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PLANTAIN MIXED PASTURES: BOTANICAL COMPOSITION AND NITROUS OXIDE EMISSIONS FROM COW URINE PATCHES

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Abstract

Plantain (*Plantago lanceolata* L.) is recognised as improving summer feed productivity and quality as well as mitigating nitrogen (N) pollution from grazed pastoral soils. A field study was conducted to monitor the proportion of plantain required in perennial ryegrass and white clover (RWC) mixed swards and to evaluate impacts of plantain mixed pastures on nitrous oxide (N_2O) emissions from urine patches. The botanical composition and persistence of plantain was monitored when it was sown at rates to provide 0%, 30%, 50% and 70% in RWC pastures under grazing with dairy cows over two growing years (2019–20 and 2020–21). The N_2O emissions from urine patches of cows grazing 0%, 30% and 50% plantain mixed pastures were measured during the summer-autumn of 2021. The proportion of plantain in mixed pastures peaked at 50% during the summer-early autumn period in both growing years. Plant density of plantain in the 30% and 50% plantain mixed swards increased by around 38% to 103 plants/m^2 and 142 plants/m^2, respectively whilst plantain population in the 70% plantain mixed pasture treatment declined to 148 plants/m^2 at the end of the first year. In the second year, the concentrations of N in the urine from cows grazing 30% (5.40 g N L^{-1}) and 50% (4.40 g N L^{-1}) plantain pastures were lower than from cows fed RWC (6.15 g N L^{-1}). A lower N content in urine and therefore lower urine N-loading rate from cows grazing 50% plantain mixed pasture resulted in 39% lower total N_2O emissions compared to the RWC urine treatment. In conclusion, 30% to 50% plantain in mixed pastures was stable throughout the two years and it reduced the grazing cattle urinary-N concentration resulting in the N_2O emissions reduction in the summer-early autumn season.

Key words: plantain, botanical composition, nitrous oxide

1. Introduction

In New Zealand, the main pastoral supply for dairying has traditionally relied on permanent pasture mixtures of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) (DairyNZ, 2016). However, these pasture species have poor tolerance of drought conditions which limits their herbage production and nutritive value and, in turn, can negatively affect grazing livestock performance (Kemp et al., 2010). Another main concern for dairy farmers relying on ryegrass–white clover (RWC) pastures is the environmental footprint of the deposited urinary nitrogen (N). Grazing animals can excrete between 75% and 90% of ingested N which is prone to be lost via leaching or gaseous losses of N. Consequently, the N excreted by dairy cattle in urine is the largest source of nitrous oxide emission (N_2O) in grazed pastoral systems.

In recent years, narrow-leaf plantain (*Plantago lanceolata* L.), a summer active pasture species, incorporated into perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) (RWC) swards has been adopted by farmers due to its drought tolerance and environmental benefits of reducing N losses. Pasture containing plantain was well documented to have herbage dry matter (DM) accumulation and quality greater than that of RWC in summer and autumn (Navarrete, 2015; Nobilly et al., 2013; Totty et al., 2013). Additionally, recent studies showed that the introduction of plantain in pastures and the animal diet can reduce N losses, including N_2O emissions from urine patches. The N_2O emitted from plantain swards were found to be 28% to 74% lower than those from RWC swards (Luo et al., 2018; Rodríguez-Gelós, 2020).

The quantity of plantain in a pasture can affect its efficacy in reducing N losses to the environment. Minnée et al. (2020) suggested that including at least 30% plantain in a cow's diet is needed to reduce the N content excreted in urine, which subsequently results in lower N losses to the environment without negative effects on milk production. Increasing the proportion of plantain in the pasture mixture and/or in the diet has resulted in greater reductions in N_2O emissions (Simon et al., 2019). Therefore, a field experiment was conducted to monitor botanical composition of RWC pasture containing different proportions of plantain (0%, 30%, 50% and 70% plantain) under a dairy cow grazing regime through years (2019/2020 and 2020/2021). Additionally, we evaluated the effects of plantain mixed pastures on reducing nitrous oxide (N_2O) emissions from cow urine patches in summer/autumn 2021. It was hypothesised that N_2O emissions may decline as the proportion of plantain in the sward increased.

2. Materials and methods

2.1. The experimental site and treatments

The field study was established in a 6.6 ha rain-fed paddock at Massey University's Dairy No. 4 Farm, Palmerston North, New Zealand, on a poorly drained Tokomaru silt loam soil, as termed in the New Zealand soil classification system.

On the 5 April 2019, the experimental plots were established by directly drilling and sowing ryegrass and white clover mixed with 0% (RWC), 30% (P30), 50% (P50) and 70% (P70) of plantain. Plant species, cultivars and sowing rates are presented in Table 1.

Table 1 Species and sowing rate (kg ha^{-1}) for each pasture treatment

Species	Cultivar	**Pasture treatments** (kg ha^{-1})			
		Ryegrass/ white clover (RWC)	30% Plantain (P30)	50% Plantain (P50)	70% Plantain (P70)
Ryegrass	One50-AR1	20	15	10	5
White clover	Emerald	3	3	3	3
Plantain	Agritonic	0	4	7	10

Pasture treatments were arranged in a randomised block design, with five replicates per treatment. In total, the research site was divided into 20 experimental plots of 800 m^2 (40 m × 20 m) each and four adaptation paddocks of 1 ha per paddock.

2.2. Grazing and pasture management

The experimental site was grazed periodically by dairy cows. The grazing interval (3–5 weeks) was decided according to pre-grazing masses targeting a cover ranging from 3,600–3,800 kg DM ha^{-1}. For each grazing, 80 dairy cows were selected from the farm herd and separated into four groups (n=20) to graze in the adaptation paddocks. Each group grazed in one treatment adaptation plot for 6 days, and then, the cows were moved to the experimental plots to graze for 2–3 days, with four cows in each replicate plot.

2.3. Botanical composition and plant density measurements

The botanical composition of the pasture treatments was monitored throughout the two growing years. In the first growing year, the botanical composition (percent of total DM) was evaluated pre-grazing in the following periods: September 2019 (spring); December 2019 (summer); March 2020 (autumn); and July 2020 (winter). During the second growing year, botanical samples from the pasture treatments were collected in September 2020 (spring); October 2020 (early summer); November 2020 (summer); February 2021 (early autumn); and May 2021 (late autumn). In May 2021, due to the application of herbicide to remove ryegrass for the reestablishment of the P70 plots, botanical samples from these plots were not taken.

In all instances, botanical samples were taken the day before grazing by cutting herbage to ground level from 10 random spots along a diagonal line within each replicate plot. The herbage was bulked in one sample per plot, sub-sampled (~100 g fresh weight per sub-sample) and manually separated into the following categories: ryegrass, white clover, plantain (leaf and reproductive stems), other grasses, weed and dead material. Each separated category was individually oven-dried at 70 °C for 48 h and weighed to determine botanical composition as the percentage of each component in the total DM sub-sample.

Following the sampling for botanical composition, plant densities (plants/m^2) of plantain in the P30, P50 and P70 pasture treatments were measured pre-grazing by counting the number of plants in a quadrat in each plot. Each plantain plant can have several shoots, but only the number of plants within the quadrat was counted towards an estimate of plant density. Plant density was counted within 0.5 m × 0.5 m quadrats along a diagonal line in each replicate plot.

2.4. Nitrous oxide emission measurements

2.4.1. Experimental design

The study of N_2O emissions and emission factor values from plantain pastures was undertaken from summer to late autumn 2021 (15 February to 1 June 2021). This experiment measured N_2O emissions from urine patches of dairy cows grazing ryegrass and white clover mixed with different proportions of plantain: 0% (RWC), 30% (P30) and 50% (P50) in the swards. In fifteen experimental plots of three pasture treatments with five replicates, a representative area (2 m × 5 m) was fenced off and excluded from grazing on 10 December 2020, nine weeks before the experiment.

Two modified polyvinyl chloride (PVC) gas sampling chambers (240 mm diameter × 150 mm deep) were set up within the fenced area in each of the P30 plots; three chambers were set up in each of the RWC plots and P50 plots. Gas chambers were inserted 50–100 mm into the soil 1 week before application of urine. One chamber was allocated to each treatment category, with five replicates per treatment.

2.4.2. Cow urine collection, analysis and application

On days 7 and 8 of the grazing period, fresh urine was collected from cows grazing pasture treatments: RWC, P30 and P50, resulting in three urine treatments: URWC, U30 and U50. The urine samples were bulked for each treatment and taken immediately for total N analysis.

On the 15th February 2021, urine (URWC, U30 and U50) treatments were applied to the corresponding pasture treatments. The RWC and P50 plots received urine collected from the cows that grazed the P50 and RWC plots. A control treatment, which did not receive any urine (NoU), was also included. In total, eight combined treatments consisted of: ryegrass/white clover treated with ryegrass/white clover urine (RWC+URWC), with 50% plantain urine (RWC+U50) and without urine (RWC+NoU); 30% plantain pasture treated with 30% plantain urine (P30+U30) and without urine (P30+NoU); 50% plantain pasture treated with 50% plantain (P50+U50), with ryegrass/white clover urine (P50+UWRC), and without urine (P50+NoU). The treatments were arranged in randomised pasture plots with five replicates.

Urine was sprayed uniformly in the chambers at the equivalent rate of 10 L m^{-2}. The control treatments received the equivalent volume of water. Urine types, total urinary-N content and application rate expressed as total N in kg ha^{-1} are presented in table 2.

Table 2 Treatments, urinary N and application rates

Treatment name	**Pasture type**	**Urine type**	**Urinary-N concentration** (g N L^{-1})	**Rate of urinary N applied** (kg N ha^{-1})
RWC+URWC	0% plantain	0% plantain	6.15	615
RWC+U50	0% plantain	50% plantain	4.40	440
P30+U30	30% plantain	30% plantain	5.40	540
P50+U50	50% plantain	50% plantain	4.40	440
P50+ URWC	50% plantain	0% plantain	6.15	615
RWC+NoU	0% plantain	N/A	N/A	N/A
P30+NoU	30% plantain	N/A	N/A	N/A
P50+NoU	50% plantain	N/A	N/A	N/A

Abbreviation. N/A, not applicable.

2.4.3. Nitrous oxide measurements

Nitrous oxide emissions were measured by using the non-vented closed chamber method (Charteris et al., 2020). Background emission samples were taken 2 days before treatment application. The N_2O flux measurements were carried out at 4, 24 and 72 h after urine application, twice per week in the first 4 weeks, weekly in the following 5 weeks and then, fortnightly, until gas flux was equal to the background level. In total, N_2O fluxes were measured 19 times over 102 days.

The hourly N_2O fluxes (mg N m^{-2} h^{-1}) were calculated based on the slope of the linear increase (R^2>0.90) in N_2O emissions within the chamber headspace, collected at different times of the sampling time (t0, t40 and t80) as described by (deKlein et al., 2003) and using Equation 1:

$$N_2O \text{ flux} = \frac{\delta N_2O}{\delta T} \times \frac{M}{Vm} \times \frac{V}{A} \qquad \text{Equation 1,}$$

where δN_2O is the increase in N_2O in the headspace over the cover period (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).

The cumulative emissions of N_2O over the experimental period were estimated by integrating the daily fluxes from each chamber on set measurement dates. The N_2O EF_3 and N_2O–N emitted were calculated using Equation 2:

$$EF_3 = \frac{N_2O\text{-N total (urine)- } N_2O\text{-N total (control)}}{\text{Urine N applied}} \qquad \text{Equation 2,}$$

where N_2O-N total (urine) and N_2O–N total (control) were the cumulative N_2O–N emissions over the measurement period from the urine-treated and control chambers, respectively (kg N ha^{-1}), and urine N applied was the rate of urine N applied (kg N ha^{-1}).

2.5. Statistical Analysis

All data were analysed using SAS version 9.4 via mixed models for a completely randomised design. Differences between treatments were analysed by one-way ANOVA followed by Least Significant Differences (LSD) test. The significant difference of the means for all analyses was established at p< 0.05.

3. Results and discussion

3.1. Botanical sustainability of plantain mixed pastures

3.1.1. Botanical composition

During the summer–autumn period of both growing years, the proportion of plantain was 40%–50%, whilst the proportion of ryegrass steadily reduced in all pasture treatments (Figure 1). The proportion of white clover in the pastures increased over time, regardless of pasture type.

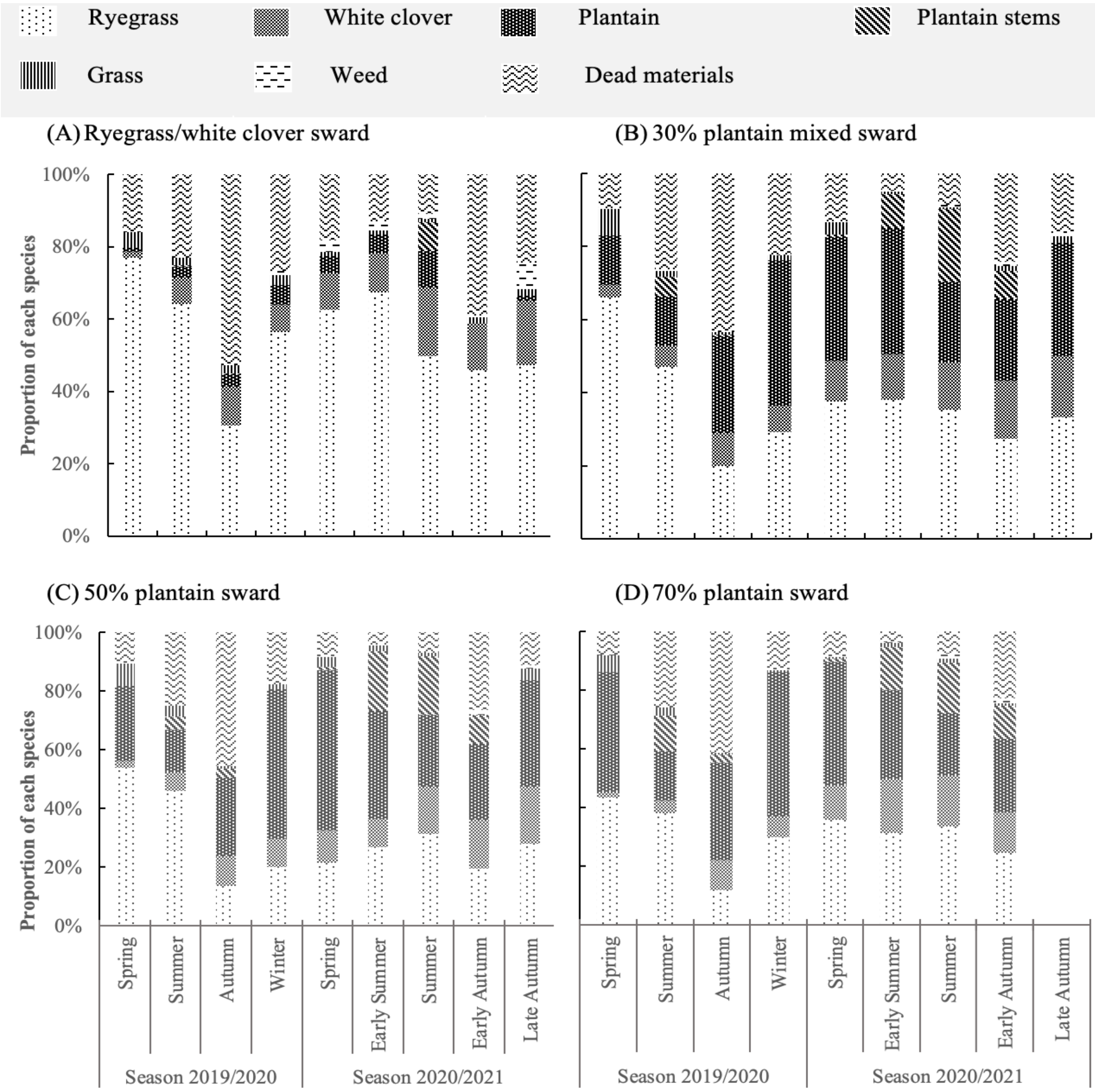

Figure 1 Botanical composition (percent of DM) in (A) RWC; (B) 30% plantain; (C) 50% plantain; and (D) 70% plantain mixed pastures in the 2019/2020 and the 2020/2021 seasons.

In the first (2019/2020) year, in the P30 pasture treatment, the proportion of plantain increased from 14% in spring 2019 to 39% in winter 2020. In the P50 pasture treatment, the proportion of plantain (leaf) in the sward was 26% in spring, but reduced to 14% in summer, before increasing in autumn and winter to 26% and 50%, respectively. Similarly, the P70 pasture treatment had 41% plantain in spring, which then decreased in summer, after which it recovered and reached 49% in winter.

In the second (2020/2021) year, plantain leaf content decreased to 22% and 25% in the P30 and P50 swards, respectively, during the summer/early autumn, along with an increased plantain stem content. In late autumn 2021, the proportion of plantain (leaf) in the P30 and P50 treatments increased to 31% and 36%, respectively. In the P70 sward, the proportion of plantain decreased over the season, ranging from 25%–30% of plantain in the sward.

The proportion of plantain in all plantain mixed-pasture treatments contributed to up to 50% of the DM during late summer and autumn, the dry period across the two growing years. This result suggests that plantain would likely not contribute more than 50% to the botanical composition in mixed pastures, even when sown at a high rate. Stewart (1996) indicated that plantain naturally represents up to 20% of a productive pasture and typically acts as minor forage in swards. Bryant et al. (2019) also observed that plantain contributed less than 30% of DM in established pastures. Therefore, plantain is unlikely to generate the majority of DM yield in mixed pastures.

3.1.2. Plant density of plantain

Through the first year, the plantain population increased from spring to autumn (Figure 2). At the end of the first grazing season (winter 2020), there was approximately a 40% increase in plantain populations in the P30 and P50 treatments compared to initial plant densities (spring 2019), while in the P70 treatment, the plantain density decreased to 148 plants/m^2.

A decrease in DM contribution of plantain in plantain mixed pasture treatments was probably associated with the plant loss observed at the beginning of the second growing years. In year 2, the plantain density tended to decline (p=0.07) in all pasture types, with plant loss ranging from 52%–62% of the plant density observed at the beginning of the season (spring 2020). In late autumn year 2, plantain populations in the three pasture treatments were similar (p> 0.05). This is in line with previous findings that stability of herbage production largely depends on plant density (Neal et al., 2009; Nie et al., 2008).

Throughout the second year, the proportions and plant density of plantain in the P50 treatment were more stable more that in the P70 treatment. Consequently, the plantain density in the P70 treatment was significantly lower than that in the P50 treatment and similar as that in the P30 treatment. These results suggested that plantain was unlikely to contribute more than 50% of the DM of a mixed RWC and plantain pastures regardless of the sowing rates of plantain, ryegrass and white clover used. The sowing rates of plantain in used for the 30% and 50% plantain pastures were effective for two years, but the decline in plantain plant density observed suggested that broadcasting or direct drilling additional plantain after two years might be required to maintain a proportion of plantain in mixed pastures of greater than 30% (Bryant et al., 2019).

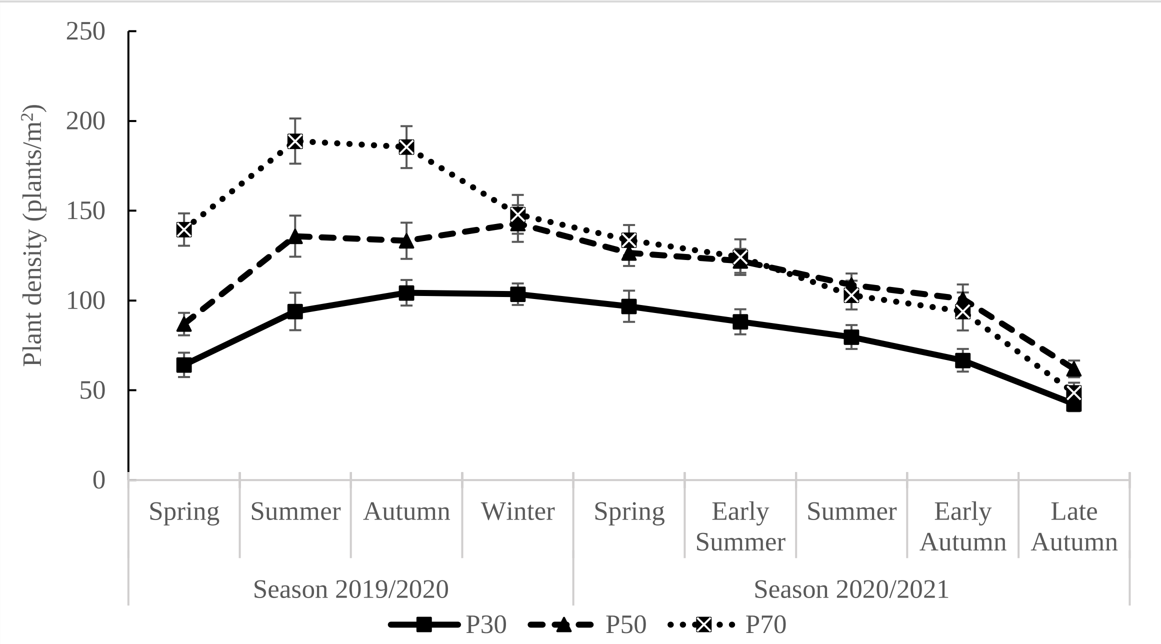

Figure 2 Plant density (plant m^{-2}) of plantain in P30, P50 and P70 treatments throughout the first year (2019/2020) and the second year (2020/2021). Error bars represent plant density standard errors for each treatment (n=5).

3.2. Nitrous Oxide Emissions

Urine application sharply increased N_2O flux from all pasture treatments (Figure 3). At 4 h, P30+U30, P50+URWC and P50+U50 produced the highest peak at 0.55, 0.62 and 0.95 mg N m^{-2} h^{-1}, respectively, followed by a decline to around 0.3 mg N m^{-2} h^{-1} on the next day. Whilst N_2O emissions from RWC pasture treatments peaked on the following day when they reached 0.5 mg N m^{-2} h^{-1}. The emissions from all the treatments declined after day 1 and remained low between day 3 and day 37 due to summer dry conditions, but small peaks occurred following rain events. The emissions from RWC+URWC were significantly higher ($p < 0.05$) than from P30+U30 and P50+U50 at day 25 and 44. N_2O emissions from plantain pastures reached the background level at 88 DAA while RWC swards obtained the background emission level at day 102.

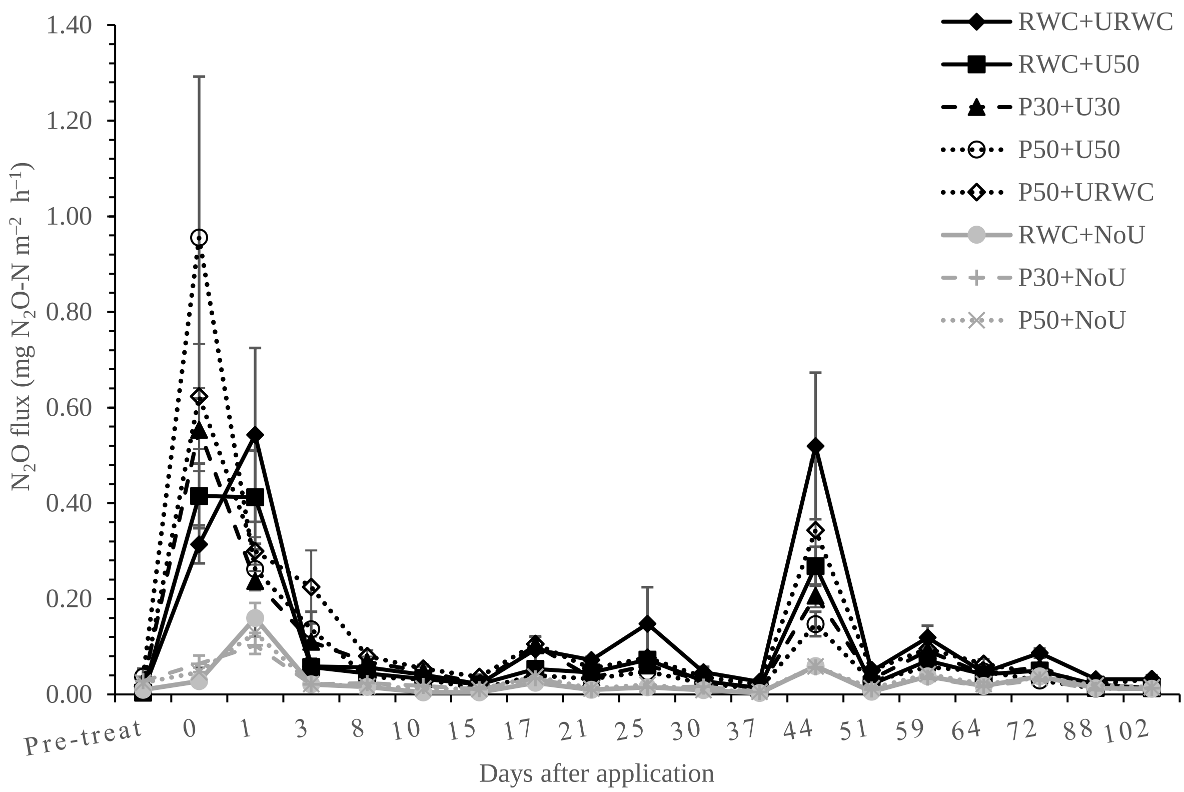

Figure 3 Hourly N_2O fluxes from urine (U) patches on plantain (P) and ryegrass/white clover (RWC) pastures. Error bars show standard error of the mean (n=5).

Pasture and urine types significantly affected ($p < 0.05$) the total N_2O emissions and the emission factors (Table 3). The higher urine loading rate of 615 kg N ha^{-1} in URWC significantly increased ($p < 0.01$) the cumulative N_2O–N fluxes from RWC and P50 pasture treatments. The total N_2O emissions from the 28% lower N loading rate in the U50 treatment were around 39% less than the emission from the URWC treatment regardless of pasture types. In contrast, there was no significant difference ($p > 0.05$) in the cumulative N_2O emissions between RWC and P50 pasture treatments treated at the same urine loading rate.

Table 3 Cumulative N_2O emissions, emission factor of the applied urine N emitted as N_2O (EF_3), change in cumulative N_2O emissions and EF_3 compared to from ryegrass/white clover pasture applied the corresponding urine type during the measurement period.

Treatments	**Urine rate** (kg N ha^{-1})	**Change in urine rate** (%)	**Cumulative N_2O emissions** (kg N ha^{-1})	**Change in N_2O** (%)	**Emission factor** (EF_3, %)	**Change in EF_3** (%)
RWC+URWC	615	N/A	2.43 (0.26)$_a$	N/A	0.31 (0.04)$_a$	N/A
RWC+U50	440	-28%	1.48 (0.16)$_b$	-39%	0.22 (0.01)$_{ab}$	-30%
P30+U30	540	-12%	1.51 (0.11)$_b$	-38%	0.18 (0.02)$_b$	-42%
P50+U50	440	-28%	1.27 (0.16)$_b$	-48%	0.15 (0.02)$_b$	-51%
P50+URWC	615	N/A	2.05 (0.25)$_a$	-16%	0.23 (0.03)$_{ab}$	-24%
RWC+NoU	N/A		0.53 (0.02)$_c$			
P30+NoU	N/A		0.53 (0.05)$_c$			
P50+NoU	N/A		0.60 (0.04)$_c$			
***p*- Value**						
Pasture type			0.0070		0.0220	
Urine type			<.0001		0.0054	
Pasture × urine			0.3014		0.8731	

Notes. Values sharing the same subscript letter do not differ significantly ($p>0.05$). Numbers in parentheses are the standard error of the mean applied to five treatments (n=5)

This study observed that the lower N-loading rate onto soil from plantain derived urine is the major effect resulting in the reduction of total N_2O emissions. The urinary-N concentration, urea-N content and subsequent N-loading rate onto soil decreased by 12% and 28%, respectively (Table 3) when mixed pastures with 30% and 50% of plantain was in grazed diet of cows, respectively. This supported earlier studies which demonstrated lower N content in urine of cows grazing pastures containing plantain compared to RWC (Box et al., 2017; Di et al., 2016; Minnée et al., 2020; Podolyan et al., 2020; Rodríguez-Gelós, 2020).

The presence of secondary compounds in urine of grazing animals is another mechanism that has been proposed to explain decreases in N_2O emissions from plantain urine type (Gardiner et al., 2018; Keir et al., 2001). However, when urine from the plantain diet was adjusted to the same N concentration as urine from ryegrass diet, N_2O emissions from two urine types did not differ at the same N loading rate (Rodríguez-Gelós, 2020; Simon et al., 2019). Therefore, it would appear that lower N concentrations in urine and resultantly lower N loading rate are the main factors in reducing N_2O emissions from plantain fed cow's urine.

The effect of the proportion of plantain in mixed pasture on N_2O emission was not obvious in this study. There was no significant differences ($p > 0.05$) in the cumulative N_2O emissions between the N-application rate of 540 kg N ha^{-1} and 440kg N ha^{-1} when these urine types were applied to the corresponding pasture types (1.27 kg N-N_2O ha^{-1} from P50+U50 treatment and 1.51 kg N-N_2O ha^{-1} from P30+U30 treatment). Similarly, Pijlman et al. (2020) did not observe the effect of increasing plantain proportion in pasture on a reduction of N_2O emission in the field experiment. This result could be related to the fluctuation in plantain composition in mixed pasture over time. The less-than-obvious effects of plantain proportions on N_2O emissions may also be associated with the size of the gas chambers used. That is, they may not have been large enough to cover representative proportions of plantain for each pasture treatment.

The EF_3 in P50 and RWC pasture treatments were similar ($p > 0.05$) with the same N-application rate of either 615 kg N ha^{-1} or 440 kg N ha^{-1} (Table 3). EF_3 values in P30+U30 treatment and P50+U50 treatment were 42–51% lower than the RWC+URWC treatment, and this difference was significant ($p < 0.05$). This may be attributed to possible release of biological nitrification inhibitors produced by plantain (Dietz et al., 2013; Luo et al., 2018; Rodríguez-Gelós, 2020). Plantain contains secondary compounds acting as nitrification inhibitors which can reduce the nitrification rates, mineral-N content and resultant N_2O emissions.

4. Conclusions

The highest contribution of plantain to pasture yield was 50% of DM in swards mixed with RWC during late summer and autumn, even when plantain sown at a rate to attain 70% of the pasture composition. The P30 and P50 plantain pasture treatments maintained more stable plantain compositions and densities than the P70 mixed pasture over two years when plantain seed was broadcast at the end of the first growing season. Therefore, sowing seed mixtures with 30%–50% of plantain, along with the broadcasting of fresh seed at the end of each growing season, could maintain a sufficient proportion of plantain in pasture mixes to extend the benefits of plantain.

In this study, the inclusion of 30% and 50% of plantain in RWC pastures reduced urine-N content and N_2O emission from cow urine patches in summer/late autumn. Nitrous oxide emission factor (EF_3) from urine from cows grazing 30% and 50% plantain in the pasture diets were significantly lower (42 to 51%) compared to ryegrass-white clover diet.

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