plays a role in stratospheric ozone depletion (Ravishankara et al., 2009). The mitigation of $N_2O$ and reactive nitrogen (N) emissions in general, are crucial challenges facing the agricultural sector due to their consequences for the climate, environment, productivity and soil fertility (Paustian et al., 2006). In Ireland, grassland-based livestock agriculture is considered as the main source of $N_2O$, with less than 24.4 % of the N applied utilised by grass (Lynch et al., 2019). This is primarily due to low N use efficiency, where livestock such as dairy cows return 75 % to 95 % of the N intake to the grassland as excreta (Van Middelaar et al., 2013).

The N content of excreta and in particular urine deposits exceeds the potential of the soil and the vegetation to assimilate it. This excess N is leached to the lower soil horizons, ground and freshwaters as nitrate and dissolved organic N, and released to the atmosphere as $N_2O$ (Chadwick et al., 2018; Saggar et al., 2015; Van Der Weerden et al., 2017b), nitric oxide and ammonia (Cai and Akiyama, 2016). Soil $N_2O$ emissions can occur from nitrification of ammonium ($NH_4^+$) to nitrate ($NO_3^-$) following hydrolysis of urea and denitrification of $NO_3^-$ to $N_2O$ (Harty et al., 2016). The principal environmental drivers of $N_2O$ emissions include soil moisture content, oxygen availability inside soil pores, soil pH, soil temperature and nutrients availability (Butterbach-Bahl et al., 2013; Giltrap et al., 2014).

The rate of urine N deposited by dairy cows can vary from 200 to 2000 kg N $ha^{-1}$ depending on the sward protein content, water content, type of breed, herd variability, age and lactation stage (Haynes and Williams, 1993; Selbie et al., 2015). Each urination event has an approximate volume of 1.5 to 2.5 l, occurs 10-12 times per day and covers an average surface area of 0.25 $m^2$ (in the range of 0.16-0.50 $m^2$) (Selbie et al., 2015; Shepherd and Carlson, 2018; Williams and Haynes, 1994). $N_2O$ emissions from urine deposits are highly variable and can result in large temporal and spatial uncertainties at plot, field, regional or national scales (Fitton et al., 2014; Milne et al., 2014; Misselbrook et al., 2011). The resulting heterogeneous distribution of the N input makes the measurements and estimations of the emissions at the field scale particularly challenging. New technologies (e.g. remote sensing, Lidar sensor) have been used to map the areas of excreta depositions that can be used to develop better estimation of the emissions (Maire et al., 2018; Roten et al., 2017).

To standardise the reporting of GHG emissions the IPCC have developed a method based on emission factors (EF), using a tiered approach. Using the Tier 1 approach (which directly estimates $N_2O$ emissions with a single value multiplied by the amount of N applied to the field), $EF_1$ refers to the percentage of N lost as $N_2O$ emissions per kg of N applied in the form of synthetic N ($EF_{1SN}$) or organic manure ($EF_{1ON}$). These EFs multiplier are set to a default value of 1 %. $EF_{3PRP}$ refers to the $N_2O$ emission produced per kg of N from animal excreta applied directly to pasture, which is set by default at 2 % (Paustian et al., 2006). In dairy systems, approximately 14-30 % of the total grazed area is potentially covered by excreta (Dennis et al., 2011; Selbie et al., 2015), but it is common practice to apply mineral fertilizer shortly after grazing, which can accumulate over deposited excreta. Consequently, a part of the mineral N applied as fertiliser is adding to the already excessive pool of urinary N in the soil and can enhance N losses. In terms of inventory reporting, emissions associated with these N applications will be additive and constant irrespective of the timing of application. There have been few studies investigating consequences of the interaction between the excreta deposit and the fertiliser applied on the $N_2O$ emissions or seasonal variability of the emissions (Anger et al., 2003; Buckthought et al., 2015a; Krol et al., 2017). Krol et al. (2016) studied the seasonal differences of EFs of urine and dung deposit applied separately and found that emissions from urine deposit were significantly higher in autumn than in other seasons. Currently, more data is needed to assess the interactive effect of N fertiliser applied to excreta deposits on $N_2O$ emissions. The understanding of this interaction is key to improve the reporting and definition of effective $N_2O$ mitigation measures.

Firstly, this study aimed to constrain the uncertainty associated with emissions of $N_2O$ following urine deposition, to improve understanding of how urine interacts with fertiliser in intensively managed dairy grassland and affects $N_2O$ emission rates at different times of the year. Secondly, this study aimed to disentangle the urine N loading effect from soil and climate effects on $N_2O$ emissions. It was hypothesised that (1) fertiliser application on a urine deposit would enhance $N_2O$ emissions with the response varying between seasons, and (2) the causes of this difference in emission rates would be mainly due to the amount and forms of N and C available under urine patch and controlled by climatic conditions and grazing practices.

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# 1.2 MATERIALS AND METHODS

## 1.2.1 EXPERIMENTAL DESIGN AND SITE DESCRIPTION

The study was designed to measure the $N_2O$ emissions from fertiliser, dairy cattle urine and the combination of both on a typical intensively managed grassland in Ireland. Work was undertaken between March and November 2017 on a clay loam soil site at the Teagasc, Johnstown Castle Research Centre, Co. Wexford, Ireland (52°18′N, 6°30′W). The experiment was conducted on established perennial ryegrass (*Lolium perenne*) dominated grassland. Livestock was excluded from grazing areas in October 2016 prior to the start of any experimentation to minimize any direct effect of the previous deposition of excreta. The experiment had three different sub-trial areas dedicated to each season. Each seasonal experiment was deployed in a randomized block of five replicate blocks of four treatments (Figure 1): i) control without N application (Control), ii) calcium ammonium nitrate fertiliser (CAN, containing 27 % N), iii) urine (U), and iv) a combination of urine and CAN fertiliser (CANU). Each trial area had designated areas for $N_2O$ sampling and additional area for grass and soil sampling throughout the experiment (Figure 1.b). Applications were made in spring (27/04/2017), summer (03/07/2017) and autumn (02/10/2017) to simulate urine deposit in the early, mid and late grazing seasons. The winter season was not included as the Nitrates Directive bans the application of inorganic N fertiliser after 15$^{th}$ September and this season is often associated with low $N_2O$ emission rates. The CAN+U treatment, which represents an addition of the effect of the urine (U) and the fertiliser (CAN) treatments applied separately, was calculated as the sum of $N_2O$ emissions from U and CAN treatments within each block. In that way it was possible to compare the CANU treatment and the composite CAN+U treatment to evaluate the interaction effect between urine and fertiliser. Urine was collected for each season from the research farm of Teagasc Johnstown Castle, Ireland. The homogenized urine was stored at 5°C prior to analysis and application. Representative sub-samples of urine were analysed for total N and carbon contents, $NH_4^+$, Total Organic Carbon (TOC), Total Oxidized Nitrogen (TON, $NO_2^-$ + $NO_3^-$) and urea-N (Table 1). The N content of the urine varied depending on the season of the collection resulting in N loading ranging from 573 to 671 kg N ha$^{-1}$ (Table 1). The grass was mechanically cut over the whole experimental area before each season trial set-up. The urine was removed from cold storage prior to application to leave enough time to attain ambient temperature. A volume of 1.5 L of urine was applied to the surface of the soil within each chamber. Urine treatments were applied to an area of 0.4 m × 0.4 m within a chamber frame to limit runoff outside of the chamber through soil pores. To facilitate infiltration, urine was applied using a watering can, which is in compliance with the work of Forrestal et al. (2016). To match fertilisation rate with surrounding grazed areas, the CAN application rates varied depending on the season, with 62 kg of N ha$^{-1}$ in spring, 108 kg of N ha$^{-1}$ in summer and 30 kg of N ha$^{-1}$ in autumn. Fertilisers were applied by hand. The rate of fertiliser application can be compared with typical intensively managed grassland.

### 1.2.2 SOIL AND CROP ANALYSES

Soil cores were sampled on a weekly basis and on the day of application in a randomized block design sampling area adjacent to the chambers receiving the same treatment as the static chambers (Figure 1). The cores were sampled from the 0-7 cm depth and then mixed, homogenized and analysed in the laboratory within 24 h. The soil N and C species concentrations (e.g. $NH_4^+$, $NO_2^-$, $NO_3$, total N, total organic C,) were analysed using 20 g of fresh soil sieved at 2 mm, extracted with 100 mL of KCl (1 M) and determined using an Aquakem 600 discrete analyser (Rigas Labs S.A). The KCl soil extracts were stored for less than 48 h at 5 °C before analysis. The gravimetric water content was determined by ovendrying soil samples at 105 °C for 24 h. Another fresh soil subsample was used to measure soil pH after the sample has being dried at 40 °C for 4 days and being rewetted. Bulk density was measured at the start of the experiment using 300 $cm^3$ bulk density rings (7 cm deep, 3.7 cm diameter) and dried at 105 °C until constant weight was reached. The grass was harvested at the end of each sampling period and used to measure the above-ground biomass, the total C and N content by elemental analysis with TruSpec Micro following drying at 70 °C for 4 days and grinding (LECO Corp., St. Joseph, MI, USA).

### 1.2.3 WEATHER DATA

Daily rainfall, soil moisture deficit (SMD) and hourly air and soil temperature were recorded at Johnstown Castle weather station (within 100 m of plots) during the experimental period (Figure 2). SMD is the quantity of rain necessary to bring the soil moisture content back to field capacity (Schulte et al., 2005). Additionally during each day of gas sampling, a frequency domain dielectric sensor Delta T WET-2 probe (Delta-T Devices, Burwell, Cambridge, UK) was used inside each static chamber to measure temperature (T, °C), bulk electrical conductivity (σ, dS $m^{-1}$) and permittivity (Ɛ), simultaneously with a 3 % accuracy.

### 1.2.4 $N_2O$ FLUX MEASUREMENTS

All $N_2O$ emission measurements were made by the closed static chamber method (De Klein and Harvey, 2015), which allows for the measurement of the accumulation of gas traces within a sealed chamber of a known volume, inserted into the soil to form an airtight seal. The chambers consisted of a 0.4 m by 0.4 m square stainless collars inserted into the soil at 5-10 cm depth at least two weeks prior to sampling, and a cover of the same dimensions (Figure 1). Chamber covers were 10 cm high which created an approximately 20-22 L headspace. Chambers were sampled one hour after treatment application then daily for the first week, every second day for the next two weeks, and every third day for the remaining experimental period of minimum 40 days. At 0, 15, 30 and 45 min from chamber closure, a 10 mL air sample was removed through a septum using a 20 mL polypropylene syringe fitted with a needle. Each sample was injected into a pre-evacuated 7 mL screw-cap septum glass vial. The gas concentration of each vial was measured in the laboratory using a gas chromatograph (GC, Varian CP 3800 GC, Varian, USA) fitted with an electron capture detector. For each sequence of gas samples from a chamber, the flux was calculated following Equation 1.

$$\mathbf{Flux\ (nmol\ m^{-2}\ s^{-1}) = dC/dt_0 * \rho V/A} \qquad \mathbf{(1)}$$

Where Flux is the gas flux from the soil, $dC/dt_0$ is the initial rate of change in concentration in nmol mol$^{-1}$ s$^{-1}$ calculated using linear or non-linear asymptotic regression methods, ρ is the density of air in mol m$^{-3}$, V is the volume of the chamber in m$^3$ and A is the ground area enclosed by the chamber in m$^2$. The choice between linear and non-linear asymptotic regression and the calculation of $dC/dt_0$ was made using RCflux package version 4.0 (Levy et al., 2011) available as an add-on package for the R software (R Development Core Team, 2019). The fluxes were calculated either using a linear regression approach or an HMR procedure based on a non-linear model proposed by Hutchinson and Mosier (1981).

### 1.2.5 DATA ANALYSIS AND STATISTICS

Data analysis was performed using R software. Hourly fluxes were assumed to be representative of the whole day emissions and were used to calculate daily emissions (De Klein and Harvey, 2015). To estimate the total $N_2O$ produced from the different treatments, cumulative fluxes were calculated by linear interpolation between the daily fluxes estimated on each sampling occasion. For the linear interpolation, chambers emissions were treated separately and uncertainty was calculated as a sum of the standard deviation of each measured replicate following a conventionally used methodology (Jones et al., 2016; Krol et al., 2016; Skiba and Smith, 2000). From the cumulative fluxes, $N_2O$ emissions factors (EFs)

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for each treatment and each season were calculated following Equation 2. EFs represent the % of N content of each treatment were emitted as $N_2O$-N.

$$\mathbf{EF = ([N_2O_{treatments} - N_2O_{Control}]/\ N\ applied) * 100} \quad (2)$$

Where $N_2O_{treatments}$ and $N_2O_{Control}$ are the cumulative mean emissions in kg $N_2O$-N $ha^{-1}$ $yr^{-1}$ for the five replicate treatment plots and the control plot respectively, and N applied is the treatment N content in kg of N $ha^{-1}$ $yr^{-1}$. The EFs were calculated on a 40 days period after application to ensure the comparability of the treatments between seasons (Skiba et al., 2013). For the composite emissions of fertiliser and urine called "CAN+U" treatments, the EF was estimated using Equation 3 where the cumulative emissions from the control treatment were subtracted from the sum of the emissions from urine ($N_2O_{Urine}$) and fertiliser ($N_2O_{CAN}$) treatments over the total N loading applied (Snell et al., 2014).

$$\mathbf{EF_{CAN+U} = ([N_2O_{CAN} + N_2O_{Urine} - N_2O_{Control}] / [N\ applied_{CAN} + N\ applied_{Urine}]) * 100} \quad (3)$$

To compare emissions between treatments while accounting for the bias caused by the difference in grass production per season, yield-scaled EFs were calculated by dividing the EF per total dry matter yield per season. The yield-scaled EFs represent the percentage of N lost per tonne of dry matter produced per hectare. To compare treatment and season effects, non-parametric statistics were applied because the data were not meeting the classical linearity assumptions, even when using common log-normal data transformation approaches. $N_2O$ emissions, in particular, are well-known to be highly variable, making the statistical difference between treatments difficult to assess. Statistical analyses were performed separately for seasonal effect and treatment effect. The significance was estimated using Kruskal-Wallis test from the agricolae package of the R software to test for differences in $N_2O$ emissions or EFs depending on treatment. A post hoc test using the Fisher's least significant difference was applied to test for significant differences between pairs of treatments. The significance threshold of all statistical tests performed was set at 0.05. The interaction between the treatment and season effects on the emissions was assessed using the aligned rank transform analysis of variance (Leys and Schumann, 2010). This method is an alternative non-parametric method to linear ANOVAs with the advantage of having a greater robustness than the parametric test when the assumption of normality is violated. This test was performed using the R package ARTool (Wobbrock et al., 2011). Drivers of $N_2O$ emissions were assessed using the method described by Krol et al. (2016), which is based on a stepwise multiple regression analysis performed in SAS (SAS Institute Inc., Cary, NC, USA). The potential drivers measured in the field were fitted as polynomial variables following the method described by Krol et al. (2016) and Minet et al. (2018). The robustness of the model was assessed by Akaike Information Criterion (AIC) and the assumptions of the analysis were checked. The model calculated correlations between $N_2O$ EFs and the influence of weather conditions at 3, 5, 7 and 10 days prior and post application as well as on the day of application. The data collected in this study were added to the datasets presented in Krol et al. (2016) and Minet et al. (2018) with a total of 80 observations applied in spring, summer or autumn (15 observations from the present study, 55 from Krol et al. (2016) and 10 from Minet et al. (2018)). Statistical analysis was performed on the urine treatment, the common treatment of the three studies, to investigate the drivers of the emissions in the case of urine deposit.

# 1.3 RESULTS

### 1.3.1 $N_2O$ EMISSIONS FOLLOWING URINE APPLICATION

While the control plots emitted approximately 80-150 g $N_2O$-N $ha^{-1}$ during the 40 days of measurement (cumulative emissions), treatments receiving N additions resulted in an immediate large increase in $N_2O$ emissions. The treatments receiving urine (i.e. U, CANU and CAN+U treatments) resulted in a major peak of $N_2O$ emissions on the first day of application in spring and summer, following an increase in soil $NH_4^+$ (Table 2). The temporal distribution of $N_2O$ emissions followed a commonly reported episodic pattern; however, the magnitude of the 'spikes' depended on the treatment and the season of application. For the CANU treatment, the maximum daily $N_2O$ emissions were measured in summer on the day of application with emissions of 1636 g $N_2O$-N $ha^{-1}$ $day^{-1}$ (Figure 2). For the CAN treatment, the highest daily emission was recorded in spring 18 days after application with 44 g $N_2O$-N $ha^{-1}$ $day^{-1}$. For the U treatment, the highest emissions were recorded 11 days after application with daily emissions of 390 g $N_2O$-N $ha^{-1}$ $day^{-1}$. In spring, a second peak of emissions was measured 16 days after application for the treatments containing urine (U and CANU) with a maximum of 480 g $N_2O$-N $ha^{-1}$ $day^{-1}$ for CANU and coincided with a rainfall event of 9.8 mm. The same pattern was observed in autumn, where the highest fluxes were observed for urine treated plots after a heavy rainfall event (12.4 mm), 11 days after application, and in summer, 9 days after application following a rainfall event of 9.4 mm (Figure 2).

### 1.3.2 TREATMENT EFFECTS ON CUMULATIVE $N_2O$ EMISSIONS

Cumulative $N_2O$ emissions were significantly lower for CAN than for U, CANU and CAN+U treatments. The treatment effect of U, CANU and CAN+U differed between the three seasons (Figure 3). In spring and summer, emissions from U and CANU treatments were significantly different, with approximately twice as much $N_2O$ emitted from the treatment containing urine during these periods compared to the autumn application. As expected, the control treatment with no N input emitted a low quantity of $N_2O$ over the experimental period. Emissions from the different treatments followed the same pattern in spring and summer with low $N_2O$ emissions from CAN and significantly higher emissions from the fertiliser applied with urine, compared to urine alone (Figure 3). Unexpectedly, the CAN treatment emitted low emissions through the year; they were not significantly different from the control in summer. The N loading applied to the treatment plots was different between seasons due to the difference of urine N content and fertiliser rates (Table 1). EFs were calculated to remove the bias stemming from the different N loading rates.

### 1.3.3 SEASONALITY OF TREATMENT EFFECTS

Studying seasonal dependency of soil $N_2O$ emissions requires a detailed analysis of the role of weather conditions for the entire experimental year. The long term average (LTA, 1981-2010, Met Éireann, 2019) from the Rosslare weather station (<15 km from experimental site) showed that 2017 was a year with lower rainfall in spring (-20 mm) and higher rainfall in both summer (+84 mm) and autumn (+32 mm) compared to the LTA. In particular, the LTA rainfall for the month of June was 54.9 mm, while in 2017 rainfall of 124.8 mm was recorded. However, July and August were drier in 2017 than the LTA. The summer experiment started in July; therefore the treatments were applied in dry conditions. The seasonal differences in soil moisture conditions can be highlighted with the daily mean soil moisture deficit measured at the experimental site, with 32.7 mm in spring, 25.5 mm in summer, and 1.1 mm in autumn, on the day of application. However, the temperature remained close to the LTA (± 3.5 degrees max) for the whole year. Every treatment had a significant seasonal influence on EFs apart from the treatment CANU, where urine and fertiliser were applied together (Table 3). Spring and summer were drier and found to correspond to lower EFs than the wetter autumn season (Figure 4). The treatment*season interaction on the EF from the five treatments and the three seasons (n=60) was not significant (p-value = 0.17). An estimation of marginal means (a.k.a. the least-squares method) was used to test for the effect of the time of application on the difference between treatments, when significant. None of the potential treatment and season interactions were significant.

### 1.3.4 INTERACTIVE EFFECT OF URINE AND FERTILISER APPLICATION ON $N_2O$ EMISSIONS AND YIELD

To assess the difference of emissions between urine application and fertiliser application separately compared with applied together, the two treatments CANU and CAN+U were compared. Adding fertiliser to urine patches significantly increased total $N_2O$ emissions in spring and summer compared to the expected total additive emissions represented by the CAN+U treatment (Table 3). In spring and summer total cumulative emissions were respectively 51.0 % and 48.4 % higher for urine and fertiliser applied together than for the sum of emissions from urine and fertiliser applied separately. For each urine deposit where fertiliser was applied, the increase of emissions represents a total of 2.5 kg and 2.0 kg of $N_2O$-N emitted per hectare in spring and summer, respectively. By contrast, for autumn applications, the cumulative emissions from CANU and CAN+U treatments were not significantly different with an average of 5.6 ±1.7 kg of $N_2O$-N emitted per hectare. The EFs from the CANU and CAN+U treatments followed the same trend with a significant difference in spring and summer which was not noticeable in autumn (Table 3). One of the observed differences in the early season compared to the autumn was the delayed peak of $N_2O$ emissions in autumn which can be observed from the daily cumulative $N_2O$ emissions (Figure 4). The initial difference in emissions from CANU and CAN+U treatments on the day of application was maintained during the whole study period. Consequently, the magnitude of the initial peaks in emissions following application can be a major driver of the differences observed. Yield-scaled EFs (Figure 5), demonstrate the percentage of N lost as $N_2O$ per N applied and per tonne of dry matter (DM) produced per hectare. Total dry matter yields of the control treatment were 3.46 t DM ha$^{-1}$, 4.29 t DM ha$^{-1}$ and 1.46 t DM ha$^{-1}$ in spring, summer and autumn (Table 3). Yield-scaled EFs were significantly different only for the summer application which could suggest a better N uptake in the case of separated applications of urine and fertiliser compared with applied together (Figure 5).

### 1.3.5 DRIVERS OF $N_2O$ EMISSIONS

Seasonal treatment applications were strongly influenced by the difference in weather and soil conditions as well as grass production. Significant relationships were observed between the EF from the urine treatment and climatic factors. The results of the stepwise multiple regressions are presented inTable 4. The model utilising weather parameters showed 73 % of the variation in the EF was explained by cumulative rainfall in the three days prior to and after application as well as the average temperature over the ten days prior to the application. The relationship with precipitation was found to be a squared relationship which is in accordance with the findings of Krol et al. (2016) (Table 4).

## 1.4 DISCUSSION

### 1.4.1 SEASONAL VARIATIONS ON $N_2O$ FLUXES

Peak $N_2O$ emissions occurred on the day of application in both spring and summer. Other studies have also observed high $N_2O$ emissions from urine treatment on the day of application (Forrestal et al., 2016; Krol et al., 2016; Qiu et al., 2015). This initial increase in emissions can be attributed to both mineralization of labile carbon and N and the increase in soil moisture that enhances soil nitrification and denitrification rates (Burchill et al., 2014; Chadwick et al., 2000; Luo et al., 2017). Moreover, the increase in soil moisture and DOC from the urine application was reported to mobilise the indigenous N pool of the soil, resulting in the production of $N_2O$ (Saggar et al., 2015). The DOC is sourced from the urine itself and released from the soil pool due to the high pH of the urine which was supported in this study by a significantly different soil pH between treatments on the day of application. However, other studies showed differences in the response to the urine application with a delay in elevated $N_2O$ emissions, which was observed during the autumn application from the urine in this study (11 days delay). Some studies observed a delay of approximately 10 days after urine application before the major emission peak (Hyde et al., 2016; Minet et al., 2018; Van Groenigen et al., 2005). The delay in emission following urine application could be explained by the high soil moisture content and the higher percentage of N leaching in autumn, (Hyde et al., 2016) and due to a less active microbial population in the soil (Anger et al., 2003). Consequently, an emission peak on the day of application could be linked to the increase of the availability of existing N pools in the soil and the dissolution of existing fertiliser pellets from the addition of water contained in the urine to dry soil. Half of the N from CAN fertiliser is in nitrate form which can be quickly lost via denitrification. In the same way, rainfall might enhance $N_2O$ emissions after a drier period (Rowlings et al., 2015; Scheer et al., 2014). Therefore, with the exception of the day of application, peaks of $N_2O$ emissions for all treatments were recorded following rainfall events and subsequent decrease in soil moisture deficit.

### 1.4.2 DRIVERS OF $N_2O$ EMISSIONS FROM URINE DEPOSIT

A simplistic statistical model used by Krol et al. (2016) and Minet et al. (2018) was applied to extract the weather parameters best explaining the EF measured from urine deposition. The urine EF was strongly influenced by short-term weather conditions before and after the day of application. The model selected a number of parameters: 1) average air temperature over 10 days after application and average soil temperature over 7 days after application; 2) cumulative rainfall 3 days prior application and cumulative rainfall 3 days after application,

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explained 73 % of the $N_2O$ emissions variations. The results reported by Krol et al. (2016) and Minet et al. (2018) are in accordance with the results presented in this study and highlight the key role of rainfall and soil temperature close to the time of urine deposit. Rainfall has been widely considered as the main driver of $N_2O$ emissions after substantial N input to the soil (Abalos et al., 2017; Rowlings et al., 2015; Scheer et al., 2014). Rainfall is a proxy of soil moisture. The soil moisture deficit at the spring and summer application was 32.7 mm and 25.5 mm, whereas in autumn the soil moisture deficit was only 1.1 mm due to a significant difference in rainfall in the 3 days before each seasonal application. Soil moisture is particularly influential when urine and fertiliser are applied to dry soil (Ambus et al., 2007; Curtin et al., 2017). Whereas, temperature affects the microbial activity with an optimal temperature for $N_2O$ production of 30 °C (Maag and Vinther, 1996) along with indirect effects of temperature on oxygen availability by increased respiration rates, it is the soil moisture effect on mineralisation rates, which limits the substrate availability, and plays an essential role in $N_2O$ production rates (Saggar et al., 2013). Adding the data from this study to the regression model from Krol et al. (2016) and Minet et al. (2018) did not change the significance of the regression and highlights the importance of the weather conditions for predicting $N_2O$ emissions from urine application.

Precipitation rates and amounts considered in this study did not reflect the past long-term seasonal trends, with a much drier spring and summer in 2017 than expected. The results of this study therefore may underestimate the ‘typical’ fertiliser induced $N_2O$ emissions in spring and summer, while overestimating it in autumn. However, these results may reflect future Irish climate influence on $N_2O$ emissions which are predicted to change with wetter autumns and winters and drier springs and summers (Nolan et al., 2017). This change in long term weather patterns suggests that if production of $N_2O$ is to be minimised, grassland management is a key element to consider. Weather conditions are variables commonly recorded and predictable in the short and long term. Linking $N_2O$ emissions to these parameters offers a great opportunity for $N_2O$ modelling over larger scales (Foltz et al., 2019).

### 1.4.3 TREATMENT EFFECT AND EMISSION FACTORS

An EF is a representative value that relates the quantity of $N_2O$ emitted to the atmosphere with the amount of N added as either fertiliser or as urine-N (Paustian et al., 2006). The IPCC Tier 1 methodology assumes a constant EF for the entire year (Paustian et al., 2006). In this study, however, the EF was calculated over a period of 40 days (for urine treatment of 0.28-0.82 %, fertiliser of 0.07-0.72 % and the combined treatment of 0.52-0.76 %). Due to the use of control plots in these studies including the current study, and the subtraction of ‘background’ emissions from the treated plots, the reported EFs are unlikely to vary from those calculated from annual studies. For most reported results, the vast majority of annual $N_2O$ emissions are emitted within 40 days after application (Buckthought et al., 2015b; Cowan et al., 2019; Skiba et al., 2013). In this study, small fluxes near the natural variability in emissions from the control treatment (after 40 days) were not considered. In Krol et al. (2016), the $N_2O$ emissions post-urine application had returned to background levels after 44 days, with comparable results in the UK (Bell et al., 2015) and New Zealand (Van Der Weerden et al., 2013). Therefore, the results of this study can be considered representative of the annual difference in emissions between treatments. However, these results should be used carefully if considered in terms of annual EF due to the well-known variability of $N_2O$ emissions which require measurements to be replicated a substantial number of times to reduce uncertainties to an acceptable level for global modelling.

The urinary-N seasonal variability was due to the natural variability of dairy cow urine composition mainly influenced by the supply of water and the N content of the grass or feeds

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(Dijkstra et al., 2013). Cumulative emissions and EFs were low for the CAN treatment in each season and not significantly different to the control treatment in summer. CAN's EF has previously been reported twice as large as that measured in this study (Bell et al., 2016; Committee on Climate Change, 2018; Harty et al., 2016) and up to 3.93±1.17 % in Hillsborough, Co Down, Northern Ireland in 2003 (Smith et al., (2012). However, the EFs for CAN of 0.33 % and 0.72 % in spring and summer, respectively are within the range of 0.3-3.0 % provided in the IPCC guidelines (Paustian et al., 2006). It is likely that the low EF from CAN treatment might be due to the weather conditions with an exceptionally dry spring and summer. Indeed, a higher EF was observed during autumn, which coincided with higher soil moisture content and could suggest a high denitrification rate as shown by Rex et al. (2018). In this study, U and CANU treatments emitted lower emissions than estimated using default EF from IPCC of 2 % or the Irish country-specific EF of 1.2% (Duffy et al., 2018). The urine EFs of 0.28 % to 1.05 % measured in this study were in the range but lower than those reported by Krol et al. (2016) of 0.30-4.81 %, by Chadwick et al. (2018) of 0.05-2.96 % and by van der Weerden et al. (2017a) of 0.30-0.75 %. These EF values are much larger than those measured by Hyde et al. (2016) who reported an EF of 0.12 % for urine application. In spring with 0.74±0.35 %, in summer with 0.52±0.18 % and in autumn with 0.76±0.19 % the EFs from CANU treatment were not significantly different between seasons and were all lower than the IPCC default.

### 1.4.4 INTERACTIVE EFFECT OF URINE AND FERTILISER APPLICATIONS

Despite the number of studies investigating N losses from urine patches (Cai and Akiyama, 2016; Chadwick et al., 2018; Li et al., 2012; Selbie et al., 2015; Van Groenigen et al., 2005), the interaction between urine and fertiliser applications to temperate grassland is limited (Buckthought et al., 2015a; Hyde et al., 2016; Krol et al., 2017). This study demonstrates the

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existence of an interactive effect between urine deposit and N fertiliser application on $N_2O$ emissions for spring and summer periods which was characterised by low soil moisture content. The application in autumn, where higher soil moisture content promotes higher $N_2O$ emissions did not show an interactive effect. It is a common practice to apply fertiliser to grassland between one and three days after grazing instead of on the same day of grazing, as done in this study. The difference between this study and common practice might have increased the effect of the urine moisture on the dissolution of the fertiliser applied. The study conducted by Krol et al. (2017) showed a potential 20 % underestimation of $N_2O$ emissions from urine and fertiliser applications when the interaction was ignored. This research also agrees with the work of Hyde et al. (2016) who showed that the cumulative $N_2O$ emissions from CAN fertiliser and urine applied together were more than double compared to the emissions from separate applications. These two studies were conducted with an application date in May and under low soil moisture conditions which is in accordance with this study. By contrast, Buckthought et al. (2015b) found no significant difference between urine applied alone and combined to N fertiliser (urea) with an application at high soil moisture content due to the soil being wetted with 800 mm of water before application of the treatment.

More data is needed to build a more robust model that can predict $N_2O$ emissions from urine deposition across seasons and soil types. Such a model could be used as a farming decision support system and might guide management decisions to reduce N loss during grazing (Minet et al., 2018). The interaction between fertiliser and urine application in grazed pastures combined with the climatic drivers influencing $N_2O$ emissions should be included in future modelling to upscale $N_2O$ losses from the chamber to field and regional scales.

### 1.4.5 YIELD-SCALED $N_2O$ EMISSIONS AND PRODUCTIVITY

Grass dry matter yield differed significantly between treatments. The grass N uptake and biomass production are major drivers of $N_2O$ emissions by controlling the nutrient pool available for nitrifier or denitrifier microorganisms and thereby could influence the interactive effect of urine and fertiliser applications. To support this hypothesis, the yield-scaled EFs from CANU and CAN+U treatment were compared.

Yield-scaled $N_2O$ emissions, also called emission intensities, represent the cumulative $N_2O$ emissions expressed as a fraction of grass yield. Emission intensities were about 0.04 to 0.22 kg $N_2O$-N $t^{-1}$ for the Control and CAN treatments, which is similar to the results of Snell et al. (2014) who found a rate of 0.13 to 0.25 kg $N_2O$-N $t^{-1}$ for fertiliser application in Nebraska, USA with rainfall and temperature conditions during the month of experimentation similar to the present study. For urine and CANU treatments, we found an emission intensity ranging from 0.40 to 3.38 kg $N_2O$-N $t^{-1}$, which was substantially higher than those found by Snell et al. (2014) which were all lower than 1.0 kg $N_2O$-N $t^{-1}$. For the autumn application, the lack of significant differences in emission intensity between CAN, CANU and U treatments suggests the increase in $N_2O$ emissions in this season could be the result of N applications exceeding the plant's requirement. Bell et al. (2016) reported a plateau effect for N applications above 240 kg N $ha^{-1}$ input to a temperate grassland on grass yields. In autumn, the N input from the U and CANU treatments exceeded this amount by at least 200 kg N $ha^{-1}$. The increase in soil moisture content and the slow grass growth rate constrained by daylight and temperature in autumn left a greater pool of available N to microorganisms to produce additional $N_2O$ emissions than in spring and summer. In terms of yield-scaled EF, the difference between CANU and CAN+U treatments was less pronounced than the comparison in terms of $N_2O$, in particular in spring, which showed that plant nutrient requirements may play an important role in the fertiliser and urine interaction between spring/summer and autumn application.

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The results of the present study emphasize the need to advise farmers on the appropriate N fertiliser inputs and application timing to match N plant needs in addition of recommendations of avoiding intense grazing or fertiliser application at high soil moisture content. This study implies the need for further replication under varying conditions, also considering the interaction between dung deposits and fertiliser applications on $N_2O$ emissions.

## 1.5 CONCLUSION

Globally, large areas of grazed grasslands are simultaneously covered by urine and N fertiliser. This study provides evidence of enhanced $N_2O$ emissions in areas of overlapping N fertiliser and urine deposit. The emission rates of urine-based $N_2O$ and fertiliser-based $N_2O$ and their interaction from grassland soil under different seasonal environmental conditions were quantified. Areas where the combined urine and fertiliser was applied are hotspots of $N_2O$ emission. Dietary and pasture management practices, which may reduce N losses as $N_2O$ emissions, could have crucial impacts on the global warming footprint linked to intensively managed grasslands. Although the EF factors measured in this study are partial and would require replicated studies before being fully validated, the higher autumn EFs for urine deposition of 0.82±0.29 and fertiliser application of 0.72±0.43 highlight the potential for carefully extending grazing during wet periods to reduce emissions. Global weather conditions are currently well modelled and this study showed the potential to use weather conditions (i.e. soil moisture content, rainfall, temperature) as proxies to model the type of interaction (additive or synergistic) between urine and fertiliser application on $N_2O$ emissions. Climate change estimations have predicted more frequent wetter autumns in European temperate climates in the future therefore favouring conditions for the increase in total N losses into the environment. The increased understanding of $N_2O$ emission drivers provides scope for adapting grassland and grazing management practices to reduce emissions.

## 1.6 AUTHOR CONTRIBUTIONS

JM, KR, GL and DK designed the experiment, JM and DP conducted the experiment and analysed the samples in the laboratories in Teagasc Johnstown Castle with the support of laboratory technicians. JM wrote the article with the contributions from all co-authors and PhD supervisory team.

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## 1.8 Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# 1.10 TABLES AND FIGURES CAPTIONS

Table 1: Rates of application per season (kg $ha^{-1}$) of total nitrogen (TN), ammonium (N-$NH_4^+$), total oxidised N (TON), urea-N, total carbon (TC) and total organic carbon (TOC) (n=60). Treatments were: untreated (Control), Urine (U), calcium ammonium nitrate (CAN), CAN and urine applied together (CANU), and CAN and urine applied separately (CAN+U).

| **Season** | **Treatment** | **Application rates (kg $ha^{-1}$)** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **TN** | **N-$NH_4$** | **TON** | **Urea-N** | **TC** | **TOC** |
| **All seasons** | **Control** | 0 | 0 | 0 | 0 | 0 | 0 |
| **Spring** | **U** | 573 | 59 | 18 | - | - | 1369 |
| | **CAN** | 62 | 31 | 31 | - | - | - |
| | **CANU / CAN+U** | 635 | 90 | 49 | - | - | 1369 |
| **Summer** | **U** | 680 | 12 | 2 | 373 | 1849 | 1569 |
| | **CAN** | 108 | 54 | 54 | - | - | - |
| | **CANU / CAN+U** | 788 | 66 | 56 | - | 1849 | 1569 |
| **Autumn** | **U** | 671 | 3 | 0 | 545 | 1582 | 1317 |
| | **CAN** | 30 | 15 | 15 | - | - | - |
| | **CANU / CAN+U** | 701 | 15 | 15 | - | 1582 | 1317 |

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Table 2: Soil $NH_4^+$, soil pH and soil dissolved organic carbon (DOC) measured right after application (n=5 for each treatment*season combination). Treatments are: untreated (Control), Urine (U), fertiliser in the form of ammonium nitrate (CAN), fertiliser and urine applied together (CANU) and CAN+U a composite treatment based on the results from U and CAN treatments.

| **Season** | **Treatment** | **Soil $NH_4^+$ (day of application)** | | **Soil pH (day of application)** | | **Soil DOC (day of application)** | |
|---|---|---|---|---|---|---|---|
| | **Units** | **mg $kg^{-1}$ dry soil** | **± *SD*** | **SU** | **± *SD*** | **mg $kg^{-1}$ dry soil** | **± *SD*** |
| **All seasons** | **Control** | 7.1-57.9 | *1.4 - 10.6* | 6.4 | *0.2 - 0.05* | 16.3-25.5 | *2.5 - 9.3* |
| **Spring** | **U** | - | - | - | - | - | - |
| | **CAN** | 39.6 | *20.8* | 6.4 | *0.2* | 26.9 | *6* |
| | **CANU** | 302.1 | *124* | 6.9 | *0.1* | 61.7 | *32.7* |
| | **CAN+U** | - | - | - | - | - | - |
| **Summer** | **U** | 278.5 | *89.3* | 6.9 | *0.3* | 56.5 | *24.2* |
| | **CAN** | 51.6 | *39.5* | 6.1 | *0.1* | 19.5 | *1.1* |
| | **CANU** | 436.3 | *104.4* | 6.6 | *0.2* | 52.2 | *10.3* |
| | **CAN+U** | 330.1 | *64.4* | - | *0.2* | 76 | *12.6* |
| **Autumn** | **U** | 309.4 | *33* | 6.8 | *0.04* | 33.1 | *12.8* |
| | **CAN** | 21.8 | - | 6.7 | - | 20.4 | - |
| | **CANU** | 736.7 | - | 7 | - | 68.3 | - |
| | **CAN+U** | 331.2 | - | - | - | 53.5 | - |

Table 3: Results of the experiment per season of the grass dry matter yield, cumulative $N_2O$ emissions and EF (n=5 per treatments*season). Treatments are: untreated (Control), urine (U), fertiliser in the form of calcium ammonium nitrate (CAN), fertiliser and urine applied together (CANU) and CAN+U a composite of the results from treatment U and CAN.

| **Season** | **Treatment** | **Grass Yield Mean** | | **Cumulative $N_2O$ emissions** | | | **Partial Emission factor** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Unit** | **t ha-1 ± *SD*** | | **kg $N_2O$-N $ha^{-1}$** | **± *SD*** | **p<0.05*** | **%** | **± SD** | **p<0.05*** |
| **All seasons** | **Control** | *1.6-2.2* | *0.2-0.5* | 0.09-0.15 | *0.02-0.10* | d c c- A A A | - | - | - |
| **Spring** | **U** | *4.1* | *0.9* | 2.06 | *1.19* | b B | 0.33 | *0.21* | ab B |
| | **CAN** | *3.5* | *1.2* | 0.33 | *0.14* | c A | 0.31 | *0.22* | b B |
| | **CANU** | *4.5* | *0.9* | 4.87 | *2.22* | a A | 0.74 | *0.35* | a A |
| | **CAN+U** | *3.8* | *1.2* | 2.39 | *1.29* | b B | 0.35 | *0.20* | b B |
| **Summer** | **U** | *5.0* | *0.3* | 2.00 | *0.50* | b B | 0.28 | *0.07* | b B |
| | **CAN** | *4.3* | *0.8* | 0.16 | *0.10* | c B | 0.07 | *0.09* | c C |
| | **CANU** | *5.0* | *0.9* | 4.18 | *1.43* | a A | 0.52 | *0.18* | a A |
| | **CAN+U** | *4.6* | *0.9* | 2.16 | *0.52* | b B | 0.26 | *0.07* | b B |
| **Autumn** | **U** | *1.4* | *0.3* | 5.60 | *1.96* | a A | 0.82 | *0.29* | a A |
| | **CAN** | *1.6* | *0.6* | 0.30 | *0.13* | b AB | 0.72 | *0.43* | a A |
| | **CANU** | *1.6* | *0.4* | 5.39 | *1.36* | a A | 0.76 | *0.19* | a A |
| | **CAN+U** | *1.7* | *0.8* | 5.90 | *2.02* | a A | 0.83 | *0.29* | a A |

** Lower case and capital letters indicates significant treatment differences between and within seasons, respectively*

Table 4: Model of stepwise multiple regression analysis for $N_2O$-N EF from urine treatment using cumulative rainfall and mean soil moisture deficit and soil temperature between 10 days before application to ten days after application.

| Parameter | Estimate | Standard Error | t Value |
|---|---|---|---|
| Intercept | -0.60 | 0.84 | -0.71 |
| Temperature 10 days average after application | 0.67 | 0.15 | 4.35 |
| Cumulative rainfall 3 days prior application | -0.12 | 0.04 | -3.36 |
| Cumulative rainfall 3 days after application ^ 2 | 0.09 | 0.04 | 2.39 |
| Soil temperature average 7 days after application ^ 2 | -0.48 | 0.09 | -5.26 |

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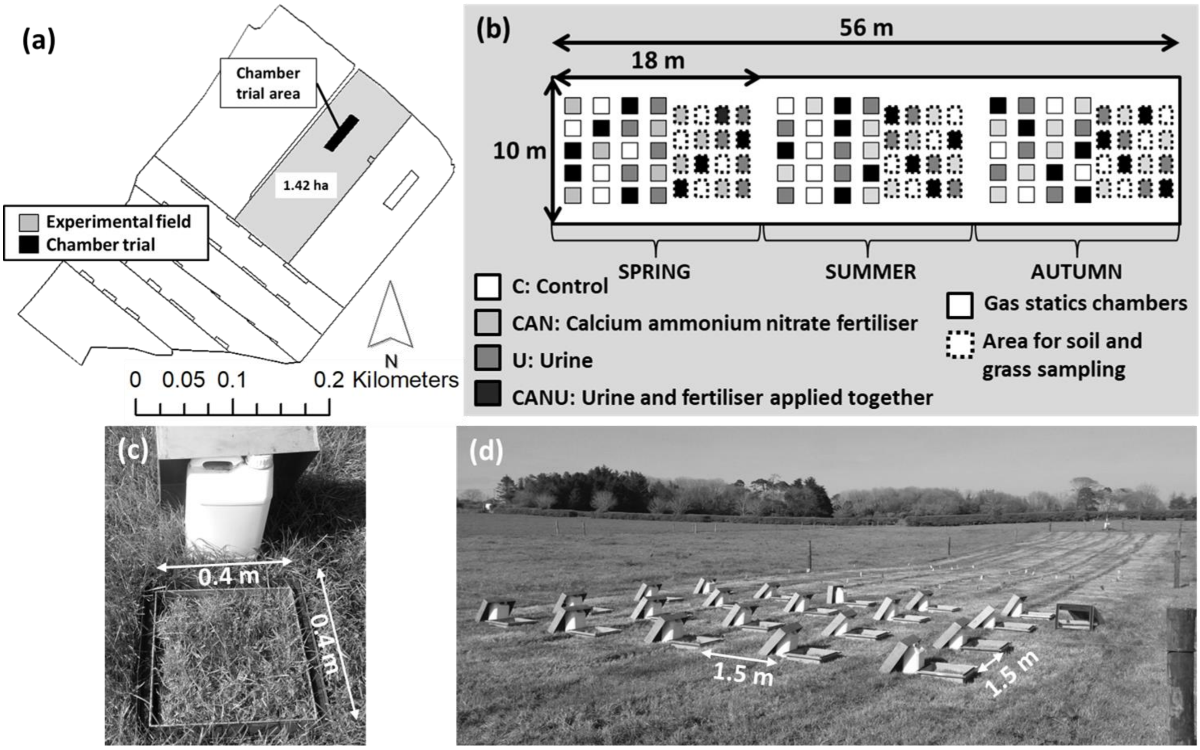


Figure 1: Experimental set-up. (a) Map showing paddocks at Johnstown Castle farm with the chamber trial and experimental field. (b) Experimental chamber trial details with designated static chamber and soil/grass sampling areas for each season of application and each treatment. (c) Photograph of the open static chamber with the square base inserted into the soil, the lead cover and the ballast weight placed on top during measurements. (d) Photograph of the chamber trial area set-up in spring.

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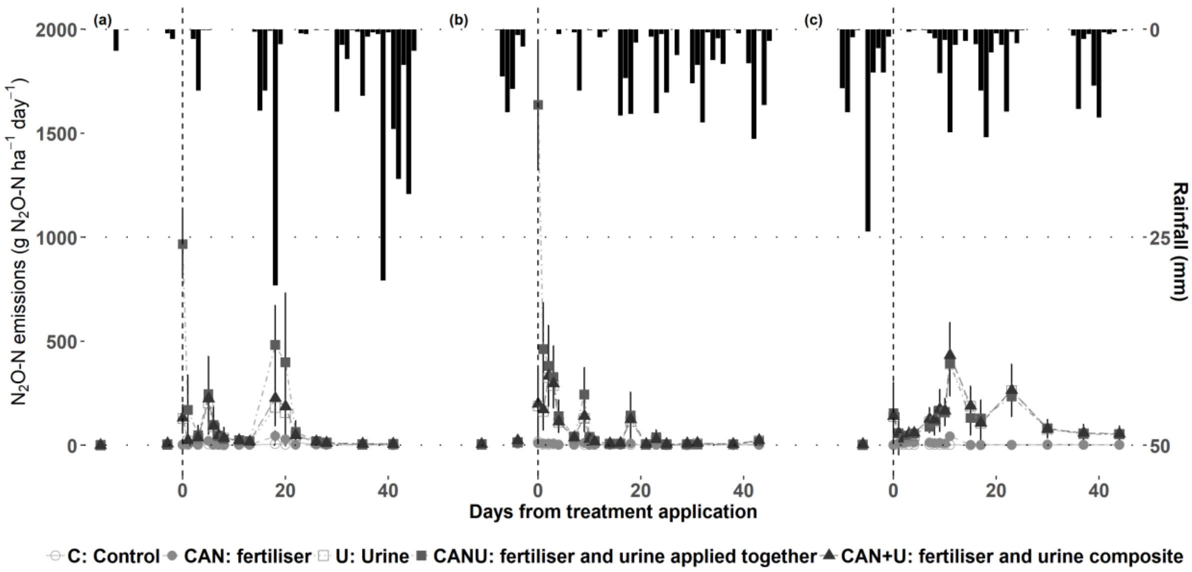


Figure 2: Daily $N_2O$ emissions over the three seasons (a- spring, b- summer, c-autumn) for all four different treatments (C-control, CAN-fertiliser, U-urine, CANU-urine and fertiliser applied together) and the urine and fertiliser aggregated data (CAN+U) (error bars represent standard deviation). Vertical black lines represent the day of application of the four treatments; points prior to these lines are background measurements. The secondary y axis is inverted and represents the daily rainfall.

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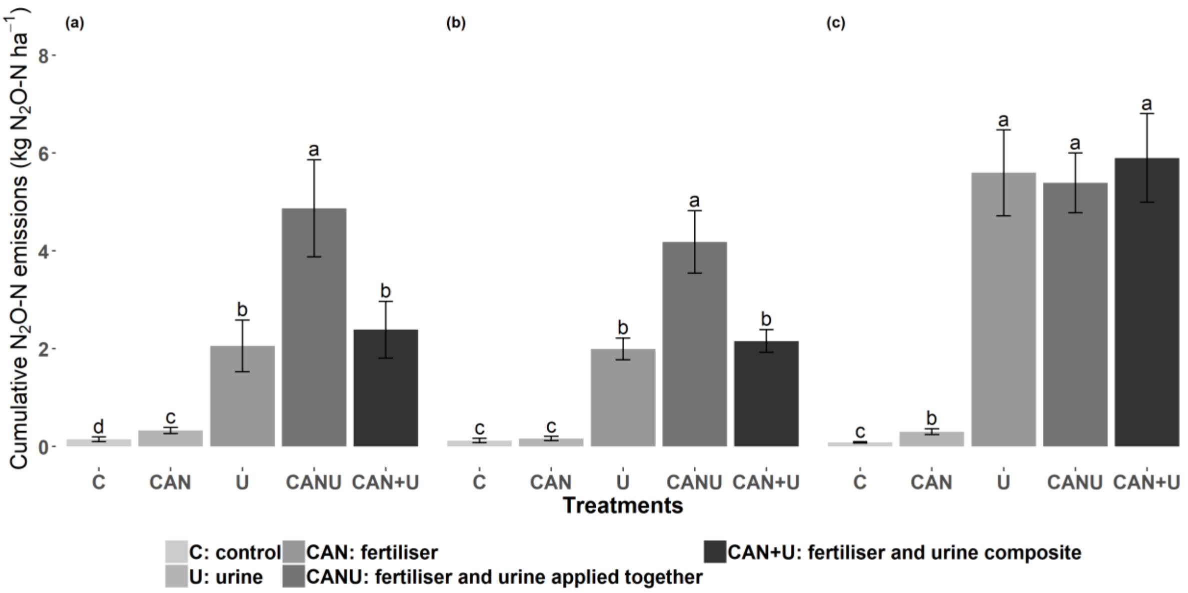
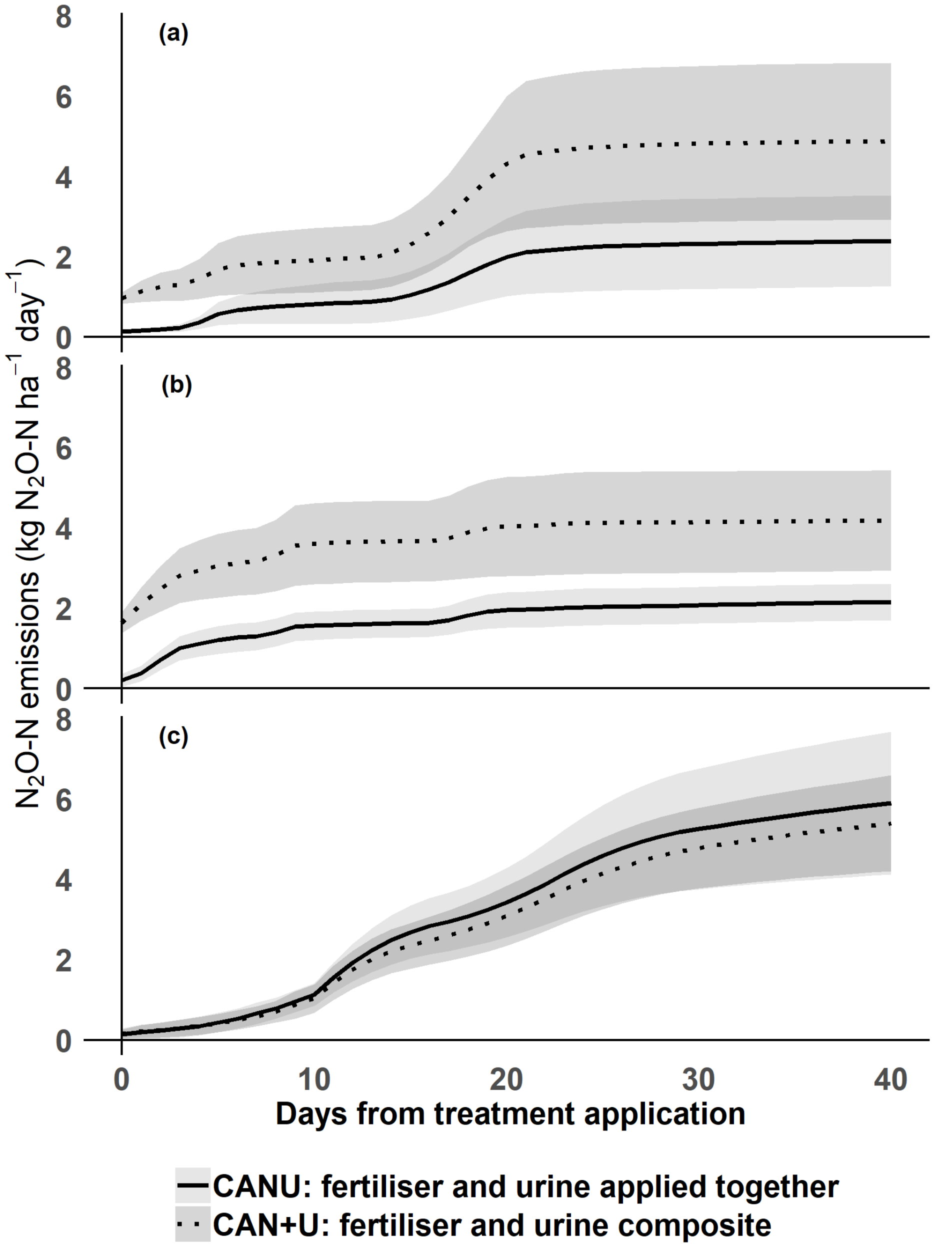



Figure 3: Cumulative $N_2O$ emissions (0-40 days after treatment application) all four different treatments (C-control, CAN-fertiliser, U-urine, CANU-urine and fertiliser applied together) and the urine and fertiliser aggregated data (CAN+U) and per season (a- spring, b- summer, c-autumn). Different letters indicate significance differences between treatments at $p < 0.05$, statistical tests run separately per season (n=60). CAN+U treatment represents aggregated data from the urine and fertiliser treatments. Error bars represents standard errors of the mean.




764

765 Figure 4: Daily cumulative $N_2O$ emissions for the CANU treatment (i.e. fertiliser and urine
766 applied together) and CAN+U (i.e. a sum of the results from U and CAN treatments) per
767 season (a- spring, b- summer and c-autumn) with the uncertainty ribbons representing the
768 daily non-cumulated 95 % confidence interval of the mean.

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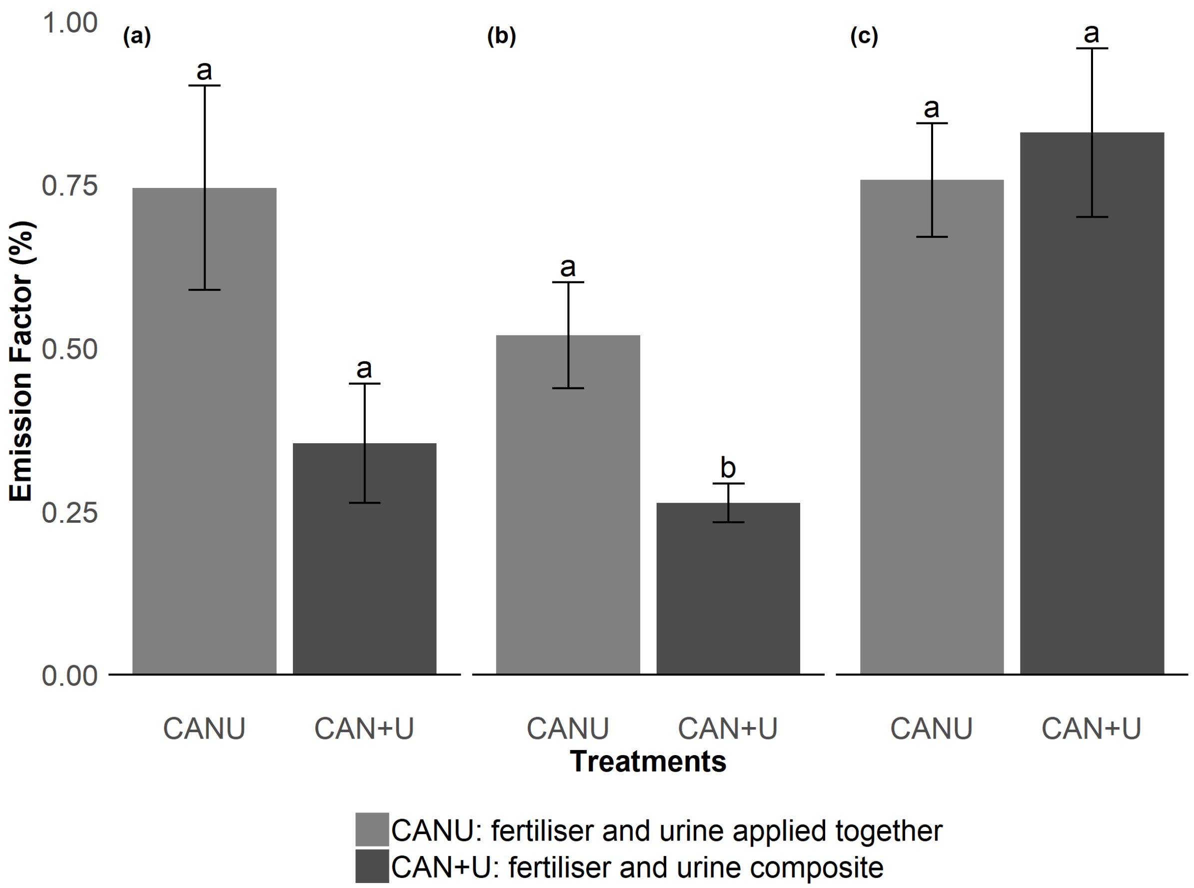


770

771 Figure 5: Yield-scaled EF for treatment fertiliser and urine applied together (CANU) and

772 CAN+U a composite of the results from treatment U and CAN per season (a- spring, b-

773 summer and c-autumn). Different letters indicate significance difference between treatments

774 at $p < 0.05$, statistical tests run separately per season.