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PLANTAIN SWARD: IS IT EFFECTIVE IN REDUCING N_2O EMISSIONS IN SPRING AND AUTUMN?

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Abstract

Several studies have indicated that the use of novel swards such as plantain (*Plantago lanceolata*) can reduce nitrogen (N) losses from grazed systems to air and water. Relative to perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) swards, cows grazing plantain excrete smaller quantities of N in urine thereby, potentially, lowering N_2O emissions from urine patches. Recently, some studies have also suggested that bioactive compounds in plantain, mainly aucubin, could play a role in inhibiting the nitrification process in the N cycle.

The potential for plantain to decrease N_2O emissions was evaluated at Massey University's Dairy Farm 4 on a Tokomaru silt loam soil by using the static chamber method in two field experiments/different seasons (spring 2017 and autumn/winter 2018). Urine treatments evaluated were, urine from cows grazing plantain, urine from cows grazing ryegrass/white clover and water (as control), which were applied to two sward treatments, plantain and ryegrass/white clover. Each treatment was replicated five times resulting in 15 soil plots for mineral N and moisture content and 15 chambers for N_2O measurements. Urine was applied using a metal ring of 0.5 m diameter at a hydraulic loading rate of 10 L m^{-2}. Soil samples were taken to determine soil mineral N and soil moisture content. In spring, urine treatment did not affect (P= 0.2665) N_2O emissions when the urine N concentration in plantain and ryegrass/white clover urine ranged between 2.81- 3.18 g L^{-1}. In autumn/winter, N_2O losses from plantain urine were lower (P< 0.003) than from ryegrass/white clover urine when the urine N concentration in the plantain urine (3.85 g L^{-1}) was 22% lower compared to ryegrass/white clover urine (4.99 g L^{-1}). In spring N_2O emissions from the ryegrass/white clover sward were higher (P= 0.0281) than from the plantain sward. However, in autumn/winter, the soil under plantain was wetter (P< 0.05) resulting in the plantain swards producing more (P< 0.0001) N_2O compared to the ryegrass/white clover sward. Therefore, in spring the plantain sward reduced N_2O emissions regardless of the urine treatment, probably due to the bioactivity of aucubin. However, in autumn/winter, N_2O losses from the plantain sward were higher than those from the ryegrass/white clover sward due to greater water filled pore space.

Introduction

In dairy systems, the high N loading in urine patches exceeds the N requirements of plants, increasing the risk to be lost to the environment as N_2O and nitrate (NO_3^-) leaching. Nitrous oxide is a greenhouse gas with a global warming potential 298 times greater than CO_2 (IPCC, 2014) that contributes to stratospheric ozone depletion (Ravishankara, Daniel, & Portmann, 2009). In New Zealand, 95% of the N_2O emissions come from agricultural sources, specifically from urine and dung patches from animals grazing all year round (Ministry for the Environment, 2019). Under the Paris Agreement, New Zealand has committed to reduce the greenhouse gases by 30% below 2005 levels by 2030 (Ministry for the Environment, 2019).

Different strategies developed to mitigate the environmental impacts of N losses from urine patches have been described in previous studies to reduce N_2O emissions from soils. Recently in New Zealand, researchers have studied plantain (*Plantago lanceolata* L.) as a potential option to reduce N_2O emissions. Plantain is a forage pasture that produces and releases bioactive compounds with potential biological nitrification inhibitor (BNI) properties. To date, research has demonstrated that a plantain sward can potentially inhibit the nitrification into the soil (Gardiner et al. 2019; Luo et al. 2018; Simon et al. 2019). The use of plantain swards on farms could potentially mitigate N losses from urine patches. Therefore, the objective of this study was to determine the effect of plantain sward on N_2O emissions following urine application onto pastures in comparison to ryegrass/wc sward.

Materials and methods

Experimental site and treatments

Two field experiments were conducted in (i) spring (29 September- 8 November 2017) and (ii) in autumn/winter (4 May- 26 July 2018). The experiments were laid out in a 2x2 factorial design with two sward treatments and two urine treatments. The sward treatments were: pure plantain, (cv Agritonic), and ryegrass/wc (cv. Trojan and Emerald, respectively); and the two urine treatments were urine of cows fed by grazing either pure plantain or ryegrass/wc, plus a control treatment where water (nil) was applied in the sward treatments.

The research area, in both experiments, comprised two areas where all treatments were applied at the same time: (i) N_2O chambers and (ii) soil plots. The plantain and ryegrass/wc swards were cut to 5 cm above ground level and the defoliated plants were removed from the plots before the urine application. Each treatment was replicated five times, resulting in 15 chambers for N_2O measurements and 15 soil plots for mineral N and moisture content analyses.

Urine was applied at a hydraulic loading rate of 10 L m^{-2} (equivalent to 10 mm) using a metal ring (0.5 m diameter) that was removed after urine infiltrated into the soil. Control treatments received the same volume of water. Urine was analysed for total N and urea-N content.

Nitrous oxide

Nitrous oxide samples were collected using the static chamber method. Each chamber had an 0.80 m internal diameter and a height of 0.30 m, therefore, the volume of the chamber was 150.7 L. Stainless-steel rings (0.80 m diameter), one for each chamber, were inserted 0.10 m into the soil; and remained into the field until the end of each experiment. On each sampling day the chambers were placed in the stainless-steel rings and sealed with bicycle tubes, which were inflated to provide a gas-tight seal. Then the chamber was covered by a wet towel to help maintain a constant temperature inside the chambers. Chambers were closed for two hours and gas samples were taken at time 0, 60 and 120 minutes, due to the large volume of the chambers. In both trials, for the first 4 weeks, N_2O samples were taken twice a week and for the remainder of the experiment, samplings were done weekly until background level was reached. On each sampling day, three air samples were also taken at each time.

The N_2O fluxes were calculated using the slope of the linear increase of N_2O concentration in the chamber over time using the following equation:

$$N_2O\ flux = \frac{\delta N_2O}{\delta T} * \frac{M}{Vm} * \frac{V}{A}$$

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

Soil sampling

Before treatment application, soil samples were collected from the area of the experiment. Following the application of the treatments, soil samples (25 mm diameter and 300 mm depth) were collected periodically from the soil plots adjacent to the N_2O. Each soil plot (0.5 m x 0.5 m) was separated by a 0.5 m buffer. The sampled soil was analysed for soil mineral-N (NH_4^+-N and NO_3^--N) and soil moisture content. Soil samples were taken 1, 7 and 14 days after application of treatments, then after a month of urine application and at the end of the experiment. At each sampling, three soil cores were bulked to produce a single sample of each plot. A sub-sample of 3 g of field moist soil was extracted with 25 mL of 2M potassium chloride (KCl). The extract was analysed for nitrate (NO_3^--N) and ammonium (NH_4^+-N) concentrations colorimetrically.

A wet soil sample per plot was weighed and then dried at 105°C for 24 hours. After drying, each sample was weighed again, and the gravimetric water content was calculated. Water-filled pore space (WFPS %) was calculated from gravimetric soil moisture content, bulk density and a particle density of 2.65 Mg m^{-3}.

Although soil moisture content in plantain sward was higher than in ryegrass/wc sward in autumn/winter, difference in WFPS between both plantain and ryegrass /wc sward were not recorded. Therefore, in autumn/winter 2019 a small field experiment was carried out, which involved taking soil samples (25 mm diameter) from 0-5 cm depth in plantain and ryegrass/wc swards. Sampling started before the rain season began and sampling continued until July. Soil samples were taken before a rainfall event and two days after it to allow the soil to drain. At each sampling, seven soil cores were bulked to produce a single sample from each plot, resulting in five replicates per sward.

Results and discussion

Urine composition

In the spring experiment, total N concentrations in the plantain and ryegrass/wc urine were 2810 and 3180 mg L^{-1}, respectively (Table 1). The urine applied in the autumn/winter experiment had higher concentrations of total N than urine collected in spring, in plantain and ryegrass/wc being 3850 and 4990 mg L^{-1}, respectively.

Table 1. Chemical composition of the dairy cow urine used in both spring and autumn experiments (*n*=3).

	Urine	Total N (mg L^{-1})
Spring 2017	Plantain	2810
	Ryegrass/wc	3180
Autumn/winter 2018	Plantain	3850
	Ryegrass/wc	4990

Nitrous oxide emissions

Daily N_2O fluxes showed temporal variations and the emissions from all treatments sharply increased after urine application for both spring and autumn/winter experiments. In both experiments, N_2O fluxes varied in response to rainfall events.

During the spring experiment, both urine treatments exhibited only one N_2O peak on day 22 after urine application regardless of the sward treatment, (Fig. 1a). On that day, the highest N_2O peak was from ryegrass/wc sward and ryegrass/wc urine (0.370 kg N_2O ha^{-1} day^{-1}). Cumulative N_2O emissions from the ryegrass/wc sward were significantly higher (P= 0.0281) than from the plantain sward (Table 2). Previous studies have reported a reduction in N_2O emissions from plantain sward compared to ryegrass/wc when the same urine type was applied (Luo et al., 2018, Simon et al., 2019). However, in this experiment there was not a significant effect of the urine treatment on N_2O emissions (P= 0.2665). In the ryegrass/wc sward, total N_2O emissions from ryegrass/wc and plantain urine were not statistically different (P= 0.3216), being 4.84 and 4.17 kg N_2O ha^{-1}, respectively. Similarly, in the plantain sward, there was no significant difference (P= 0.3027) in cumulative N_2O emissions between the ryegrass/wc and plantain urine, being 3.49 and 2.79 kg N_2O ha^{-1}, respectively.

During the autumn/winter experiment in both the ryegrass/wc and plantain swards, N_2O emissions exhibited major peaks on three days, 11, 19 and 43, after urine application (Fig. 1b). After day 57, N_2O emissions reached the background level. Nitrous oxide emissions from plantain urine were reduced by 22% (P< 0.003) compared to ryegrass/wc urine as plantain urine had lower N concentration than ryegrass/wc urine. Contrary to the results from spring, in autumn cumulative N_2O emissions from both urine treatments applied to the plantain sward were significantly higher (P< 0.0001) than from ryegrass/wc sward.

Nitrous oxide fluxes from the water control treatments in both experiments ranged between 0.001 and 0.161 kg N_2O ha^{-1} d^{-1}, remaining constant and low during both trial periods.

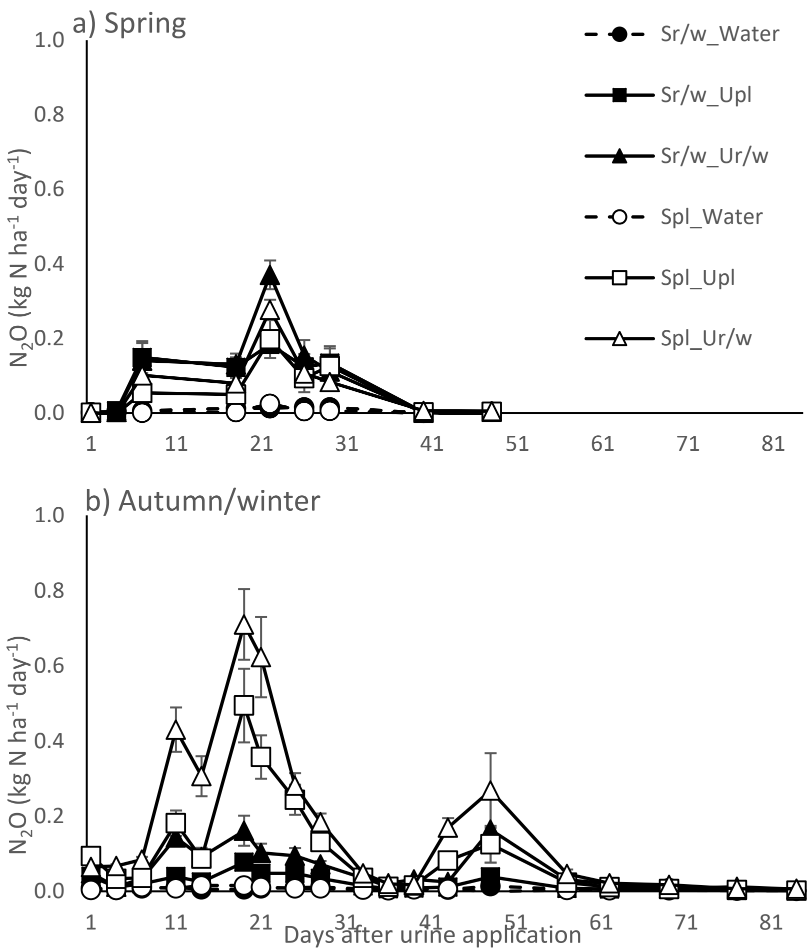

Fig. 1. Nitrous oxide fluxes from urine patches in pure plantain and ryegrass/ white clover swards, treated with urine from cows fed on pure plantain and ryegrass/ white clover pastures; a control was also included were water was applied. (a) Spring 2017 experiment; (b) Autumn/winter experiment 2018. Error bars are SEM (n= 5). $S_{r/w}$_Water = ryegrass/WC sward with water; $S_{r/w}$_ U_{pl} = ryegrass/WC sward with plantain urine; $S_{r/w}$_$U_{r/w}$ = ryegrass/WC sward with ryegrass/WC urine; S_{pl}_Water= plantain sward with water; S_{pl}_ U_{pl} = plantain sward with plantain urine; S_{pl}_$U_{r/w}$= plantain sward with ryegrass/WC urine.

Table 2. Cumulative N_2O emissions (kg N_2O ha^{-1}) during spring 2017 and autumn/Winter 2018.

	Spring 2017		Autumn/Winter 2018	
	Cumulative N_2O (kg N ha^{-1})	Reduction (%)	Cumulative N_2O (kg N ha^{-1})	Reduction (%)
$S_{r/w}$_$U_{r/w}$	4.84 ± 0.77	n.a.	4.71 ± 1.03	n.a.
$S_{r/w}$_U_{pl}	4.17 ± 0.57	-13.8	1.74 ± 0.23	-63
$S_{r/w}$_Water	0.35 ± 0.12	n.a.	0.53 ± 0.13	n.a.
S_{pl}_$U_{r/w}$	3.49 ± 0.45	-28	13.07 ± 1.99	+177
S_{pl}_U_{pl}	2.79 ± 0.55	-42	7.41 ± 0.92	+157
S_{pl}_Water	0.18 ± 0.08	n.a.	0.48 ± 0.10	n.a.
<u>Significance</u>				
Sward type	0.0281		<.0001	
Urine type	0.2665		0.0030	
Interaction	0.8544		0.2871	

$S_{r/w}$_Water = ryegrass/WC sward with water; $S_{r/w}$_ U_{pl} = ryegrass/WC sward with plantain urine; $S_{r/w}$_$U_{r/w}$ = ryegrass/WC sward with ryegrass/WC urine; S_{pl}_Water= plantain sward with water; S_{pl}_ U_{pl} = plantain sward with plantain urine; S_{pl}_$U_{r/w}$= plantain sward with ryegrass/WC urine. %Reduction: % of reduction in comparison with RG/WC urine applied to ryegrass soil for each season. n.a. not applicable

Soil moisture content

In spring the soil WFPS values ranged from 22% to 78% in plantain sward and from 25% to 71% in RG/WC soil during the trial period (Fig. 2a). Generally, plantain soil showed higher soil moisture content than RG/WC soil, but it was not statistically different.

During the autumn/winter experiment (Fig. 2b), soil WFPS from plantain sward were similar but numerically higher than ryegrass/wc, but only at the end of the experiment were those values significantly higher ($P< 0.05$).

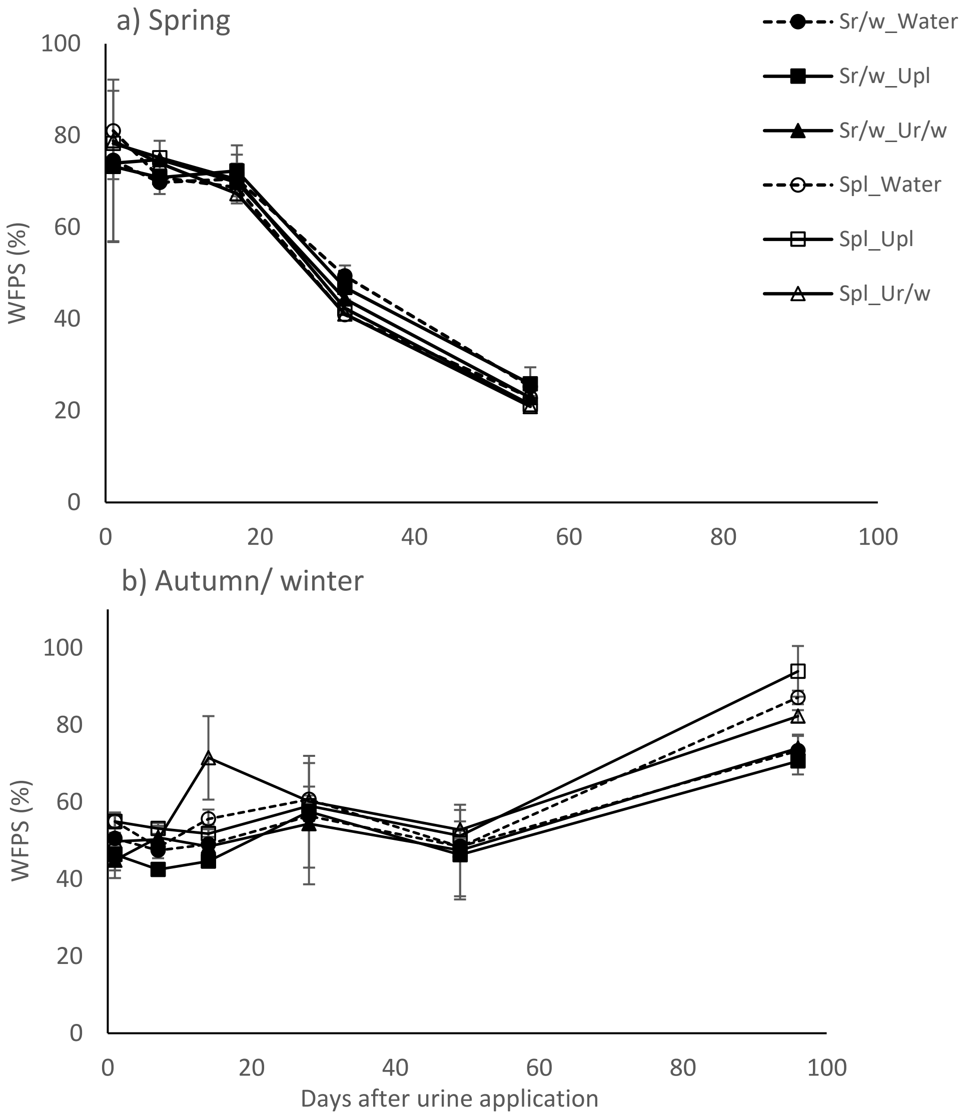

Fig. 2. Soil water filled- pore space (%) in pure plantain and ryegrass/ white clover swards, treated with urine from cows fed on pure plantain and ryegrass/ white clover pastures; a control was also included where water was applied. (a) Spring 2017 experiment; (b) Autumn/winter 2018 experiment. Error bars are standard error of the mean (SEM) (n= 4). $S_{r/w}$_Water = ryegrass/WC sward with water; $S_{r/w}$_ U_{pl} = ryegrass/WC sward with plantain urine; $S_{r/w}$_$U_{r/w}$ = ryegrass/WC sward with ryegrass/WC urine; S_{pl}_Water= plantain sward with water; S_{pl}_ U_{pl} = plantain sward with plantain urine; S_{pl}_$U_{r/w}$= plantain sward with ryegrass/WC urine.

Figure 3 shows the WFPS from soil samples taken in autumn 2019. Water filled pore space was significantly higher (P= 0.0496 and P = 0.0398) in plantain than ryegrass/ white clover on 8/7 and 14/7.

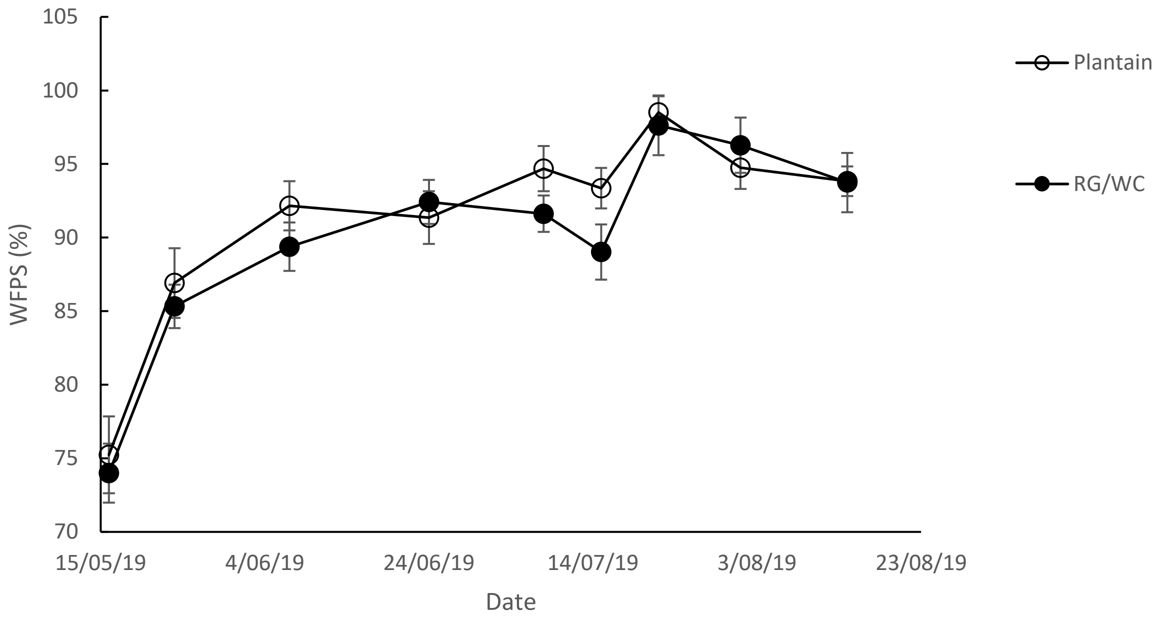

Fig. 3. Soil water filled pore space (WFPS%) in plantain and ryegrass/WC sward sampled in autumn 2019. Error bars indicate the SEM (n=5)

Mineral N

For the spring experiment, soil mineral N peaked on day 7 after urine application, reaching 443.17 mg N kg^{-1} in ryegrass/wc sward and plantain urine ($S_{r/w}$_U_{pl}) treatment, followed by plantain sward and plantain urine (S_{pl}_U_{pl}), plantain sward and ryegrass/wc urine (S_{pl}_$U_{r/w}$) (374.60 and 367.55 mg N kg^{-1}, respectively) and ryegrass/wc sward and ryegrass/wc urine ($S_{r/w}$_$U_{r/w}$) (305.29mg N kg^{-1}). Following day 7 after urine application, soil mineral N in both swards gradually decreased reaching water control treatment concentrations 55 days after urine application (Fig. 4a).

For the autumn/winter experiment, soil mineral N peaked between days 7 and 14 after urine application (Fig. 4b). On day 7, mineral N was higher from plantain sward than ryegrass/wc sward (P= 0.0416) and from ryegrass/wc (P< 0.0001) urine than plantain urine. On day 14, mineral N concentration was higher (P= 0.0002) from ryegrass/wc urine than plantain urine and there were no differences (P= 0.1942) between swards.

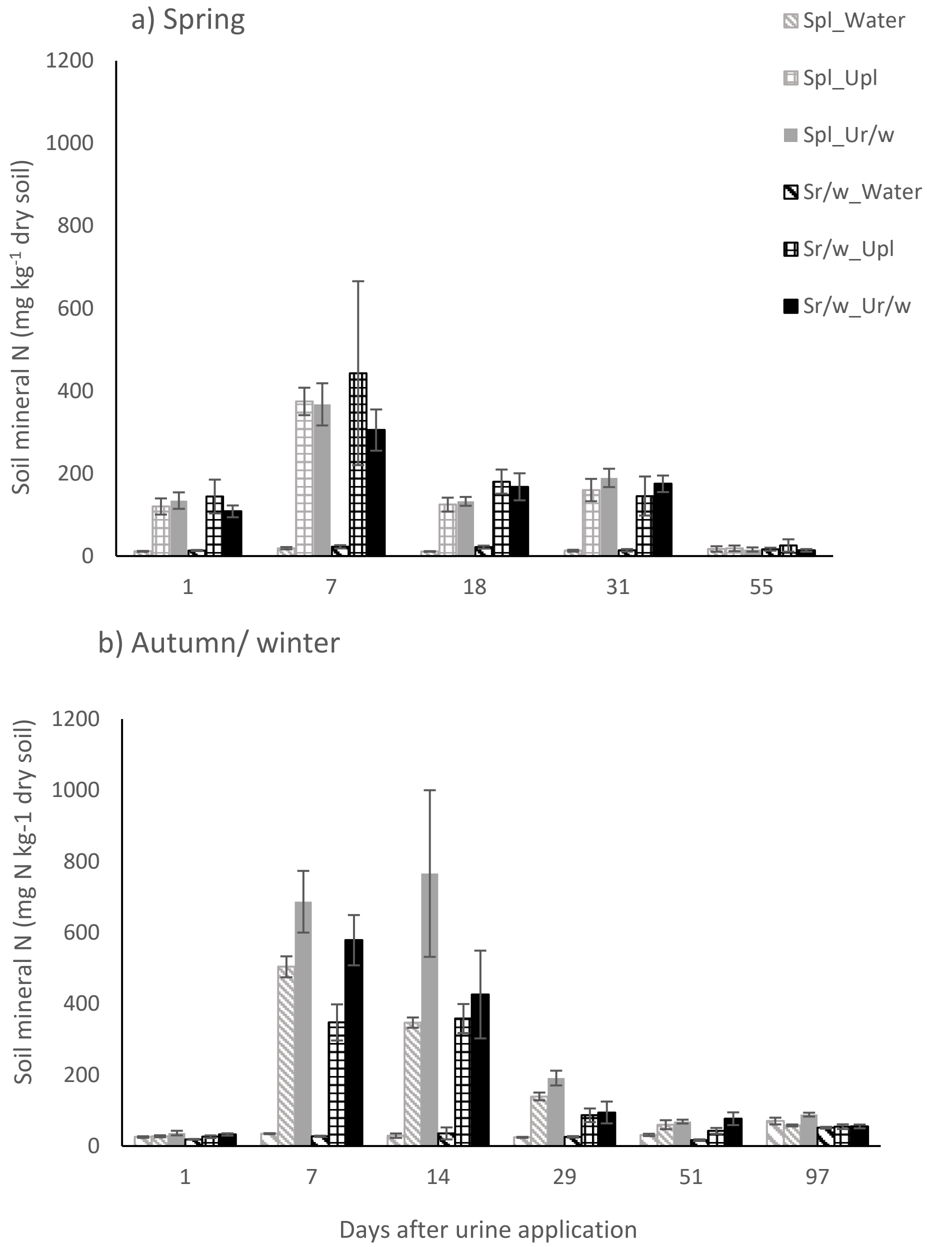

Fig. 4. Soil mineral (mg N kg^{-1}) at 0-30cm soil depth in pure plantain and ryegrass/ white clover swards, treated with urine from cows fed on pure plantain and ryegrass/ white clover pastures; a control was also included were water was applied. (a) Spring 2017 experiment; (b) Autumn/winter 2018 experiment. Error bars are SEM (n= 4). $S_{r/w}$_Water = ryegrass/WC sward with water; $S_{r/w}$_ U_{pl} = ryegrass/WC sward with plantain urine; $S_{r/w}$_$U_{r/w}$ = ryegrass/WC sward with ryegrass/WC urine; S_{pl}_Water= plantain sward with water; S_{pl}_ U_{pl} = plantain sward with plantain urine; S_{pl}_$U_{r/w}$= plantain sward with ryegrass/WC urine.

Conclusions

In spring plantain sward reduced N_2O emissions, regardless of the urine treatment, suggesting a sward effect. However, in autumn/winter, N_2O losses were higher from plantain than ryegrass/wc due to a greater WFPS and N_2O emissions from plantain urine were lower than ryegrass/wc due to an urine effect.

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