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EFFECT OF PLANTAIN USE ON REDUCTION OF NITROUS OXIDE EMISSIONS FROM A WAIKATO FARM

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Abstract

The objectives of this study were to compare the nitrous oxide (N_2O) emission factors for urine (EF_3, N_2O-N emitted as % of N applied) from animals grazing two pasture types: conventional ryegrass (*Lolium perenne*) /white clover (*Trifolium repens*) (RGWC) or a mixed sward of 60% plantain (*Plantago lanceolate* L.) and 40% ryegrass/ white clover (PRGWC), and to assess whether any differences in EF_3 were due to a "urine composition" effect or a "sward" effect. This work was carried out on a free-draining alluvial soil near Waharoa in the Waikato region during the winter of 2019. A static chamber method was used to measure N_2O fluxes from urine, collected from cows which had been fed on RGWC or PRGWC diets, applied to five plots of each pasture type. Plots that received no urine were also included. Gas sampling took place once before urine application, and then over the following 3 months until N_2O fluxes from the urine-applied soil returned to background levels. The N_2O fluxes were integrated over time to estimate the total emissions. EF_3 values were then calculated for each combination of urine and pasture type.

The mean net N_2O emission from the RGWC urine was 0.57 kg N_2O-N ha^{-1} when applied to the RGWC pasture and 0.40 kg N_2O-N ha^{-1} when applied to the PRGWC pasture. The mean net N_2O emission from the PRGWC urine was 0.60 kg N_2O-N ha^{-1} when applied to the RGWC pasture and 0.41 kg N_2O-N ha^{-1} when applied to the PRGWC pasture. Overall, the differences in net N_2O emission between the two urine types were not significant ($P > 0.05$), but the net mean N_2O emissions from the PRGWC pasture were lower compared with those from the RGWC pasture ($P < 0.05$). Accordingly, the EF_3 values were not different when either urine type was applied to either RGWC soil or PRGWC soil, but the EF_3 values for the PRGWC sward (0.10-0.12%) were lower than those for the RGWC sward (0.15-0.17%) ($P < 0.05$). These results indicate that the effect that plantain had on reduction of N_2O emissions was not a "urine composition" effect but a "sward" effect. Possible explanations for this sward effect include biological nitrification inhibition from plantain root exudates, or plantain plant effects on the soil microclimate such as soil moisture levels.

Introduction

Nitrous oxide (N_2O) is a long-lived greenhouse gas (GHG) with a global warming potential 265 times higher than carbon dioxide (CO_2) over a 100-year horizon (IPCC, 2013). In 2017, N_2O emissions from agriculture contributed 10.6% (8,566 kt CO_2-equivalent) to the New

Zealand gross GHG emission profile. These levels have increased by 28.1% (1,877 kt CO_2-equivalent) above those in 1990 (MfE, 2019). Key drivers for increasing N_2O emissions in New Zealand since 1990 were the growth of the national dairy herd size by 89.6% and the accelerated application of synthetic N fertilisers (650%), which are consistent with global trends (MfE, 2019).

An estimated 80% of the total N_2O emissions come from livestock excreta-N (mainly urine) deposited onto the soil during grazing (de Klein et al. 2003; MFE 2019). N_2O production in agricultural soils is linked to the processes of microbial nitrification and denitrification (Di et al. 2016). Introduction of plantain into grazed pastures has been suggested as an effective farm management strategy for reducing nitrate leaching, and potentially N_2O emissions (Di et al. 2016; Luo et al. 2018). The proposed mechanisms of N-loss reductions have been reviewed in de Klein et al. (2019). These mechanisms can be loosely categorized as being due to a "urine composition" effect or to a "sward" effect. Proposed urine composition effects include lower N-content of plantain containing forage (Box et al. 2017); increased N portioning into dung relative to urine (Carulla et al. 2005); diuretic effect of plant secondary metabolites (PSM) leading to more frequent urination (O'Connell et al. 2016) and biological nitrification inhibitor effects of PSM delivered through urine to the soil (Balvert et al. 2017). Sward effects include a biological inhibitor effect of PSM in root exudates (Byrnes et al. 2017); increased N immobilisation through an increase in soil carbon in the rhizosphere (Bowatte et al. 2018) and /or changes in the soil microclimate (de Klein et al. 2019; Simon et al. 2019).

The aim of this research was to quantify N_2O emissions from urine of animals grazing two pasture types: conventional ryegrass/ white clover (RGWC) or a mixed sward of 60% plantain and 40% ryegrass / white clover (PRGWC), and to assess whether any differences in EF_3 were due to a "urine composition" effect or a "sward" effect.

Materials and Methods

Site Description and Preparation

This study was conducted on a commercially operating 199 ha dairy farm near Waharoa (37.78 °S, 175.80 °E) in the Waikato Region. The experimental site was established in a paddock containing ryegrass / white clover-based pasture with plots which had 0% or 60% plantain (i.e. RGWC or PRGWC). The trial site was sown in March 2018, and once established the pastures were grazed by dairy cows. This dairy pasture had a stocking rate of 3.2 cows ha^{-1} and was managed under a typical rotational grazing regime (i.e. cows graze on a paddock for a day or two and are then moved to a fresh paddock to allow pasture to regrow). Cows were excluded from the experimental site for 12 weeks before the commencement of the measurement period. The trial site was on a Te Puninga soil, which is an imperfectly drained, moderately gleyed yellow-brown loam (McLeod 1992). Soil properties of the upper 75 mm of the soil profile were: total C of 8.5%, Olsen Phosphorus of 51 mg/l, pH of 6.3, bulk density of 0.79 Mg m^{-3}, and cation exchange capacity of 24 me/100g.

In each of 40 individual plot areas of 1.25 x 1.25 metres a 25 cm diameter stainless steel gas collection ring was installed in the ground. Each plot also had a 50 cm circular area for destructive soil sampling.

Treatments

Treatment application of urine was carried out on 21 May 2019. Urine for application to the gas rings was obtained from cows fed diets of 0% (i.e. RGWC) and 60% plantain (i.e. PRGWC) at DairyNZ's Lye Farm. Artificial cow urine (Fraser et al. 1994) was applied to soil plots at the same volume and N concentration as the urine applied to the gas rings. The urine was applied at 10 L m^{-2} to represent the average hydraulic loading rate in a urine patch (Selbie et al. 2015), resulting in N loadings equivalent to 388 and 343 kg N ha^{-1} for the urine from the cows fed 0% and 60% and plantain diets, respectively (Table 1).

Table 1. Treatments for determining N_2O emission factors (EF_3) for dairy cow urine applied to 0% and 60% plantain pastures. RGWC= ryegrass / white clover, PRGWC = plantain / ryegrass / white clover, n=5.

Sward Type	% Plantain in sward	Urine	Urine volume applied (L/m^2)	Urinary N load applied (kgN ha^{-1})
RGWC	0	RGWC	10	388
		PRGWC	10	343
		Nil	0	0
PRGWC	60	RGWC	10	388
		PRGWC	10	343
		Nil	0	0

Nitrous oxide measurements and analysis

A soil chamber technique was used to measure N_2O emissions, and the methodology was based on the guidelines developed by de Klein and Harvey (2015). Stainless steel gas sampling bases (25 cm diameter), with a water channel around the circumference of each base, were placed in each plot a week before the gas measurements commenced. Sampling took place one day before treatment application, on the day of treatment application (21 May 2019), and the day following treatment application. Samples were then taken twice per week for the next four weeks, weekly for another four weeks and then fortnightly until completion of the measurements on 8 August 2019. On each sampling occasion plastic gas sampling chambers, with a volume of approximately 10 L, were fitted to the sampling bases, and the base channels filled with water to provide a gas-tight seal. The chambers were left for a period of one hour between 10 am and noon (representing the daily average N_2O flux rate) (van der Weerden et al. 2013). At times 0, 30 and 60 minutes, gas samples were extracted from a sampling port on the chambers using a plastic syringe and injected through a septum into evacuated 5.6 mL glass sample vials. After 6 weeks, when linearity of the increase in N_2O concentration over 60 minutes had been confirmed, sampling was reduced to times 0 and 45 minutes. Chamber temperatures were also recorded at each sampling occasion for use in flux calculations. The samples were analysed for N_2O concentration at Lincoln University using gas chromatography (GC; SRI 8610 gas chromatograph; ^{63}Ni electron capture detector, SRI Instruments, CA, USA).

The hourly N_2O fluxes (mg N m^{-2} h^{-1}) were calculated from the increase in head space N_2O over the sampling time (de Klein et al., 2003):

$$N_2O\ flux = \frac{\delta N_2O}{\delta T} * \frac{M}{Vm} * \frac{V}{A} \quad (1)$$

where δN_2O is the increase in head space N_2O concentration over time (μL/L); δT is the enclosure period (hours); M is the molar weight of N in N_2O; Vm is the molar volume of gas at the sampling temperature (L mol^{-1}); V is the headspace volume (m^3); and A is the area covered (m^2).

These hourly emissions were integrated over time, for each chamber, to estimate the total emission over the measurement period. The N_2O emission factors (EF, N_2O-N emitted as % of N applied) were then calculated for the urine treatments using Equation 2.

$$EF = \frac{N_2O\text{-}N\ total\ (treatment) - N_2O\text{-}N\ total\ (control)}{N\ applied} \times 100\% \quad (2)$$

where N_2O-N *total (treatment)* and N_2O-N *total (control)* are the cumulative N_2O-N emissions from the treatment and control plots, respectively (kg N ha^{-1}), and *N applied* is the rate of N applied in the urine (kg N ha^{-1}).

Soil measurements and analysis

Adjacent to each gas sampling ring a 0.2 m^2 soil sampling area was established within a 50 cm ring. The corresponding urine treatments were applied to these areas, but using artificial urine formulated to match the N concentrations of the real urine applied to the gas rings. Soil samples (75 mm depth) were taken on each gas sampling occasion, with 2 cores (25 mm diameter) bulked per plot. Alkathene sleeves were placed into the holes resulting from soil coring to minimise disturbance to the rest of the plot.

Gravimetric moisture contents were determined on each gas sampling occasion by drying samples of soil at 105°C overnight. Volumetric water contents were calculated by multiplying gravimetric water contents by soil bulk density. Soil particle density was assumed to be 2.65 mg m^{-3} (Danielson and Sutherland 1986). Soil water-filled pore-space (WFPS) was calculated by dividing volumetric water content by total soil porosity.

Soil N was measured on samples taken on nine occasions. The soils were sieved to 4 mm and extracted for two hours with 2 M KCl at a soil to extractant ratio of 1:10. The extracts were analysed for nitrate-N and ammoniacal-N using a Skalar SAN++ segmented flow analyser.

Results

Soil and climatic conditions

The summer and autumn preceding the trial were dry and warm. Rainfall for the period 1 January to 21 May 2019 at Matamata Airport (5 km distance from the trial site) totalled 159 mm which was 43% of the 30-year average rainfall of 369 mm for this period. During the trial period of 21 May to 13 August 2019 rainfall totalled 244 mm, which was 84% of the 30-year

average rainfall of 293 mm of this period. The daily air temperature averaged 14.1°C in the 1 January to 13 August period; the 30-year average is 13.6°C.

Soil WFPS values ranged from 55% to 81% throughout the trial period (Figure 1). The soil WFPS increased to around 70% after the first two weeks of the trial period. For the rest of the trial period the soil WFPS values were above 70%.

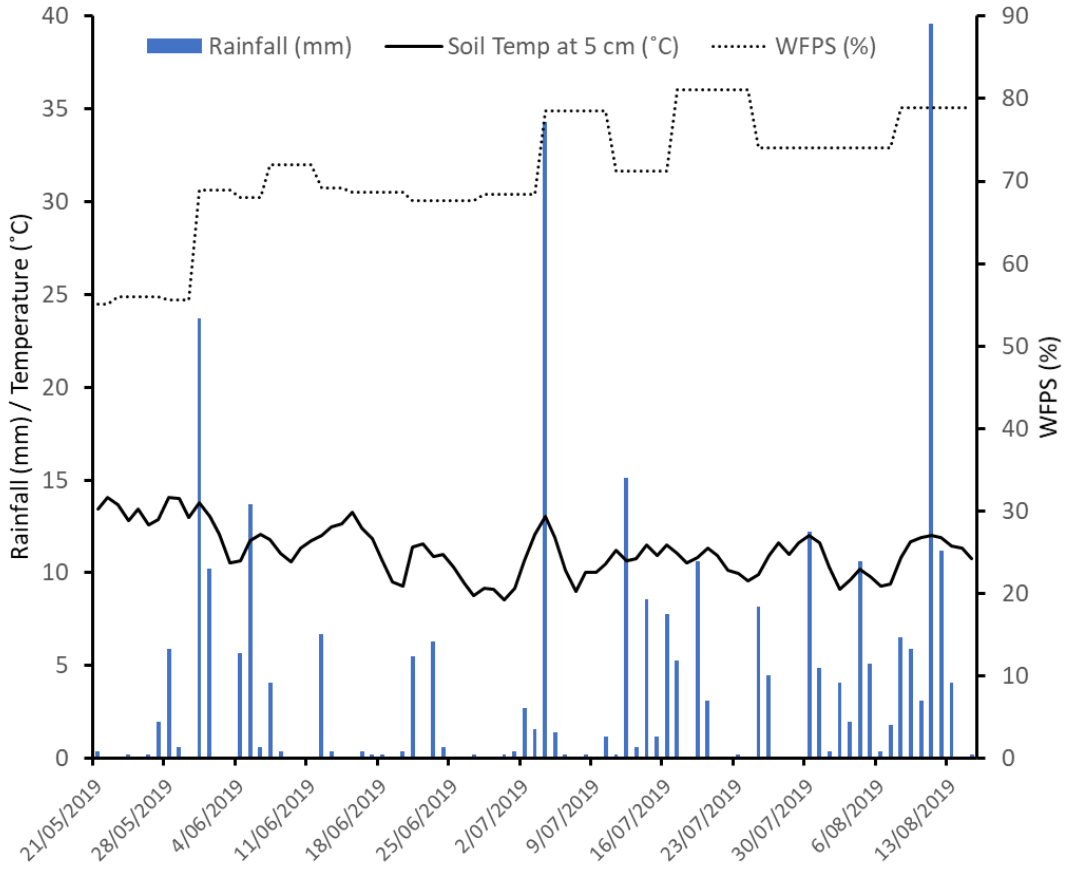

Figure 1: Daily rainfall, soil temperature at 5 cm depth and water filled pore space (WFPS) at 0-75 mm depth at a trial site for measuring N_2O emissions pastures.

Soil mineral N concentrations

Soil ammonium and nitrate N concentrations are shown in Figure 2. Concentrations of ammonium in the urine-treated soil reached their peak at three days post application of urine. The PRGWC urine-applied to RGWC pasture had the highest NH_4^+ levels at 318 kg N ha^{-1} and then declined over the following week to levels close to the nil urine treatments. The ammonium concentrations in the Nil urine RGWC and PRGWC pastures were about 10 and 3 kg NH_4^+-N ha^{-1}.

Soil nitrate concentrations in the urine treated plots reached their peak at ten days post application of urine. The RGWC urine-applied PRGWC pasture had the highest NO_3^- levels at 142 kg N ha^{-1} and then declined over the following four weeks to levels close to the nil urine treatments. The soil nitrate concentrations of the Nil urine PRGWC and the RGWC pastures were close to 0 kg NO_3^--N ha^{-1} throughout the trial.

It should be noted that artificial urine was used for application to the soil sampling plots, so the only difference between RGWC and PRGWC urine was the amount of N applied.

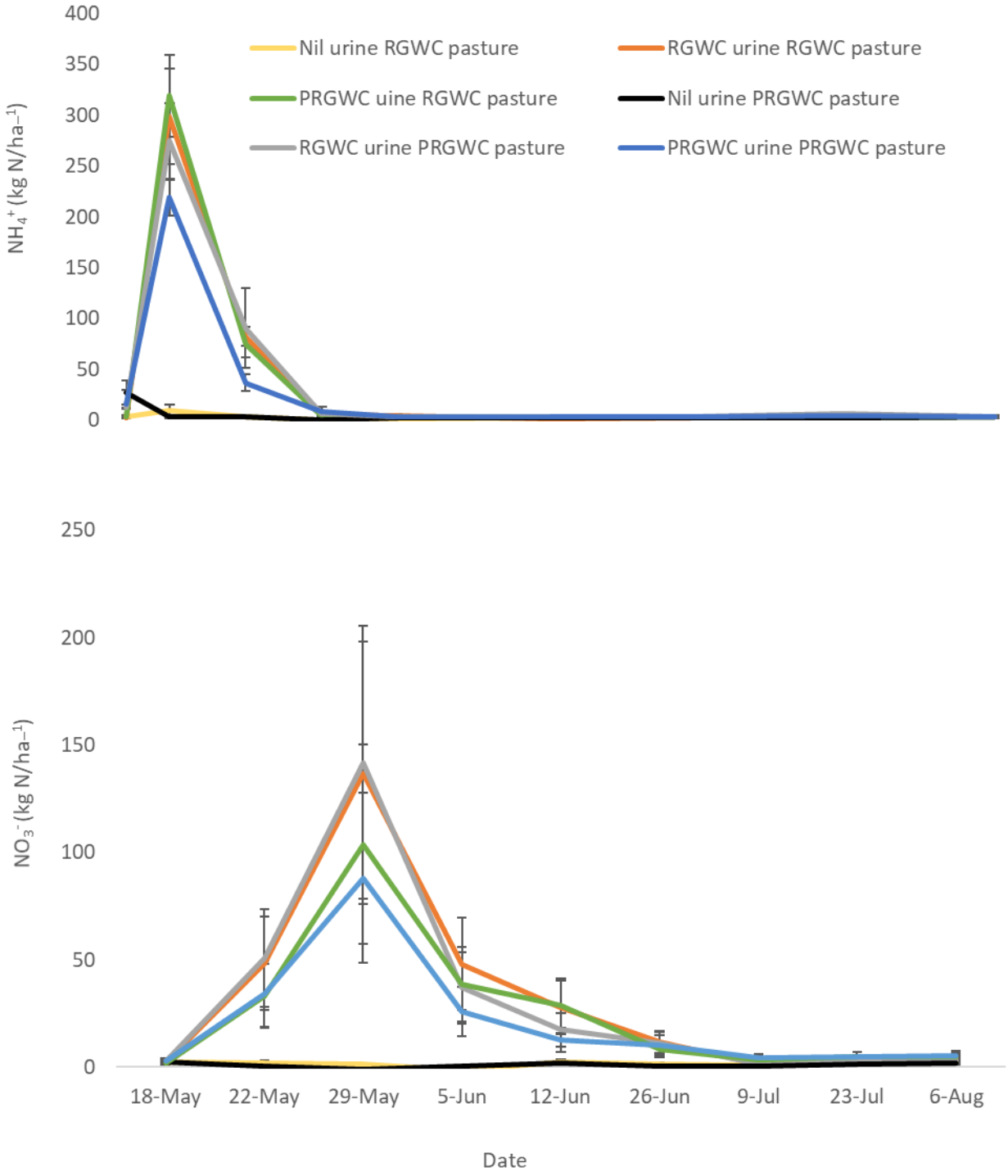

Figure 2: Changes in soil NH_4^+-N and NO_3^--N levels at 0-75 mm, treatment means and standard error (bars), n=5. Treatments were 100% white clover pasture (RGWC) or 60% plantain/40% ryegrass white clover pasture (PRGWC) receiving no urine (nil urine) or artificial urine with a urinary-N application rate of 388 kgN ha $^{-1}$ (RGWC) or 343 kgN ha $^{-1}$ (PRGWC) Treatments were applied on 19 May.

N_2O emissions

Background N_2O fluxes, measured on one occasion before the urine treatments were applied, were low (< 0.01 mg N m^{-2} hr^{-1}) with a range of 0.001 to 0.007 mg N m^{-2} hr^{-1}. N_2O fluxes from nil urine treatments on both pasture types were low (less than 0.01 mg N_2O-N m^{-2} hr^{-1}) throughout the measurement period (Figure 3). Changes in the fluxes in the nil urine treatments were not significantly correlated to changes in soil moisture levels, mineral nitrogen (N) concentrations or temperature.

Nitrous oxide fluxes from all RGWC and PRGWC plots sharply increased when sampled two hours after urine application. The mean fluxes for all urine treated plots were 1.881 and 0.593 mg N_2O-N m^2 hr^{-1} for RGWC and PRGWC respectively. The mean fluxes for the RGWC urine and PRGWC urine treated plots were 0.957 and 0.1.437 mg N_2O-N m^2 hr^{-1} respectively. The mean flux for all non-urine control plots was 0.006 mg N_2O-N m^2 hr^{-1}. By the following day the mean flux of all urine treated plots was 0.022 mg N_2O-N m^2 hr^{-1}.
The mean net cumulative N_2O emissions from the RGWC urine were 0.57 kg N_2O-N ha^{-1} when applied to the RGWC pasture and 0.40 kg N_2O-N ha^{-1} when applied to the PRGWC pasture (Figure 3). The mean cumulative net N_2O emissions from the PRGWC urine were 0.60 kg N_2O-N ha^{-1} when applied to the RGWC pasture and 0.41 kg N_2O-N ha^{-1} when applied to the PRGWC pasture. Overall, there was no difference in net cumulative N_2O emissions between the two urine types ($P > 0.05$), but the net cumulative N_2O emissions from the PRGWC pasture were significantly lower compared with those from the RGWC pasture ($P < 0.05$). Accordingly, the EF_3 values were not different when either urine type was applied to either RGWC soil or PRGWC soil (Table 2). However, the EF_3 values for the PRGWC sward (0.10-0.12%) were significantly lower than those for the RGWC sward (0.15-0.17%) ($P < 0.05$) (Table 2). The emission factors ranged from 0.10% to 0.17%, which is lower than the NZ default EF_3 value of 1% for deposited urine (MfE, 2019). The lower EFs observed in our study are possibly due to the relatively dry conditions prior to and during the experimental period.

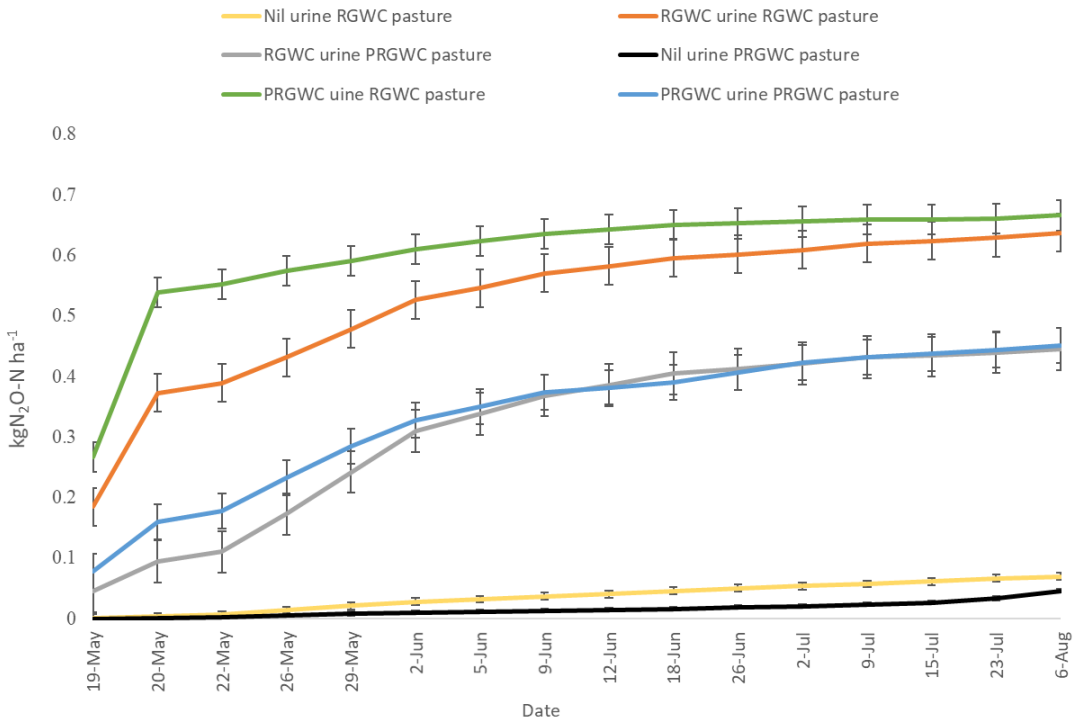

Figure 3: Cumulative N_2O emissions, treatment means and standard error (bars), n=5. Treatments were 100% white clover pasture (RGWC) or 60% plantain/40% ryegrass white clover pasture (PRGWC) receiving no urine (nil urine) or urine collected from animals on a RGWC or a PRGWC diet. Treatments were applied on 19 May.

Table 2. Average cumulative N_2O emissions, emission factors and standard error of the mean (SEM) of the cumulative emissions, n=5) of urine collected from animals on a 100% ryegrass white clover diet (RGWC) or on a 60% plantain/40% ryegrass white clover pasture (PRGWC) applied to RGWC pasture or PGRWC pasture.

	N_2O emissions (kg N_2O-N ha^{-1})	EF_3 (%)*	SEM
RGWC urine applied to RGWC pasture	0.57	0.15a	0.023
RGWC urine applied to PRGWC pasture	0.40	0.10b	0.033
PRGWC urine applied to RGWC pasture	0.60	0.17a	0.017
PRGWC urine applied to PRGWC pasture	0.41	0.12b	0.018

*EF_3 values followed by the same letter are not statistically different ($P < 0.05$).

Conclusions

Although the PRGWC urine contained 12% less N than the RGWC urine there was no measurable effect of animal diet on the N_2O EF_3 values of urine applied. However, N_2O EFs were significantly higher from urine applied to RGWC pasture than from PRGWC pasture. These results indicate that the effect of plantain on the reduction of N_2O emissions, observed in this study, was not a "urine composition" effect but a "sward" effect. This supports earlier findings by Simon et al. (2019), who concluded that possible explanations for this sward effect included biological nitrification inhibition from plantain root exudates, or plantain plant effects on the soil microclimate.

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