CAN NITROUS OXIDE EMISSIONS FROM SOIL BE REDUCED BY FEEDING LAMBS WITH FRESH FORAGE RAPE (BRASSICA NAPUS L.)?

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Abstract
In New Zealand, agriculture is predominantly based on animal grazing systems. Animal excreta have been identified as a major source of nitrous oxide (N_2O) emissions. Forage brassicas (*Brassica* spp.) have been increasingly used due to their faster growing ability, higher dry matter yield, higher nutritional value and having less neutral detergent fibre and more non-structural carbohydrates than perennial ryegrass (*Lolium perenne L.*). The aim of this study was to determine the partitioning of dietary N between urine and dung in the excreta from sheep fed forage brassica rape (*B. napus* subsp. *Oleifera* L.) and perennial ryegrass, and then to measure N_2O emissions when the excreta from the two different feed sources are applied to the soil. A sheep metabolism study was conducted to determine urine and dung outputs from sheep fed forage rape or perennial ryegrass and urine and dung N contents analysed for estimation of N partitioning between two types of excreta. Urine and dung collected simultaneously from another set of animals were then used in a field plot trial for measuring N_2O emissions. The experimental site contained perennial ryegrass and white clover (*Trifolium repens* L.) pasture on a poorly-drained silt-loam soil. The treatments included urine from sheep fed forage rape or ryegrass, dung from sheep fed forage rape or ryegrass, and a control without dung or urine applied. N_2O emission measurements were carried out using a static chamber technique and gas samples analysed by gas chromatography. Total N_2O emissions and the emission factors (EF3; N_2O-N emitted as a % of animal excreta N applied) for each excreta type were calculated. Excreta N transformations in the soil were also regularly measured. The results indicated that N use efficiency by sheep was equivalent for both forage rape and ryegrass. Urine from sheep fed forage rape showed higher N transformation rates in soil from organic N to ammonium-N and to nitrate-N, compared with that from those fed ryegrass. As a consequence, the EF3 was lower for urine from sheep fed forage rape compared to ryegrass. However, dung for sheep fed forage rape showed lower N transformation rates from organic N to ammonium-N and to nitrate-N and as a result, the EF3 was higher, although the difference was not significant. EF3 for sheep dung was substantially lower than that for sheep urine.

Keywords: Excreta N partitioning, sheep, brassicas, nitrous oxide

Introduction

In New Zealand, agriculture is predominantly based on pasture-based grazing systems. Agricultural soils have been identified as a major source of nitrous oxide (N_2O) emissions (Ministry for the Environment, 2011). N_2O is problematic as it contributes to global warming

by its action as a greenhouse gas and it is also involved in the destruction of stratospheric ozone (McTaggart *et al.* 1997).

In New Zealand N_2O emissions from agricultural soils account for approximately one-third of all greenhouse gas emissions made by the agricultural sector (Ministry for the Environment 2011). N_2O emissions in New Zealand are generally higher in winter when soils are wet and the emissions increase after grazing, due to the large amount of nitrogen (N) in the dung and urine that is excreted by grazing animals (Luo *et al*. 2008a, b).

Forage brassicas (*Brassica* spp.) are widely used in animal agriculture due to their fast growing ability, high dry matter yield and high nutritional value (Belesky *et al*. 2007). Forage brassicas also have less crude protein, less neutral detergent fibre and more non-structural carbohydrates than perennial ryegrass (*Lolium perenne L.*) (Sun *et al*. 2012). The lower crude protein content and the greater readily fermentable carbohydrate content of brassicas compared to ryegrass, is expected to potentially improve the efficiency of N utilisation in the rumen and consequently reduce N losses. However, little data is available describing the partitioning of dietary N between urine and dung from animals fed forage brassica rape, and the quantification of N_2O emissions from urine and dung when applied to the soil.

The aim of this study was to measure N output in excreta (urine and dung) from sheep fed forage rape (*B. napus* subsp. *Oleifera* L.) or perennial ryegrass, and to quantify N_2O emissions when these excreta were applied to a perennial ryegrass pasture soil.

Materials and methods

1) Animal experiment

A sheep metabolism study at the AgResearch Grasslands in Palmerston North, New Zealand, was used to collect urine and dung from sheep fed forage rape or perennial ryegrass. Twelve Romney male lambs at the age of 9-months and liveweight of 39.3 ±1.4 kg (mean ± S.D.) were randomly allocated to two groups fed either forage rape or ryegrass. The experiment was conducted in two periods. The first period of the experiment included 41 days of outdoor acclimation, 3 days in pens and 10 days in metabolism crates for further acclimation and 7 days of measurement. Animals were subsequently released to outdoors to graze the same forage-type they ate before for 38 days. The process was then repeated for the second period of the experiment, with 3 days in pens and 9 days in crates, followed by a further 7 days of measurement.

To minimise internal parasite burdens, the sheep were drenched three times. On the first day of the experiment, each sheep received 4 ml of parasiticide containing 8 mg abamectin, 320 mg Levamisol, 20 mg cobalt and 4 mg selenium (Intervet Schering-Plough Animal Health Ltd., Wellington, New Zealand). Another two drenches were performed on the day they were transferred indoors during the two indoor periods of the experiment. On these two occasions each animal was given a dose of 9 ml parasiticide containing 9 mg abamectin, 360 mg levamisole, and 204 mg oxfendazole via mouth (Merial New Zealand Ltd, Auckland, New Zealand). Minerals were supplemented according to their contents in the forage. On the third day of the experiment, 1.5 ml of iodised peanut oil containing 390 mg of organically bound iodine (Flexidine, Bomac Export Limited Auckland, New Zealand) and 4 g of CuO (Bayer New Zealand Limited, North shore, New Zealand) were supplied to sheep in the rape group. Salt (sodium chloride) was freely available to all animals during the outdoor periods and supplied about 5-10 g per day per animal.

In the indoor periods of the experiment, the animals were provided fresh cut forage twice a day in equal amounts at 09:00 and 16:00 h and water *ad libitum*. Feeding level was set at 1.5 times the metabolic energy (ME) maintenance requirement according to Australian Agricultural Council (1990). Feed allowance was calculated from animal liveweight, feeding level and the amount of ME in the forage. The latter was estimated using near infrared reflectance spectroscopy (NIRS; Bruker Optics, model MPA, Ettlingen, Germany). Forage chemical composition during two metabolism experiments is in Table 1.

Table 1 *Chemical composition (g/kg dry matter) of forage rape (Brassica napus subsp. Oleifera L.) and perennial ryegrass (Lolium perenne L.).*

	Period 1		Period 2	
	Rape	Ryegrass	Rape	Ryegrass
Organic matter	860	863	921	903
Crude protein	194	169	156	150
Readily fermentable carbohydrates*	195	71	319	149
Neutral detergent fibre	243	506	163	450
Acid detergent fibre				
Lignin	50	30	36	17

*Readily fermentable carbohydrates include hot water-soluble carbohydrates plus pectin.

Pure forage swards were established in paddocks at AgResearch Grasslands for providing feed for both the grazing and indoor experimental periods. Forage rape (*var*. Titan) was sown at a rate of 4.7 kg/ha together with diammonium phosphate at the rate of 140 kg/ha in a Manawatu fine sandy loam soil on 3 March 2011. Perennial ryegrass (*var*. Ceres One 50 containing endophyte AR1) was established in autumn 2008. Before the experiment, 200 kg/ha of superphosphate containing P 93 g/kg, S 108 g/kg and Ca 200 g/kg (Ravensdown Limited, Hornby, New Zealand) was applied on 12 April 2011, and 60 kg/ha of urea containing 460 g N/kg on 5 May 2011 and the paddock was grazed on 9 May 2011.

During the indoor experimental periods, forage for animals was harvested daily in mornings (10:30 to 12:00 h) using a sickle bar mower with a stubble height at *ca*. 10 cm for rape and *ca*. 5 cm for ryegrass. During both experimental periods, forage rape and perennial ryegrass were in a vegetative state. Harvested forage was stored in a cold room (4°C) until presented to the animals. The dry matter (DM) content of each forage-type was determined daily in triplicate at 105^{o}C for 24 h. One forage sample per forage-type per day was collected daily, dried at 65^{o}C for 48 h, pooled and ground in a Wiley mill through a 1 mm sieve for chemical analysis.

After acclimation, sheep were transferred to metabolic crates with a wire mesh floor. Harness and faecal bags were installed a day before collection. The crate was installed with a stainless steel tray placed below the wire mesh in order to capture and collect urine into a bucket. Each bucket had 100 ml of 6 M H_2SO_4 added in advance, to prevent ammonia volatilisation and stirring the contents was conducted twice daily (in the morning and in the afternoon). Uneaten forage (refusal), faecal and urine samples were collected once daily in the morning before feeding from all animals over the 7 day measurement period. Refusal samples were dried at 65°C for 48 h, weighed and pooled over the 7 days for each animal. Faecal samples were weighed and a 10% aliquot subsampled and stored at -20°C. Faecal subsamples were pooled over the 7 days for each animal, freeze-dried and then oven dried at 65°C for DM determination. Refusal and faecal samples were ground in a Wiley mill through a 1 mm sieve for N content determination. Collected urine was weighed daily and 50 ml aliquot sample

was taken and then stored at -20°C. At the end of the experiment, urine samples from each individual animal were thawed and pooled according to the proportion of urine output each day and the N content determined.

Forage samples were analysed for neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), N, ash and readily fermentable carbohydrates including hot water-soluble carbohydrate and pectin, using the same procedure as described in Sun et al. (2011). Crude protein (CP) was calculated from N content multiplied by 6.25. Organic matter (OM) was calculated from ash content.

2) Field experiment

Urine and dung simultaneously collected from another animal experiment in the second period were used in a field plot trial. The field plot experiment was established at the AgResearch Ruakura Farm in Hamilton, New Zealand. The experimental site contained perennial ryegrass and white clover (*Trifolium repens* L.) pasture, on a poorly-drained silt-loam soil (Te Kowhai soil). The Te Kowhai soil is classified as a Typic Orthic Granular soil in the Soil Classification system (Hewitt, 1998).

Excreta (urine and dung) were separately collected from 24 lambs that had been fed forage rape for 15 wks and from 18 lambs fed perennial ryegrass for the same period of time. Collection was conducted twice a day prior to morning and afternoon feedings for 2 consecutive days. No acid was added to urine. After each collection, urine and dung were kept in sealed containers in a cool room (4^oC) and after the final collection they were immediately transferred to the experimental site in iced chilly bins for application to the pasture.

The treatments, with four replicates of each, included: 1) urine from sheep fed forage rape, 2) urine from sheep fed ryegrass, 3) dung from sheep fed forage rape, 4) dung from sheep fed ryegrass and 5) control (without urine or dung).

The plots were approximately 0.5 m x 0.5 m set-up in a randomised block design, with 5 plots in each of 4 blocks. The individual treatments were randomly assigned to the treatment plots in each block and each plot containing an area for gas sampling and an area for destructive soil sampling. These plots received the same treatments (fresh sheep dung and urine) as for N_2O measurement.

Sheep urine was applied at a rate equivalent to 4 L/m^2. The application rate of urine for the sheep fed ryegrass was 441 kg N/ha and for the sheep fed forage rape was 155 kg N/ha. The urine was evenly applied to the entire 24 cm diameter area of the gas chamber. Sheep dung was applied at a rate equivalent to 5 kg/m^2. The application rate of dung for sheep fed ryegrass was 430 kg N/ha and for sheep fed forage rape was 890 kg N/ha. The dung was evenly spread to a plot 20 cm in diameter inside the 24 cm diameter gas chamber.

A soil chamber technique was used to measure N_2O emissions, and the methodology was based on that from previous studies on N_2O emissions (Luo et al., 2008c). The sampling chambers were modified PVC "sewer-hatches" attached to a section of PVC pipe. The chambers were 200 mm deep and with a 240 mm internal diameter. The "sewer-hatch" rim had an internal half-turn locking system and a greased rubber O-ring, which formed a gas-tight seal. Chambers were inserted 50-100 mm into the soil one day before excreta application. Chambers remained in place throughout each individual measurement period.

Chamber heights were measured and each chamber volume calculated. On each sampling day, the chamber was closed with a lid for 1 h, and the air above the soil surface was sampled through a three-way tap on the chamber lid, using a 60-ml syringe. A 25-ml air sample was taken from each chamber at 0 minutes (T_0) and 60 minutes (T_{60}).

Following the treatment application in early September 2011, N_2O emission measurements were carried out at least weekly. More frequent sampling occurred in the first month and following rainfall. On each sampling day, headspace gas samples were taken from each chamber during a cover period of 60 minutes, as explained above. Gas sampling from the two urine treatments was completed in December 2011 and sampling from the dung treatments continued until May 2012 when background levels were reached for both dung treatments.

Gas samples were analysed using a gas chromatograph equipped with a ^{63}Ni-electron capture detector. The measured hourly N_2O fluxes were calculated for each chamber, from the linear increase in head space N_2O concentration over the sampling time. The hourly fluxes were integrated over time to estimate the total daily emission and the emissions over the measurement period.

Emission factors (EF3) were then calculated from the difference in total emissions from each excreta treatment and the control treatment, divided by the rate of urine N or dung N applied, as described by equation 1:

$$EF3 = \frac{N_2O \text{ total (urine/dung)} - N_2O \text{ total (control)}}{\text{Urine/Dung N applied}} \times 100\% \quad (1)$$

Where EF3 is emission factor (N_2O-N emitted as % of urine-N or dung-N applied), N_2O total (urine/dung) and N_2O total (control) are the cumulative N_2O emissions from the urine/dung and control plots, respectively (kg N ha^{-1}), and Urine/Dung N applied is the rate of urine N or dung N applied (kg N ha^{-1}).

Soil samples (75 mm deep, 25 mm diameter) were taken from all plots for determination of soil nitrate, ammonium and water content. Immediately after sampling the hole was back-filled with sealed PVC tubes to minimise any effects on soil aeration. Back in the laboratory on the same day or the following day, the samples were thoroughly mixed and about 15 g of fresh soil (about 10 g dry soil equivalent) was extracted for 1 h in 100 ml of 2 M KCL. The filtered (using filter paper No 42 or equivalent) solutions were then frozen until analysed for nitrate (plus nitrite) and ammonium using a Skalar SAN^{++} segmented flow analyser (Skalar Analytical B.V., Breda, Netherlands). The nitrate method involves cadmium reduction to nitrite followed by diazotisation with sulphanilamide and coupling with N-(1-naphthyl) ethylenediamine dihydrochloride to form an azo die measured colourimetrically at 540 nm. The ammonium method is based on the modified Berthalot reaction. Ammonia is chlorinated to monochloramine which reacts with salicylate and is then oxidised to form a blue/green coloured complex which is measured colourimetrically at 660 nm.

The remainder of the mixed soil was dried at 105ºC for 24 h, to determine gravimetric soil water content. Water-filled pore space (WFPS) was calculated by dividing volumetric water content by total porosity. Total porosity was calculated as follows: 1–(bulk density/particle density). Volumetric water content was calculated by multiplying gravimetric water content by bulk density. Air and soil temperatures (at 10 cm depth) were monitored on each sampling day and rainfall was monitored continuously over the sampling period.

Results and discussion

1) Nitrogen excretions

In the first experimental period, DM intake of sheep in the rape group was 8% higher than that in the ryegrass group ($P<0.001$) (Table 2). With the higher N content in rape than in ryegrass, daily N intake of sheep fed rape was 23% higher compared with ryegrass ($P<0.001$). Although animals in the rape group ate 10% less DM than those in ryegrass group in the second period, rape had a slightly higher (4%) N content. As a result, the two groups of animals had similar N intake per day ($P=0.266$). Consequently, daily N output from urine was higher by 18% from animals fed rape than those fed ryegrass ($P=0.021$) in the first period, but was similar ($P=0.475$) for the two groups in the second period. In terms of N output from urine per unit of N intake (g/kg N intake), no differences were found between the two groups, with averages at 814 in the first period ($P=0.505$) and 485 in the second period ($P=0.297$).

Dung output from sheep fed rape was 36-39% less than that from those fed ryegrass ($P<0.0001$). Although dung from sheep fed rape was richer in N content than ryegrass, by 24% in the first period ($P<0.001$) and 8% in the second period ($P=0.085$), dung N output was still lower from sheep fed rape than those fed ryegrass by 25% and 31% ($P<0.001$) in the first and second periods, respectively. When dung N output is expressed as per unit of N intake, sheep fed rape excreted less N than those fed ryegrass in both periods ($P<0.01$).

In terms of N balance, sheep fed rape retained N, but those fed ryegrass lost N from their body in the first period. In the second period, sheep in both groups had positive N retention. Sheep fed rape kept 77 g more N in the body than those fed ryegrass, although due to a large variation among animals the difference was not statistically significant ($P=0.193$). The total N excreted as urine and dung was similar from sheep fed either rape or ryegrass ($P>0.118$), averaging 25.8 and 18.4 g N/d for the first and second periods, respectively. However, the proportion of urine N in total N excretions was higher by 0.07 ($P=0.001$) with sheep fed rape (0.83) than ryegrass (0.76) in the first period. In the second period, feeding rape (0.68) to sheep still had a higher (0.09, $P=0.001$) proportion of urine N in total N excretions than feeding ryegrass (0.59).

In this study, urine N output from sheep was higher (18%) for rape than for ryegrass in the first period of the experiment. This might result from higher (23%) N intake by sheep fed rape than those fed ryegrass. In terms of urine N output in total N intake, the ratio was 0.80 vs. 0.83 for rape and ryegrass, respectively. In the second period, sheep achieved similar amounts of N intake and excreted similar amounts of urine N for both forages. Although forage rape contained more readily fermentable and less structural carbohydrates than ryegrass, N utilisation had not been improved. However, dung N output from sheep fed rape was reduced by 25-31% compared with ryegrass. Therefore, urine N output per unit of N intake was similar for both forages (Table 2). Dung N output from sheep fed forage rape was slightly less than that from those fed ryegrass, but the difference was not statistically different ($P>0.05$). This suggests that N use efficiency by sheep was equivalent for both forage rape and ryegrass.

Table 2 *N excretions, partitioning in urine and dung, and N balance from sheep (n=6 per forage) fed fresh forage rape or perennial ryegrass.*

	Period 1			Period 2		
	Rape	**Ryegrass**	***P***	**Rape**	**Ryegrass**	***P***
Feed						
DM intake (kg/d)	0.895	0.826	<0.001	0.932	1.041	0.003
N content (g/kg DM)	31	27		25	24	
N intake (g/d)	27.9	22.7	<0.001	24.1	24.7	0.266
Urine						
Urine output (L/d)	4.89	3.98	<0.001	4.89	3.4	0.001
N content (g/L)	4.55	4.75	0.494	2.43	3.48	0.002
N output (g/d)	22.2	18.8	0.021	11.9	11.8	0.475
Dung						
Dung output (g/d)	0.179	0.293	<0.001	0.167	0.261	<0.001
N content (g/kg DM)	25.4	20.5	<0.001	33	30.5	0.085
N output (g/d)	4.5	6	<0.001	5.5	8	<0.001
N partitioning (Proportion of urine N in total N excretions)						
	0.83	0.76	0.001	0.68	0.59	0.001
N balance (g N/kg N intake)						
Urine N	797	831	0.505	493	476	0.297
Faeces N	163	264	0.001	228	321	<0.001
Retention N	40	-94	0.014	279	202	0.193

* DM = dry matter. n=6 each treatment each period. 6M H_2SO_4 was added into urine sample to prevent ammonia evaporation during collection.

2) Nitrous oxide emissions

The impacts of urine excreted by sheep fed forage rape compared to ryegrass on the fate of urine N in the soil are shown in Figures 1 and 2. The initial urine N transformation rates (organic N to ammonium-N to nitrate-N) were higher when urine from sheep fed forage rape was applied to the soil, compared to urine from sheep fed ryegrass. The peak nitrite and nitrate-N level occurred 5 days after the urine had been applied to the soil from the sheep fed forage rape, compared to 26 days for the urine from sheep fed ryegrass. The reason for this difference is unknown. It may be related to urine composition in the two types of urine or different urine-N loading rates used in this trial.

The duration of N_2O flux peaks from the application of urine from sheep fed forage rape was much shorter compared to the urine from sheep fed ryegrass (Figures 1 and 2). The difference in the duration of the N_2O flux peaks was closely associated with the different concentrations of mineral N, particularly nitrate-N, in the soil after the application of the two types of urine.

The highest N_2O fluxes recorded were generally associated with rainfall events (Figures 1 and 2). An increase in the water filled pore space (WFPS) of the soil creates anaerobic conditions, and this along with high levels of nitrogen and carbon availability in the soil led to a greater opportunity for N_2O production and emissions. Studies (e.g., Luo *et al*. 2008a, b, c) have shown that soil WFPS, of all measured variables, has the strongest influence on N_2O emissions from excretal N input. Generally, N_2O emissions are highest when soil WFPS is above soil field capacity.

The impacts of sheep fed ryegrass compared to forage rape on the fate of dung N in the soil are shown in Figure 3. The initial dung N transformation rates were slower when dung from sheep fed forage rape was applied to the soil, compared to dung from sheep fed ryegrass. A greater soil ammonium-N concentration was found where dung from sheep fed forage rape was applied to the soil. The N transformation rates from dung applied by the two different types of feeds were in contrast to that happened when urine N was applied to the soil from the two different forage types.

In the dung treated soil the duration of N_2O flux peaks from the dung of sheep fed forage rape was longer than that of the flux peaks from the application of dung from sheep fed ryegrass, again in contrast to what was shown when urine from the two different types of fed was applied to the soil. However, as in the urine treatments, the difference in the duration of the N_2O flux peaks was closely associated with or probably caused by the different concentrations of mineral N in the soil after the application of the two types of sheep dung (Figure 3).

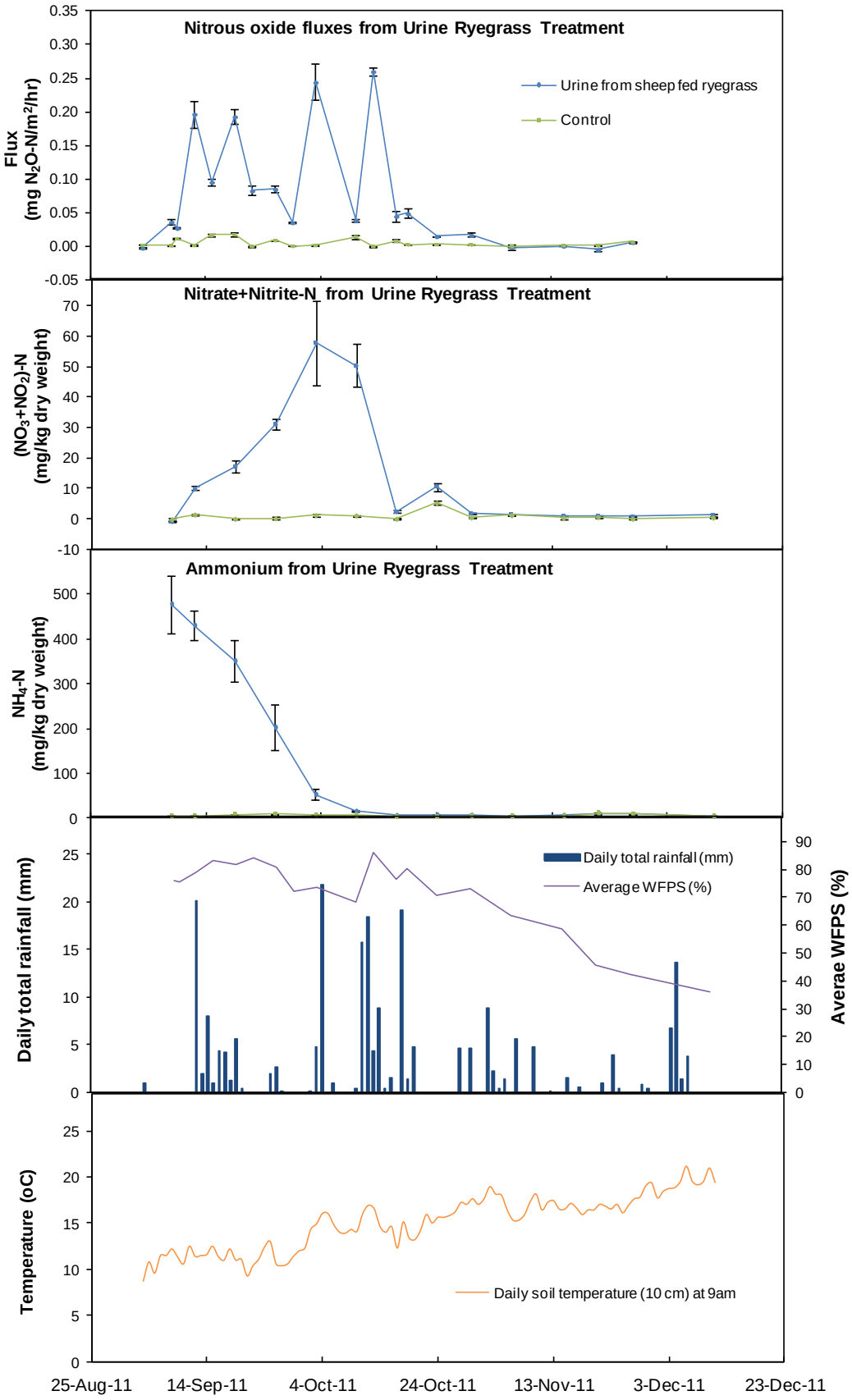

Figure 1

Nitrous oxide emissions, mineral N concentrations and soil and climatic conditions after application of urine from sheep fed ryegrass.

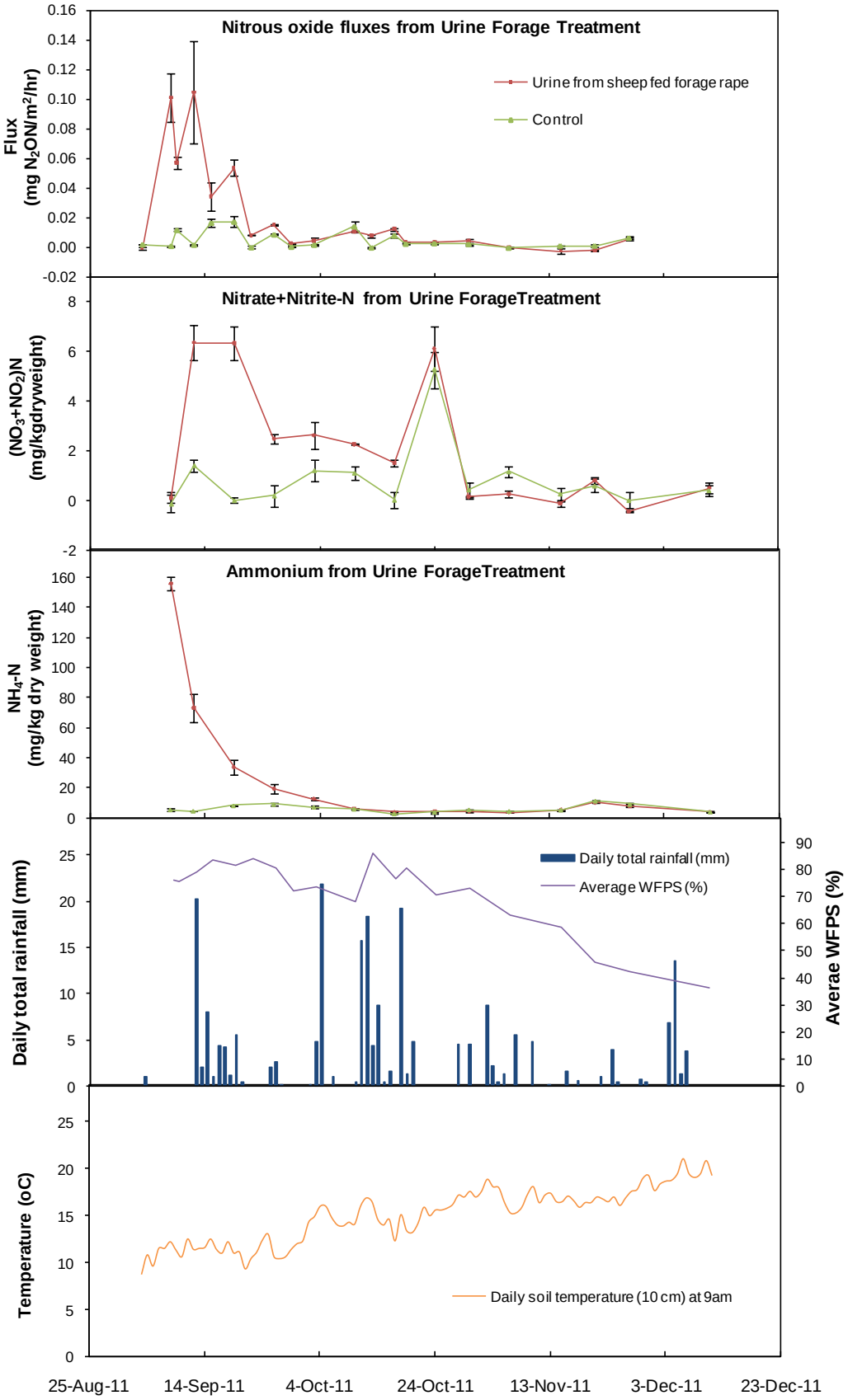

Figure 2 *Nitrous oxide emissions, mineral N concentrations and soil and climatic conditions after application of urine from sheep fed forage rape.*

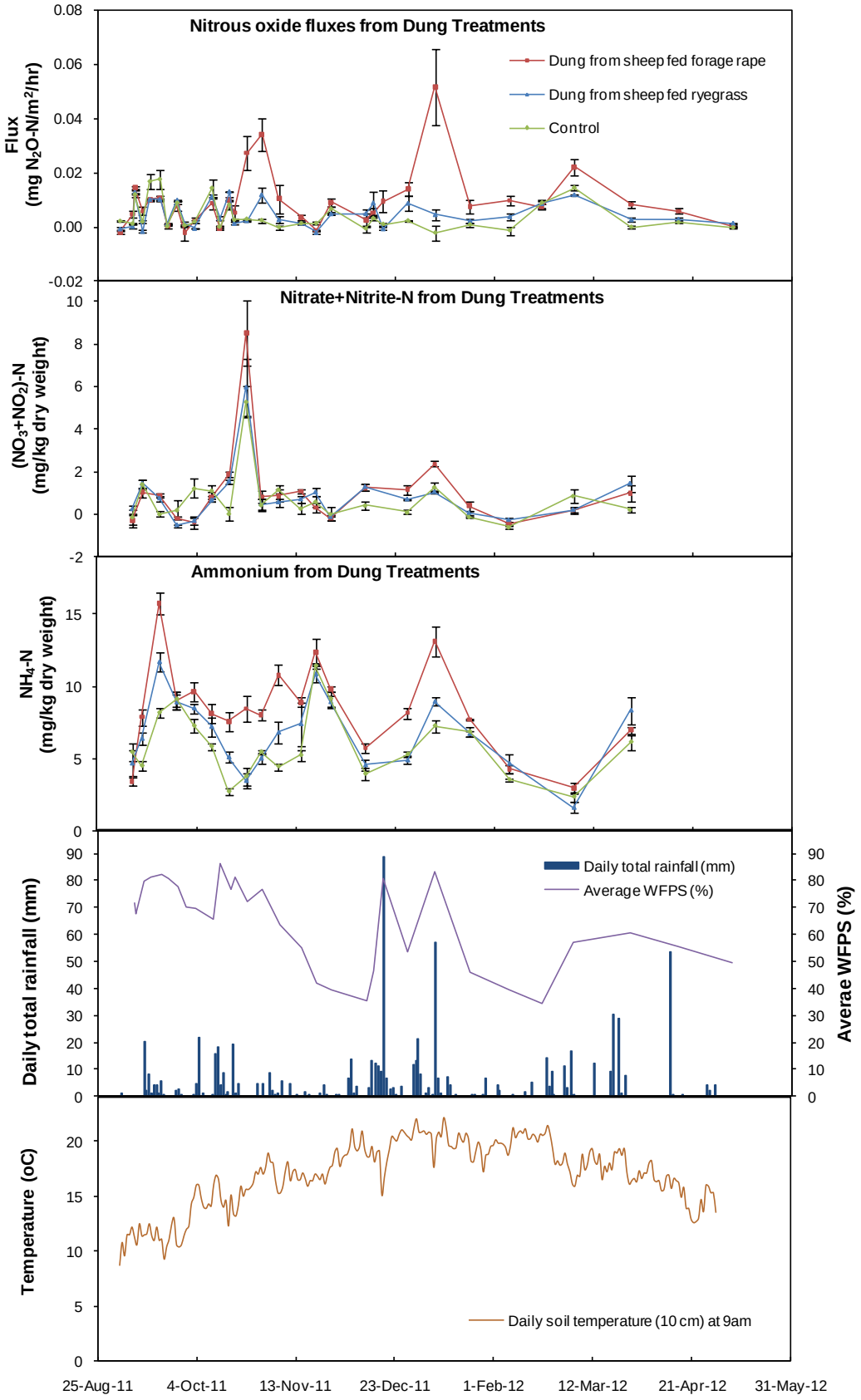

Figure 3 *Nitrous oxide emissions, mineral N concentrations and soil and climatic conditions after application of dung from sheep fed ryegrass and forage rape.*

The emission factors (EF3) from the urine and dung treatments are an important factor to consider in terms of the success of the two different types of feed at reducing N_2O emissions. Table 3 shows the EF3 from each treatment and the associated standard error. The emission factor for urine from the sheep fed ryegrass (0.27%) was higher ($P<0.05$) than that from forage rape (0.11%). Accordingly, the use of forage rape reduced EF3 for sheep urine by about 60%, compared to the use of ryegrass. The finding is significant for developing mitigation measures to reduce EF3 for animal urine. If this finding is confirmed, the use of forage rape could be promoted as one method for reducing N_2O emissions from animal production systemsFurther field trials need to be conducted to confirm this finding. As well, the reason for such effect needs to be explored.

In this trial urine was applied at the same volume for the two types of urine, rather than the same N loading rate, to mimic animal urination in the grazing situations. This led to different N loading rates between the two types of urine which may affect the EF3 results. However, the effect of urine N loading rate on EF3 is not conclusive (de Klein and Luo unpublished). In contrast the emission factor for dung from the sheep fed ryegrass (0.03%) was lower than that from forage rape (0.08%), although the difference was not statistically significant ($P>0.05$). As observed in other trials (e.g. Luo *et al.* 2012), the N_2O emission factor for sheep dung was much lower than that for sheep urine.

Table 3 *Emission factors from the various treatments comparing sheep fed ryegrass and forage rape*

Treatment	Emission Factor (%)	Standard Error
Urine from sheep fed ryegrass	0.27	0.026
Urine from sheep fed forage rape	0.11	0.056
Dung from sheep fed ryegrass	0.03	0.028
Dung from sheep fed forage rape	0.08	0.026
LSD* at $P<0.05$	0.10	

*Least significant difference

Conclusions

The efficiency of N use by sheep was equivalent for both forage rape and ryegrass. The use of forage rape reduced EF3 for sheep urine by about 60%, compared to the use of ryegrass. The findings from this study are important to assess the effect of diet on N_2O emissions. However, before any mitigation can be claimed/recommended the results of the current study must be evaluated in the context of a whole farm system. Further field trials under different climate and soil conditions need to be conducted for the confirmation of these findings. Additionally, the mechanisms behind the effects observed in the present study need to be explored.

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