



Do sward age and pasture renewal affect the potential of plantain (*Plantago lanceolata*) to reduce N₂O emissions from urine patches?

Priscila L. Simon · Pablo Lacerda Ribeiro ·
Cecile A. M. de Klein

Received: 26 March 2025 / Accepted: 14 November 2025 / Published online: 22 January 2026
© The Author(s) 2025

Abstract Plantain (*Plantago lanceolata*) has shown the potential to reduce nitrous oxide (N₂O) emissions from urine patches. However, inconsistent results have been reported in previous studies. In this context, the sward age might be a factor regulating plantain effectiveness in mitigating N₂O emissions. Thus, this study aimed to assess the effect of sward age on N₂O emissions from urine patches under different sward composition ratios of ryegrass/white clover (RG/WC) and plantain (PL). We also examined the potential impact on N₂O emissions of transitioning a RG/WC based pasture to PL swards. Two field experiments were undertaken to: (i) compare N₂O emission from urine applied to 2-year (Old plantain) and 3-month (New plantain) old swards with sowing densities aiming at four proportions of PL mixed with RG/WC (0; 30; 45 and 100% PL) and (ii) assess N₂O emissions during soil cultivation and sowing following transition from RG/WC to PL swards. New plantain tended to decrease N₂O emissions from urine patches by ~31% relative to RG/WC pastures, but this

effect was significant only in the 45% PL. In contrast, there were no significant effects of the Old plantain sward, probably because of increased clover biomass relative to the new sward. In addition, the transition from RG/WC pasture to PL swards increased N₂O emissions from 0.1 to 1.53 kg N ha⁻¹. Finally, our results suggest that plantain's inhibitory effect on N₂O emissions from cattle urine is more effective in a 3-month-old sward than a 2-year-old sward. However, it was not possible to disentangle effects of sward age and clover share in the sward in this study. Pasture renewal increased N₂O emissions, and therefore, other approaches to renew swards should be considered to minimize N₂O emissions.

Keywords Biological nitrification inhibition · Denitrification · Emission factors · Pasture renovation · Plantain proportion · Clover

Introduction

Nitrous oxide (N₂O) is a powerful greenhouse gas (GHG) with a radiative potential that is about 300 times greater than that of carbon dioxide (CO₂) (IPCC 2021). The agricultural sector contributes with ca. 50% to global N₂O emissions, with 22% deriving from soil in pastoral dairy farming systems (van der Weerden et al. 2017). In grazed livestock systems, animal urine patches are the largest source of N₂O,

P. L. Simon · P. L. Ribeiro (✉) · C. A. M. de Klein
Bioeconomy Science Institute – AgResearch Group,
Invermay Research Centre, Private Bag 50034,
Mosgiel 9053, New Zealand
e-mail: pablo.ribeiro@agresearch.co.nz

C. A. M. de Klein
e-mail: cecile.deklein@agresearch.co.nz

P. L. Simon
S&P Commodity Insights, Sao Paulo, Brazil

and there is ongoing research on developing options to reduce N_2O emissions from urine.

Strategies to mitigate N_2O emissions from soil can be broadly categorised as those that reduce the total amount of urinary nitrogen (N) excreted by animals and those that reduce the proportion of urine N lost through manipulating the soil processes nitrification or denitrification (de Klein et al. 2020). A recent review suggested that plants can help mitigate N_2O emissions from urine patches, with plantain (*Plantago lanceolata*) showing particular promise (de Klein et al. 2022). Results to date have shown that plantain can not only reduce the total amount of urinary-N produced by grazing animals (Minnée et al. 2020; O'Connell et al. 2016), but also reduce the proportion of N lost from urinary N (Bowatte et al. 2018; Byrnes et al. 2017). Studies comparing N_2O emissions following urine application on plantain swards with emissions from urine applied to standard ryegrass and white clover pasture found a reduction in N_2O emissions of up to 30% (Luo et al. 2018; Simon et al. 2019). Although the exact mechanisms by which plantain reduces N_2O emissions are still being investigated, there is increasing evidence that plantain can inhibit the soil microbial nitrification process through root exudation of secondary metabolites (Gardiner et al. 2017; Judson et al. 2020; Rodriguez et al. 2021; Simon et al. 2020, 2019). By inhibiting nitrification, the production of nitrate-N from N excreted by animals is delayed. This is beneficial because nitrate-N is more susceptible than ammonium-N to losses via leaching and emission of gaseous N forms such as N_2O , N_2 , and NO (Cardenas et al. 2016).

The inhibition of nitrification through plant root exudates (biological nitrification inhibition, BNI) has been demonstrated in a range of plant species, largely from tropical systems (e.g. signal grass, sorghum, maize, rice, and wheat) (Subbarao et al. 2009, 2012). Similarly, plantain is known for producing the iridoid glycosides aucubin and catalpol, and the polyphenol verbascoside/acteoside, which are bioactive compounds with potential BNI effects (Gardiner et al. 2016; Wichtl 2004).

Previous studies suggest that plantain age can affect its ability to inhibit N_2O emissions after the deposition of cattle urine. For instance, Nyameasem et al. (2021) found a minimal effect of a newly established plantain sward in reducing the microbial enzyme activity in the soil and N_2O emissions. It was

suggested that this effect is likely due to the young age of plantain. In contrast, Carlton et al. (2019) reported a reduced ammonia-oxidizing bacteria (AOB) abundance for a 2-year-old plantain-rich pasture (ca. 30% PL), with a strong impact of contrasting soils and environmental conditions. Another study also found that a 2-year-old plantain presented nearly double the concentration of the BNI compound aucubin ($6.9 \text{ mg g}^{-1} \text{ DM}$) in plantain leaves compared with a 1-year-old plantain ($3.8 \text{ mg g}^{-1} \text{ DM}$) (Navarrete et al. 2016).

Establishing plantain in a sward could influence N_2O emissions due to the required soil cultivation and planting. For instance, a study in northern Germany has shown that pasture renewal by ploughing and harrowing in spring and autumn emitted 3.73 and $14.08 \text{ kg N ha}^{-1}$ as N_2O per year, respectively, while an uncultivated permanent grassland emitted $0.84 \text{ kg N ha}^{-1}$ (Reinsch et al. 2018). In New Zealand, Thomas et al. (2008) indicated that pasture conversion from ryegrass/clover to a triticale crop with soil tillage increased N_2O emissions when the soil was subject to treading and urine-N application at soil moisture levels above the field capacity. Also in New Zealand, Wall et al. (2023) found that pasture renewal by direct drill following herbicide application increased N_2O emissions, probably due to increased net soil N mineralisation, which provides substrate for nitrification and denitrification microbial processes. Although plantain has shown potential to reduce emissions from urine patches, transitioning ryegrass/clover to plantain-containing swards may increase N_2O emissions and research on this topic is lacking.

Results on the effect of plantain proportion in the sward on reducing N_2O emissions from grazing systems have been inconsistent. A linear decrease in N_2O emissions was observed with increasing the proportion of plantain swards (Simon et al. 2019), while Pijlman et al. (2020) found no obvious link between the proportion of plantain and N_2O emission reduction. Therefore, more work is needed to explore the effect of plantain proportions on its ability to reduce N_2O emissions.

Based on results reported so far, we hypothesize that a 2-year-old established plantain would present a greater potential to reduce N_2O emissions compared with a 3-month-old established plantain sward. We also hypothesized that the transition of a ryegrass to a plantain-containing pasture can increase N_2O

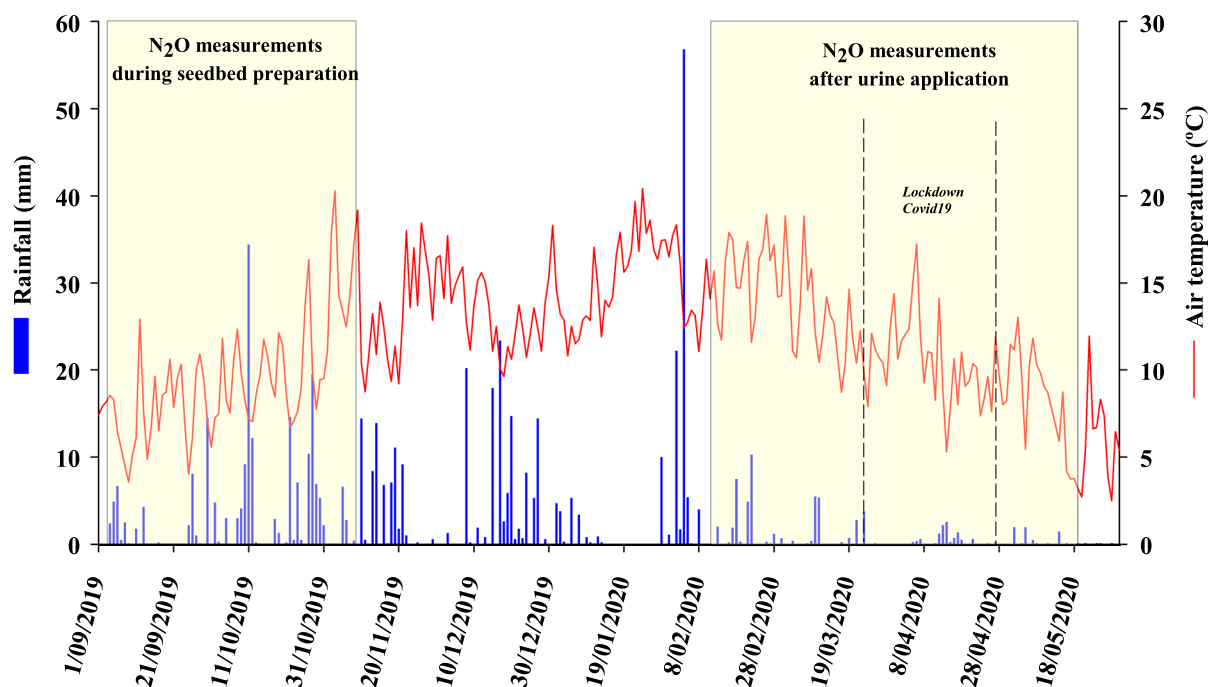


Fig. 1 Rainfall (mm) and air temperature (°C) during the measurement periods (2019–2020)

Table 1 Means and standard error (between parentheses) of actual plantain, ryegrass, and clover share (%) in swards subject to sowing with different nominal proportions of plantain (0%, 30%, 45% and 100%) in 2-year-old (Old plantain) and 3-month-old (New plantain) swards

	Plantain (%)	Ryegrass (%)	Clover (%)
0% Plantain			
Old plantain	0 Da	66 (7) Ab	34 (7) ABa
New plantain	0 Da	85 (5) Aa	15 (5) Ab
30% Plantain			
Old plantain	35 (5) Ca	24 (8) Bb	41 (7) Aa
New plantain	40 (8) Ca	45 (6) Ba	15 (5) Ab
45% Plantain			
Old plantain	57 (2) Ba	19 (3) Bb	25 (4) ABa
New plantain	64 (4) Ba	25 (4) Ba	11 (3) Ab
100% Plantain			
Old plantain	79 (7) Aa	0 Ca	21 (7) Ba
New plantain	91 (2) Aa	0 Ca	9 (2) Ab

Means followed by different uppercase letters differ statistically between nominal plantain sward proportions within the same age. Means followed by different lowercase letters represent significant differences between old and new plantain swards within the same nominal plantain proportion according to Tukey's test ($P < 0.05$)

emissions. The objectives of this study were to: i) assess the effect of plantain sward age on its ability to reduce N_2O emissions from urine patches applied to pastures with increasing proportions of plantain mixed with ryegrass/white clover, and ii) conduct an assessment of the potential for increased N_2O emissions during plantain sward establishment.

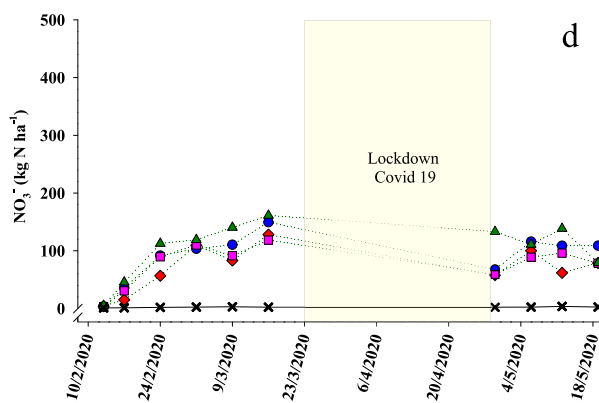
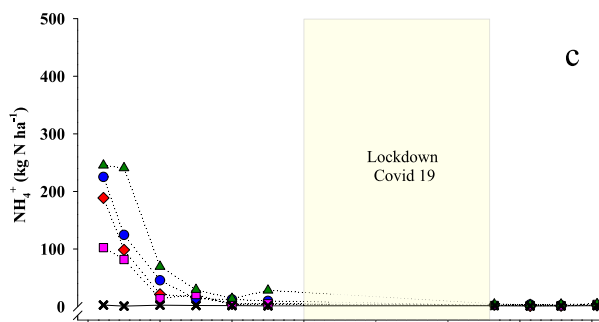
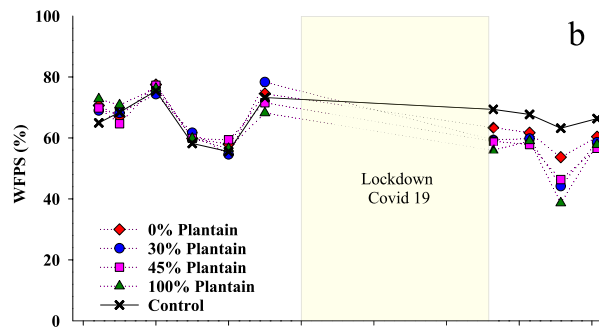
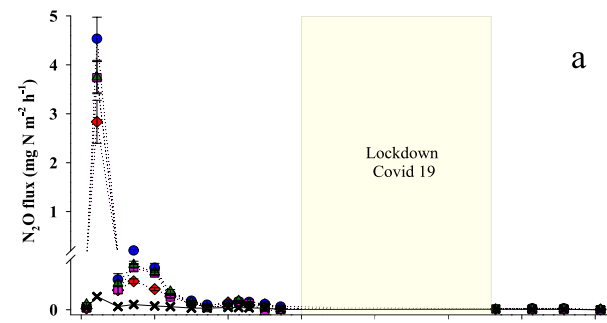
Material and methods

Experimental site

Two experimental areas containing plots with varying proportions of plantain (*Plantago lanceolata* L.) were established at Invermay Agricultural Centre near Mosgiel, New Zealand (45° 52' 30" S and 170° 20' 50" E). The climate is temperate with a mean annual precipitation of 750 mm. The mean air temperature for the region is 9 °C, with the warmest months usually being January/February and the coldest being June/July (NIWA 2018).

The trial sites are located in an area formerly under a perennial pasture of ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.), rotationally

Old plantain



New plantain

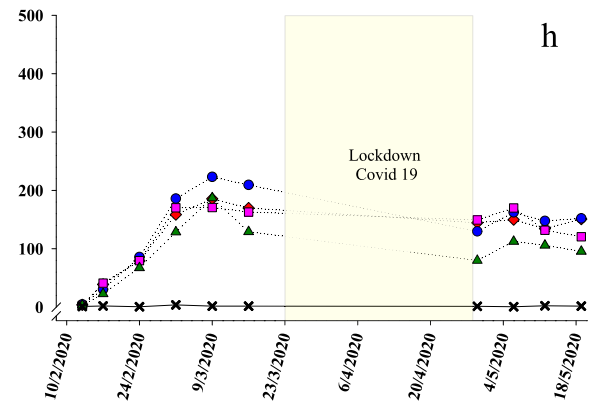
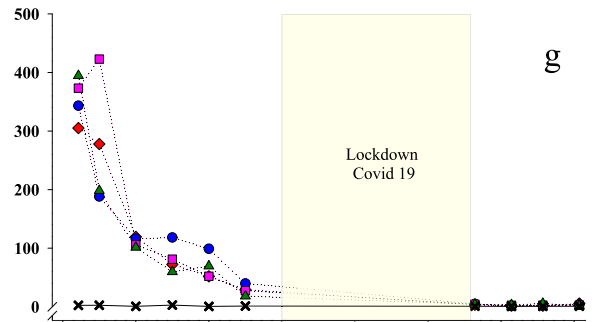
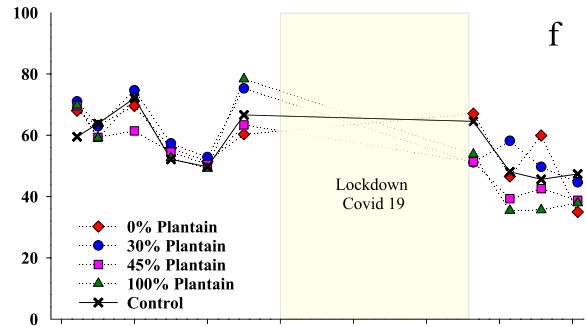
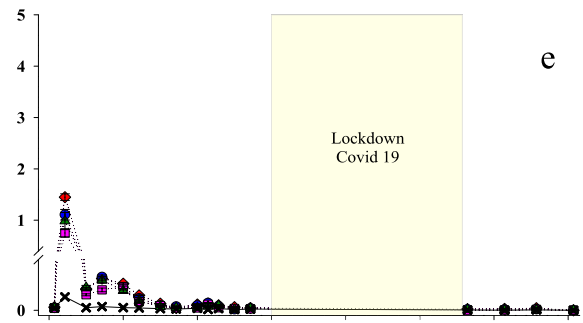


Fig. 2 Fluxes of N_2O (a, e), water filled-pore spaces (WFPS%) (b, f), soil NH_4^+ (c, g) and soil NO_3^- (d, h) concentrations following cattle urine application to 2-year-old (a to d) and 3-month-old (e to h) plantain swards, under different nominal proportions of plantain mixed with ryegrass and white clover. Vertical bars denote LSD according to Tukey's test ($P < 0.10$). Please note that the control treatment in this graphic represents the mean values of all control plots used for 0, 30, 45 and 100% nominal plantain proportions

grazed by sheep. The soil is a moderately well-drained Wingatui silt loam (NZ Taxonomy, weathered Fluvial Recent soil; USDA Taxonomy, Dystrudepts; (Hewitt 2010; Soil Survey Staff 2022)). This soil is generally deep, has a high plant-available water capacity and is naturally fertile with a high base saturation. The soil's physicochemical attributes at 0–0.20 m soil depth are: $\text{pH}_{\text{CaCl}_2} = 5.9$, organic C = 5.4%, organic matter = 9.2%, total N = 0.54%, clay = 37%, silt = 54%, fine sand = 7%, bulk density = 0.99 Mg m^{-3} , cation exchange capacity = $21.1 \text{ cmolc dm}^{-3}$, total base saturation = 75.7%, and Olsen P = 23 mg L^{-1} .

Trial set-up and treatment application

The experimental areas contained plots with four sown proportions (0, 30, 45 and 100%) of plantain cv. Agritonic (PL) mixed with perennial ryegrass cv. One50 AR37, and white clover, cv. Tribute, (RG/WC). Trial 1, hereinafter referred to as “Old plantain”, was sown in November 2017, and trial 2, hereinafter referred to as “New plantain”, was sown in November 2019. To establish both trials, a site containing a ryegrass/clover sward (ca. 75/25% proportion) was sprayed with the herbicide glyphosate twice, ploughed at a 25 cm depth, harrowed three times to create a seedbed, and sowing was performed with a seed drill. Fertilisers were not applied at least 3 months prior to soil cultivation. The respective sward types PL0%, PL30%, PL45%, PL100% were obtained with the following sowing densities:

PL0%: 20 kg ha^{-1} RG and 4 kg ha^{-1} WC.

PL30%: 4 kg ha^{-1} PL, 16 kg ha^{-1} RG, and 4 kg ha^{-1} WC.

PL45%: 6 kg ha^{-1} PL, 12 kg ha^{-1} RG, and 4 kg ha^{-1} WC.

PL100%: 12 kg ha^{-1} PL and 4 kg ha^{-1} WC.

Notably, the labels 0, 30, 45 and 100% of PL refer to the sowing density of plantain (nominal plantain proportion) relative to ryegrass only and may deviate from the actual plantain proportion during the trials. This does not consider clover, as all treatments were sown with 4 kg ha^{-1} WC. Moreover, we also present the actual plantain proportion and dry matter yield in the results section, which refers to the plantain above-ground biomass measured 2 days before urine-N application.

The treatments were arranged in a randomised block design with five replicates. The plots were mown at 30, 50, and 80 days after sowing to stimulate vegetative growth and to enable a standardization of the plots. The clipped material was removed from the plots to avoid plant residues interfering with soil properties. A stainless-steel ring of 25 cm diameter (0.049 m^2) was inserted 7.5 cm into the soil. This was used to delimit an area for urine application and as a base for the chambers used for N_2O flux measurements. Adjacent to each ring was a 0.5 m^2 area that received the same urine treatments and was utilized for soil mineral N and soil moisture content determinations. Each of the plots also had another circular plot of 0.049 m^2 and an adjacent 0.70 m^2 area that did not receive any urine (control or no urine treatments). These areas were used as the control treatments for the N_2O flux and soil parameter measurements, as well as for calculating urine-N emissions factors.

The measurements for assessing the impact of grassland renewal on N_2O emissions were performed in plots in the New plantain area and an undisturbed adjacent area during the trial establishment from September to November 2019.

In February 2020, fresh urine was collected from Friesian dairy cows grazing RG/WC pasture. The urine collection occurred during cows' milking using plastic containers, and the volume obtained was stored at -4°C for 24 h. A subsample of the urine was sent to the laboratory for total N concentration analysis. On the day of application, 0.49 L of cattle urine (equivalent application rate of 682 kg N ha^{-1}) was evenly poured onto each gas measurement area (0.049 m^2). Simultaneously, the 0.5 m^2 soil plots received 4.9 L of synthetic urine (Clough et al. 2003) with the same N content as the real urine applied to the gas measurement plots. The urine volume applied to the plots was equivalent to 10 L m^{-2} , considered as

Table 2 Cumulative emission (kg N ha^{-1}) and emission factor ($\text{EF}_3\%$) of nitrous oxide (N_2O) ($n=5$), from cattle urine patches deposited to different nominal proportions of plantain

swards (0, 30, 45 and 100%) of a 2-year-old (Old plantain) and a 3-month-old (New plantain) swards

	0%PL	30%PL	45%PL	100%PL
<i>Cumulative N_2O emission (kg N ha^{-1})—Urine treatments</i>				
Old plantain	2.47 (0.668) Aa	3.94 (0.736) Aa	3.18 (0.556) Aa	3.26 (0.530) Aa
New plantain	1.54 (0.099) Ba	1.24 (0.133) Bab	0.86 (0.107) Bb	1.09 (0.281) Bab
<i>Cumulative N_2O emission (kg N ha^{-1})—Control (no urine treatments)</i>				
Old plantain	0.08 (0.008) Bb	0.17 (0.031) Aa	0.11 (0.011) Aab	0.21 (0.076) Aa
New plantain	0.22 (0.093) Aa	0.16 (0.024) Aa	0.11 (0.025) Aab	0.08 (0.023) Bb
<i>Emission factor (%)</i>				
Old plantain	0.35 (0.10) Aa	0.55 (0.11) Aa	0.45 (0.08) Aa	0.45 (0.07) Aa
New plantain	0.19 (0.02) Ba	0.16 (0.02) Bab	0.11 (0.02) Bb	0.15 (0.04) Bab

Means followed by different uppercase letters differ statistically between old and new plantain swards within the same nominal plantain proportion. Means followed by different lowercase letters represent significant differences between nominal plantain sward proportions within the same age according to Tukey's test ($P < 0.05$ for urine treatments, and $P < 0.10$ for no urine treatments). Values between parentheses refer to the standard error of the mean

Note: Emission factor and cumulative emission values need to be interpreted with caution considering the interrupted period of measurements due to Covid19

the average of an adult cow's urination ($8\text{--}14 \text{ L m}^{-2}$) (Haynes and Williams 1993).

N_2O gas measurements from transitioning pasture to plantain swards

Pre- and post-sowing measurements were taken during the establishment of the New plantain plots to enable an assessment of the effect of transitioning from RG/WC to PL swards on N_2O emissions. A stainless-steel ring of 25 cm diameter (0.049 m^2) was inserted 7.5 cm into the soil, serving as a base for the chambers used for N_2O flux measurements.

N_2O gas measurements from the New and Old plantain and ryegrass/white clover plots

Two days before urine application, pre-treatment N_2O measurements were conducted to determine the variability of background N_2O fluxes between the plots. The first post-treatment N_2O measurement was conducted immediately after urine application. Thereafter, samplings occurred twice a week, at 4, 7, 11, 14, 18, 21, 25, 27, 29, 32 and 35 days after urine application. The measurements were then discontinued due to the Covid19 pandemic. After the Covid19

lockdown, we continued the gas measurements weekly on days 76, 83, 89 and 96 following urine application.

On each sampling event, N_2O measurements were carried out between 10 am and 1 pm, which allows extrapolation to a daily flux without bias (van der Weerden et al. 2013). Chamber headspace samples were taken by a 20 mL syringe, using the sampling system described by de Klein et al. (2003), and stored in 7.5 mL evacuated vials (Exetainer, Labco Ltd., High Wycombe, UK).

For the first 12 sampling occasions, chamber headspace gas samples were collected during an enclosure period of 60 min, at times t_0 , t_{30} and t_{60} . After the linearity of the increase in N_2O concentration during the enclosure period was confirmed, gas samples were collected at t_0 and t_{60} for the remainder of the sampling occasions. On each sampling event, two background atmosphere samples were also collected. Gas analysis was conducted at Lincoln University (Lincoln, New Zealand) by gas chromatography using a Shimadzu GC-17a gas chromatograph equipped with a 63Ni-electron capture detector with oxygen-free N_2 as a carrier gas.

The hourly N_2O emissions ($\text{mg N m}^{-2} \text{ h}^{-1}$) were calculated for each chamber from the increase in headspace N_2O over the sampling period. The hourly

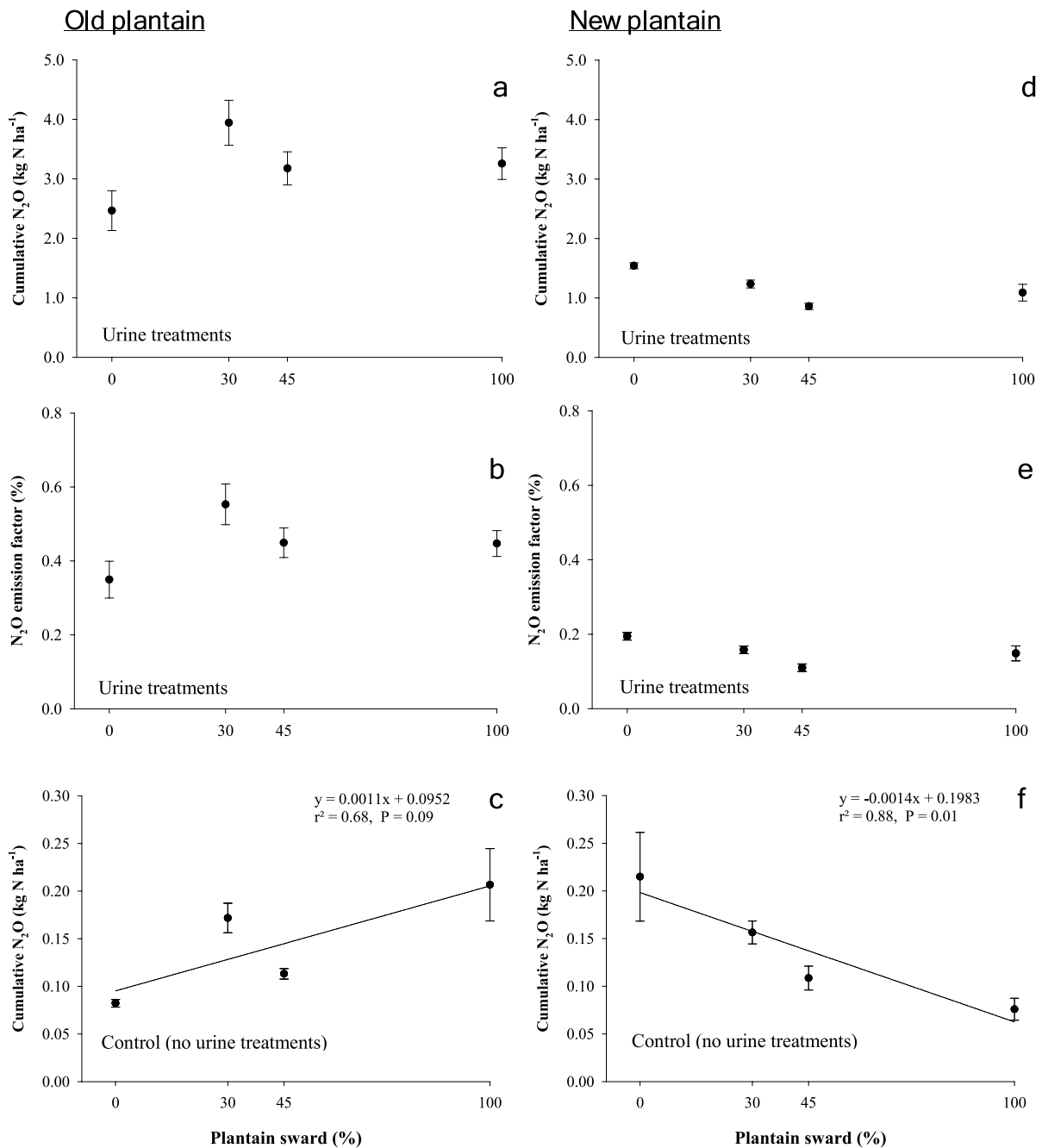


Fig. 3 Cumulative N_2O emissions (**a**, **d**) and emission factors (**b**, **e**) (mean $\pm$ standard error, $n=5$) from urine applied to swards of ryegrass and white clover with increasing nominal

proportions of plantain (0, 30, 45 or 100%). The effect of the nominal proportion of plantain on N_2O emissions in non-urine treatments is presented in (**c**) and (**f**)

N_2O emissions ($mg\ N\ m^{-2}\ h^{-1}$) were calculated as follows (de Klein et al. 2003):

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

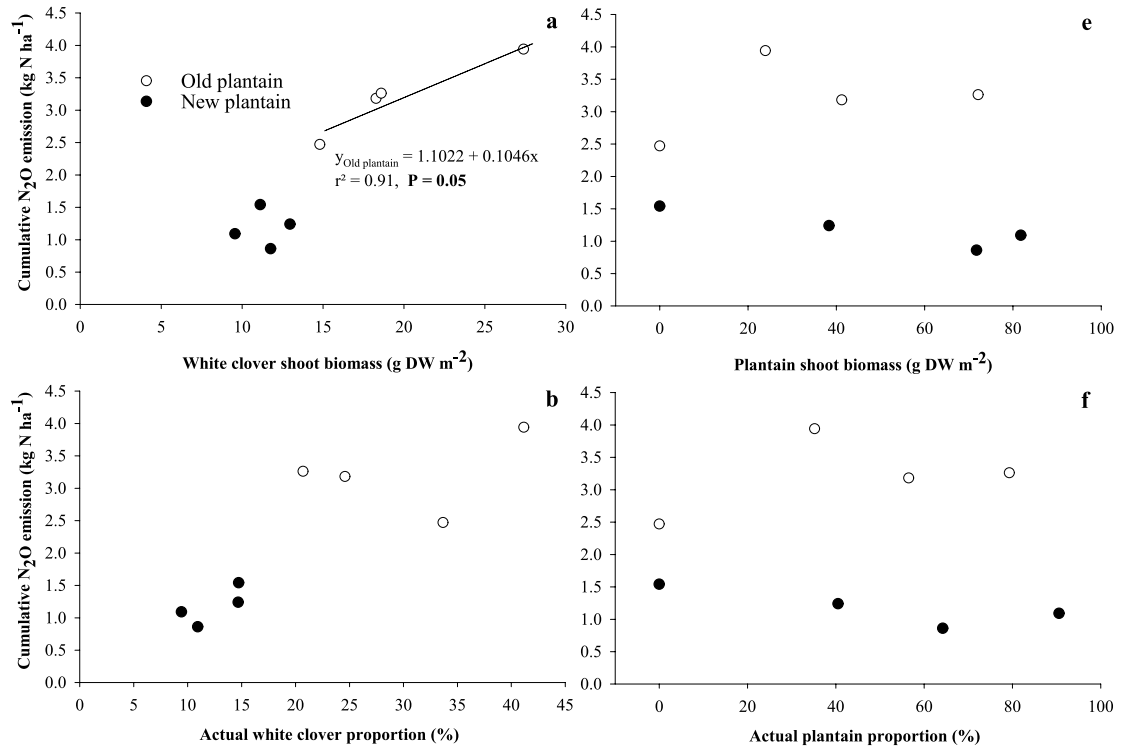
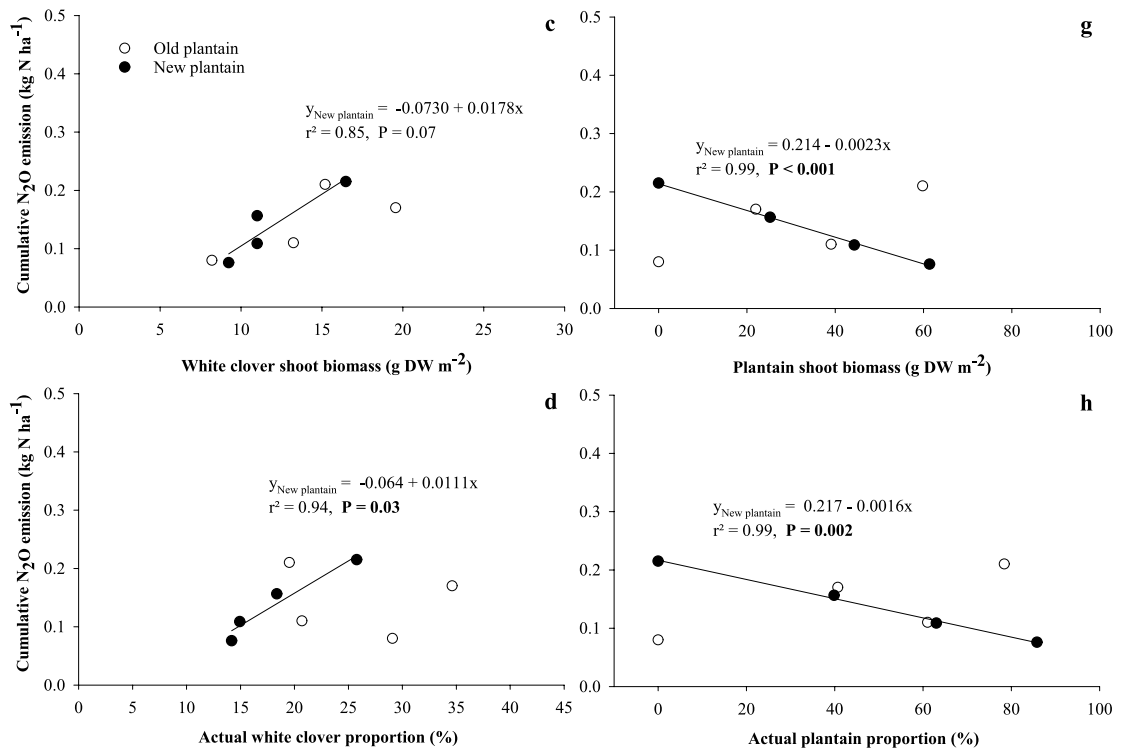
Urine treatments**Control (no urine treatments)**

Fig. 4 Relationship between cumulative N_2O emissions and shoot biomass (**a**, **c**, **e**, and **g**) or actual proportion (**b**, **d**, **f**, and **h**) of white clover and plantain in 2-year-old (Old plantain) and a 3-month-old (New plantain) swards, subjected to the application of urine and no urine (control)

where: δN_2O is the increase in headspace N_2O over time ($\mu L/L$); δT is the enclosure period (hours); M is the molar weight of N in N_2O ; V_m is the molar volume of gas at the sampling temperature (L/mol); V is the headspace volume (m^3); and A is the area covered (m^2).

These hourly emissions were integrated over time for each enclosure to estimate total emissions over the measurement period. Emission factors (EF_3 , N_2O -N emitted as % of N applied) were calculated using Eq. (2).

$$EF_3 = \frac{N_2O \text{ total}_{(urine)} - N_2O \text{ total}_{(control)}}{\text{Urine N applied}} \times 100 \quad (2)$$

where EF_3 is the emission factor, N_2O total (urine) and N_2O total (control) are the cumulative N_2O emissions from the urine and control plots, respectively ($kg \text{ N ha}^{-1}$), and urine N applied is the rate of urine N applied ($kg \text{ N ha}^{-1}$).

Weather, soil, and sward composition measurements

Daily total rainfall and mean ambient air temperatures were obtained from a weather station located within 500 m of the trial site. A manual rain gauge was also used to monitor total rainfall between sampling dates. Manual soil temperature probes were used on each gas sampling event to measure soil temperature at 5 cm depth.

Soil cores (0–7.5 cm depth, 25 mm diameter) were collected once a week from the 0.5 m^2 soil plots. From each plot, three cores were taken to the lab and thoroughly mixed to provide one composite sample per plot. Approximately 15 g of soil was utilized for soil mineral N extraction with 2 M KCl. After extraction, samples were frozen and sent to the Ruakura AgResearch campus for ammonium and nitrate determination via flow injection analysis (FIA). The remainder of the soil was weighed and dried at 104°C for 48 h to determine gravimetric soil water content. Mineral N was converted to

$kg \text{ N ha}^{-1}$ considering the soil N concentration ($mg \text{ kg}^{-1}$), bulk density (0.99 Mg m^{-3}), and the sampling depth (7.5 cm). Water-filled pore space (WFPS %) was calculated from gravimetric soil moisture content, bulk density, and an assumed particle density of 2.65 Mg m^{-3} (Linn and Doran 1984).

Sward biomass was measured 2 days before urine application. This was done by placing a 0.05 m^2 quadrat in a representative area of the plot. Plants inside the quadrat were cut at a height of 3.5 cm and transported to the laboratory for dissection according to plant species. Subsequently, plant samples were oven-dried at 65°C for at least 24 h to measure the biomass dry weight (DW).

Statistical analysis

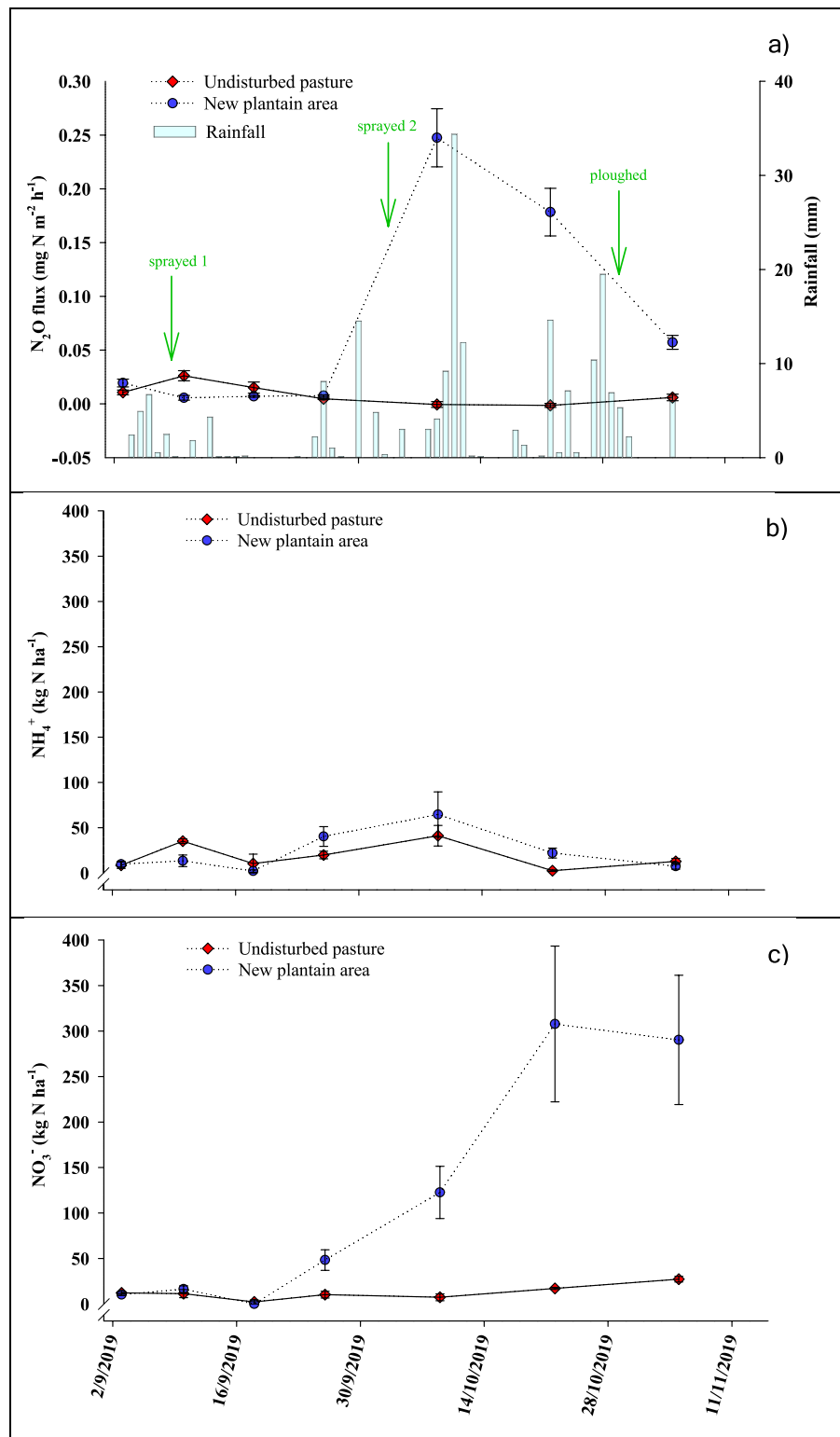
Analysis of variance (two-way ANOVA) was performed on the cumulative emissions and emission factors of N_2O for the factors of PL age and proportion in the sward. If significant differences were found, means were compared using Tukey's test ($P < 0.05$). Pearson's correlation and regression analyses were carried out to compare differences in cumulative N_2O emissions between proportions of PL sward (0, 30, 45 and 100%), using the statistical software R, version 4.3.0 (R Core, 2022).

Results

Weather

Cumulative rainfall during seedbed preparation of the New plantain trial (September to November 2019) was 200 mm over the 64 days, while air temperature ranged from 3.6 to 20.3°C (average 9.1°C) (Fig. 1). In contrast, rainfall during the N_2O measurement period after cattle urine application (Feb to May 2020) was considerably lower, totaling 124 mm over a 99-day period. Air temperature ranged from 3.2 to 18.9°C (average 11.6°C) (Fig. 1). The accumulated rainfall during the 36 days of Covid19 lockdown was very low, totaling 42 mm, while air temperature ranged between 5.3 and 17.2°C (average 10.5°C).

Fig. 5 Nitrous oxide flux (a), soil ammonium (b), and nitrate (c) during the transition of ryegrass/white clover pasture to a plantain-containing sward. Arrows indicate the timing of spraying and ploughing in the New plantain area. Vertical bars refer to the standard error of the mean



Pasture composition

Actual plantain proportions did not significantly differ between new and old swards (Table 1). Old plantain swards contained higher clover and lower ryegrass proportions relative to the new swards. As expected, increasing the proportion of plantain resulted in decreased proportions of ryegrass for old and new swards. Only in the old sward, the 30% PL increased the proportion of clover relative to the 100% PL.

Nitrous oxide emissions after cattle urine application: Effect of sward age

Nitrous oxide fluxes peaked on the day of urine application in both trials, with fluxes ranging from 2.83 mg N m⁻² h⁻¹ (0% PL) to 4.53 mg N m⁻² h⁻¹ (30% PL) in the Old plantain trial (Fig. 3a) and from 1.45 mg N m⁻² h⁻¹ (0% PL) to 0.75 mg N m⁻² h⁻¹ (45% PL) in the New plantain trial (Fig. 3e).

A second small increase in N₂O fluxes occurred 6 days after urine application (Fig. 2a, e). This second peak was associated with an increase in soil water-filled pore space (WFPS) generated after urine application and rainfall (Fig. 2b, f). In addition, the soil NO₃⁻ concentration increased over this period (Fig. 2d, h).

Cumulative N₂O emissions varied from 0.86 kg N ha⁻¹ (45% PL) to 1.54 kg N ha⁻¹ (0% PL) in the New plantain swards and from 2.47 kg N ha⁻¹ (0% PL) to 3.26 kg N ha⁻¹ (100% PL) in the Old plantain swards (Table 2). Cumulative N₂O emissions in the New plantain plots were 20% (30% PL), 44% (45% PL), and 29% (100% PL) lower than those from the RG/WC sward (0% PL). In contrast, in the Old plantain, N₂O emissions were 22–37% higher than in the RG/WC sward (0% PL) (Table 2), although this effect was not statistically significant, except for 45% PL in the new sward.

The N₂O emission factors (EFs) in the New plantain treatments ranged from 0.11% (45% PL) to 0.19% (0% PL), with an average EF for all PL plots of 0.14%. This was a reduction of 26% compared with the EF from urine deposited to RG/WC (Table 2). In the Old plantain plots, the EFs ranged from 0.35% (0% PL) to 0.55% (30% PL). The average EF over all PL plots was 0.48%, which was 27% higher than the EF from urine deposited to RG/WC (Table 2).

Effect of plantain proportion in the sward

There was no significant effect of PL treatment on cumulative N₂O emissions after urine application in the old sward ($P > 0.05$) (Table 2). However, when urine was applied to newly established PL swards, there was a significant decrease of 44% in N₂O cumulative emissions from 45% PL sward compared with RG/WC clover sward ($P < 0.05$) (Table 2). At 30% and 100% PL in the newly established sward, there were 20% and 30% reductions, respectively, in cumulative N₂O emissions, but these were not statistically significant reductions from 0% PL ($P > 0.05$) (Table 2, Fig. 3d). For emission factor values, the new sward presented lower values than the old sward (Table 2). In addition, a trend of reducing N₂O emission factors with increasing PL proportions in the sward was observed between the 0, 30 and 45% PL treatments in the newly established trial (Fig. 3e).

The results indicated that the higher the clover biomass, the larger the N₂O emissions in the Old plantain treatments subjected to urine application ($P = 0.05$). However, this relationship was not significant in the new sward (Fig. 4a). Plantain shoot biomass and actual proportions were not significantly correlated to N₂O emissions (Fig. 4e, f).

Effect of sward age and proportion of plantain on N₂O emissions from non-urine plots

Cumulative N₂O emissions were also assessed from treatments without urine application (control), and emissions were significantly lower in the 100% PL treatment of the New plantain swards ($P < 0.05$) (Table 2). We also found a significant decrease in N₂O emissions with increasing PL proportions (Figs. 3f, 4g, 4h). White clover shoot biomass had a positive relationship with N₂O emissions in the new sward when no urine was applied (Fig. 4d, $P = 0.03$). In the control plots of the Old plantain swards, an opposite effect was observed, with cumulative emissions being significantly higher in 30 and 100% PL treatments compared with the 0% PL treatment ($P < 0.10$) (Table 2). Furthermore, there was a marginally significant trend of increasing N₂O emissions with increasing nominal PL proportions ($r^2 = 0.68$, $P = 0.09$) (Fig. 3c). The same trend was observed for actual plantain proportions and shoot biomass of the

Old sward, but it was not statistically significant in this case (Fig. 4g and h).

Potential impact on N₂O emissions during the establishment of plantain in the sward

Measurements were taken during the establishment of the New plantain trial to assess the effect of transitioning from RG/WC clover to PL swards on N₂O emissions. During the first three weeks after spraying out the RG/WC sward with the herbicide glyphosate, N₂O fluxes were low and similar to the undisturbed pasture (Fig. 5a). However, five days after a second herbicide spraying (30 days after the first application), N₂O emissions were significantly greater in the PL sward (0.25 mg N m⁻² h⁻¹) compared with emissions from the undisturbed pasture (−0.001 mg N m⁻² h⁻¹) ($P < 0.05$) (Fig. 5a). Considering cumulative N₂O emissions, the new plantain area and the undisturbed pasture emitted 1.53 kg N ha⁻¹ (± 0.30 SE) and 0.1 kg N ha⁻¹ (± 0.07 SE) of N₂O, respectively.

Soil ammonium content slightly increased in the New plantain area 9 days following the first herbicide application and peaked 5 days after the second herbicide application (65 kg N ha⁻¹) (Fig. 5b). Soil nitrate content increased 9 days after the first herbicide application and was further augmented significantly after a heavy rainfall event of 34 mm, reaching 308 kg N ha⁻¹ (Fig. 5c).

Discussion

Plantain reduced N₂O emissions only in the new sward with a 45% nominal proportion

Our results showed large peaks in N₂O fluxes shortly after urine application, which is consistent with what has been observed in previous studies (e.g. Hoogenboom et al. 2016; Simon et al. 2019; van der Weerden et al. 2017). These authors have associated these events with several factors, such as the priming effect of urine, resulting in a rapid mobilisation of N and carbon.

The potential of PL to reduce N₂O emissions from urine was observed in newly established swards, but not in older swards (Table 2). The N₂O emissions measured in the Old plantain swards were up to ca. 3 times higher than the emissions in the New plantain

swards, even though the trials were side-by-side and measurements were conducted under the same environmental conditions. These larger N₂O fluxes in the Old plantain swards could have been due to higher clover contents observed in the Old plantain swards, as we found a significant linear relationship between above-ground clover biomass and N₂O emissions following urine application (Fig. 4a; $r^2 = 0.91$, $P < 0.05$). The higher clover contents could have resulted in higher indigenous soil N content, soil microbial populations, and thus increased N₂O fluxes (Sturite et al. 2021). Biological N fixation is not considered a source of N₂O emissions according to the IPCC guidelines. For example, Cashman et al. (2025) found that biologically fixed N by clover did not contribute to N₂O emissions from an unfertilized perennial pasture in Ireland. It is also unlikely that biological N fixation contributes to N₂O emissions from urine patches, as high soil mineral N suppresses the formation of root nodules. Despite this, previous studies carried out in New Zealand investigating N₂O emissions from urine patches found larger emissions in white clover monocultures compared to plantain and perennial ryegrass (Bowatte et al. 2018; Luo et al. 2018). The exact mechanisms behind this effect are unclear; however, Luo et al. (2018) indicated that elevated soil moisture in white clover swards, exudation of labile C, and rhizosphere acidification are among the potential factors to explain it. This also may explain why our results differ from Navarrete et al. (2016), who found higher concentrations of plantain bioactive compounds possibly associated with its BNI effect, such as aucubin, in older plantain swards.

Previous studies assessing the persistence and quality of PL in grazed systems showed a significant decrease in PL persistence from a 1-year-old to a 3-year-old established PL swards (Moorhead and Pigot 2009). This reduces the interaction between PL and soil N, thus decreasing its potential for nitrification inhibition and N₂O release mitigation. However, there were no statistical differences between actual plantain proportions in new and old swards in our study (Table 1). Thus, low plantain persistency in the old sward is unlikely to explain the different effect of plantain in old and new swards in the present study.

These contrasting results on the effect of the proportion of PL on N₂O emissions in the Old versus New plantain swards may suggest that the inhibitory effect of PL on N₂O emissions reduces as sward age

increases. Thus, apart from the confounding effects of different white clover contents in New and Old plantain swards in our study, it is also possible that the capacity of plantain to reduce N_2O emissions decreases over time. It is known that the belowground allocation of C in plants, including root exudates with potential BNI effect, changes according to plant phenological stages (Buss et al. 2026; Pausch and Kuzyakov 2018). For instance, Warembourg and Estelrich (2001) demonstrated that the belowground C allocation in a perennial grass (*Bromus erectus*) increases until ca. 100 days after planting, then declines gradually over time. Therefore, further research is necessary to identify if plantain follows this pattern and how this could influence its potential to mitigate N_2O emissions.

When no urine was applied to the soil, the New plantain swards also showed a significant trend of decreasing cumulative N_2O emissions with increasing proportions of PL in the sward (Figs. 3f, 4g, and h). This suggests that even under low N (non-urine) concentrations in the soil, PL had the potential to reduce N_2O emissions. In contrast, in the non-urine plots of the Old plantain swards, a marginally significant increase in cumulative N_2O emissions was observed ($P=0.09$) with increasing nominal PL proportions in the sward (Fig. 3c). This trend was also observed for actual plantain proportions and shoot biomass, although it was not statistically significant in those cases (Fig. 4g and h). Again, these results could have been due to the confounding effect of variations in clover content between the different PL treatments in the swards, as higher clover shoot biomass tended to present higher N_2O emissions in no urine plots (Fig. 4c and d).

Pasture renewal to establish plantain in the swards increased N_2O emissions

Our measurements of N_2O emissions during the transition phase from RG/WC to PL swards suggested that the emissions were higher in the PL compared to the undisturbed area. These higher emissions were possibly due to soil exposure and increased N mineralisation during soil cultivation, which is known to lead to increased N_2O emissions (Luo et al. 2017). Moreover, the intense rainfall after the second herbicide spraying probably contributed to exacerbating the N_2O emissions in the presence of higher concentrations

of substrates for denitrification due to cultivation. Similarly, Thomas et al. (2008) found that soil water content above the field capacity led to a significant increase in N_2O emissions from a renewed grassland relative to uncultivated soil; however, the authors did not observe this effect of cultivation when the soil water content was below the field capacity. Our results indicate that pasture renewal can lead to elevated N_2O emissions. Therefore, other approaches for grassland renewal should be considered to decrease N_2O emissions. Alternatives to be considered include N fertilization management, tillage methods, and timing of sward renewal that aim at reducing N surplus, minimizing intensive soil disturbance and avoiding periods of high soil moisture, respectively (Seidel et al. 2009; van 't Hull et al. 2025).

Conclusions

The results of this study show that plantain's inhibitory effect on N_2O emissions from cattle urine is effective in a newly established sward (3 months) but not in an older sward (2 years). In this regard, cumulative N_2O emissions from urine applied to a new plantain sward decreased, although this effect was significant only for the 45% plantain proportion. In contrast, there was no significant effect of increasing plantain proportions on N_2O emissions from urine in the old sward. Importantly, the old plantain sward had higher clover biomass than the new sward, and this is likely to have influenced the results. This is because a significant relationship between N_2O emissions from urine and the aboveground clover biomass of the old sward was observed. Therefore, it was not possible to disentangle sward age and clover share in the sward effects in this study, and the results need to be interpreted with caution. Finally, transitioning from a ryegrass/white clover to a plantain-containing sward can increase N_2O emissions following soil cultivation. Therefore, alternative cultivation approaches that consider the timing of grassland renewal, tillage methods, and N fertilisation management may be considered in future research.

Acknowledgements We thank the Ag Emissions Centre, formerly known as the New Zealand Agricultural Greenhouse Gas Research Centre (NZAGRC), for funding this work, Agri-com Seeds for providing the seed mixes, and the AgResearch

technical team for their support with urine collection and gas sampling.

Author's contribution P.L.S. and C.A.M. de K. contributed to the study conceptualisation; P.L.S. and C.A.M. de K. performed the experiments; P.L.S. performed the data analyses and wrote the manuscript's first draft; all authors performed data interpretation, discussion and reviewed the manuscript; P.L.S. and C.A.M. de K. were responsible for funding acquisition.

Funding Open access funding provided by Bioeconomy Science Institute.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding authors on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Bowatte S, Hoogendoorn CJ, Newton PCD, Liu Y, Brock SC, Theobald PW (2018) Grassland plant species and cultivar effects on nitrous oxide emissions after urine application. *Geoderma* 323:74–82. <https://doi.org/10.1016/j.geoderma.2018.03.001>
- Buss W, Verburg K, Garba II, O'Sullivan CA, Wang E, Gupta VV, Bentley AR (2026) Harnessing biological nitrification inhibition to reduce soil nitrogen losses—systematic quantification of plant and soil factors to maximise field-scale benefits. *Agric Ecosyst Environ* 396:110002. <https://doi.org/10.1016/j.agee.2025.110002>
- Byrnes RC, Nunez J, Arenas L, Rao I, Trujillo C, Alvarez C, Arango J, Rasche F, Chirinda N (2017) Biological nitrification inhibition by *Brachiaria* grasses mitigates soil nitrous oxide emissions from bovine urine patches. *Soil Biol Biochem* 107:156–163. <https://doi.org/10.1016/j.soilbio.2016.12.029>
- Cardenas LM, Misselbrook TM, Hodgson C, Donovan N, Gillespy S, Smith KA, Dhanoa MS, Chadwick D (2016) Effect of the application of cattle urine with or without the nitrification inhibitor DCD, and dung on greenhouse gas emissions from a UK grassland soil. *Agric Ecosyst Environ* 235:229–241. <https://doi.org/10.1016/j.agee.2016.10.025>
- Carlton AJ, Cameron KC, Di HJ, Edwards GR, Clough TJ (2019) Nitrate leaching losses are lower from ryegrass/white clover forages containing plantain than from ryegrass/white clover forages under different irrigation. *N Z J Agric Res*. <https://doi.org/10.1080/00288233.2018.1461659>
- Cashman O, Casey I, Sorley M, Forrestal P, Styles D, Wall D, Burchill W, Humphreys J (2025) Lowering the greenhouse gas and ammonia emissions from grassland-based dairy production. *Agric Syst* 222:104151. <https://doi.org/10.1016/j.agsy.2024.104151>
- Clough TJ, Sherlock RR, Mautner MN, Milligan DB, Wilson PF, Freeman CG, McEwan MJ (2003) Emission of nitrogen oxides and ammonia from varying rates of applied synthetic urine and correlations with soil chemistry. *Aust J Soil Res* 41(3):421–438. <https://doi.org/10.1071/SR02105>
- de Klein CAM, Barton L, Sherlock RR, Li Z, Littlejohn RP (2003) Estimating a nitrous oxide emission factor for animal urine from some New Zealand pastoral soils. *Aust J Soil Res* 41(3):381–399. <https://doi.org/10.1071/sr02128>
- de Klein CAM, van der Weerden TJ, Luo J, Cameron KC, Di HJ (2020) A review of plant options for mitigating nitrous oxide emissions from pasture-based systems. *N Z J Agric Res*. <https://doi.org/10.1080/00288233.2019.1614073>
- de Klein CAM, Bowatte S, Simon PL, Arango J, Cardenas LM, Chadwick DR, Pijlman J, Rees RM, Richards KG, Subbarao GV, Whitehead D (2022) Accelerating the development of biological nitrification inhibition as a viable nitrous oxide mitigation strategy in grazed livestock systems. *Biol Fertil Soils*. <https://doi.org/10.1007/s00374-022-01631-2>
- Gardiner CA, Clough TJ, Cameron KC, Di HJ, Edwards GR, de Klein CAM (2016) Potential for forage diet manipulation in New Zealand pasture ecosystems to mitigate ruminant urine derived N₂O emissions: a review. *N Z J Agric Res* 59(3):301–317. <https://doi.org/10.1080/00288233.2016.1190386>
- Gardiner CA, Clough TJ, Cameron KC, Di HJ, Edwards GR, de Klein CAM (2017) Potential inhibition of urine patch nitrous oxide emissions by *Plantago lanceolata* and its metabolite aucubin. *N Z J Agric Res*. <https://doi.org/10.1080/00288233.2017.1411953>
- Haynes RJ, Williams PH (1993) Nutrient cycling and soil fertility in the grazed pasture ecosystem. *Adv Agron* 49:119–199. [https://doi.org/10.1016/S0065-2113\(08\)60794-4](https://doi.org/10.1016/S0065-2113(08)60794-4)
- Hewitt AE (2010) New Zealand soil classification, vol 1, 3 edn. Manaaki Whenua Press, Landcare Research Science Series, Hamilton
- Hoogendoorn CJ, Luo J, Lloyd-West CM, Devantier BP, Lindsey SB, Sun S, Pacheco D, Li Y, Theobald PW, Judge A (2016) Nitrous oxide emission factors for urine from sheep and cattle fed forage rape (*Brassica napus* L.) or perennial ryegrass/white clover pasture (*Lolium perenne*

- L./*Trifolium repens*). Agric Ecosyst Environ 227:11–23. <https://doi.org/10.1016/j.agee.2016.04.029>
- IPCC (2021) Climate Change 2021: The physical science basis. In: Masson-Delmotte V, Zhai P, Pirani A, Connors SL, Péan C, Berger S, Caud N, Chen Y, Goldfarb L, Gomis MI, Huang M, Leitzell K, Lonnoy E, Matthews JBR, Maycock TK, Waterfield T, Yelekçi O, Yu R, Zhou B (eds) Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change.
- Judson R, Houck K, Paul Friedman K, Brown J, Browne P, Johnston PA, Close DA, Mansouri K, Kleinstreuer N (2020) Selecting a minimal set of androgen receptor assays for screening chemicals. Regulat Toxicol Pharmacol, 117. <https://doi.org/10.1016/j.yrtph.2020.104764>
- Linn DM, Doran JW (1984) Effect of water filled pore space on carbon dioxide and nitrous oxide production in tilled and non-tilled soils. Soil Sci Soc Am J 48:1267–1272. <https://doi.org/10.2136/sssaj1984.03615995004800060013x>
- Luo J, Balvert SF, Wise B, Welten B, Ledgard SF, de Klein CAM, Lindsey S, Judge A (2018) Using alternative forage species to reduce emissions of the greenhouse gas nitrous oxide from cattle urine deposited onto soil. Sci Total Environ 610:1271–1280. <https://doi.org/10.1016/j.scitotenv.2017.08.186>
- Luo J, Wyatt J, van der Weerden TJ, Thomas SM, de Klein CAM, Li Y, Rollo M, Lindsey S, Ledgard SF, Li J, Ding W, Qin S, Zhang N, Bolan N, Kirkham MB, Bai Z, Ma L, Zhang X, Wang H et al (2017) Chapter Five - Potential Hotspot Areas of Nitrous Oxide Emissions From Grazed Pastoral Dairy Farm Systems. In: Sparks DL (ed) Advances in agronomy (Vol. 145, pp. 205–268). Academic Press, New York. <https://doi.org/10.1016/bs.agron.2017.05.006>
- Minnée EMK, Leach CMT, Dalley DE (2020) Substituting a pasture-based diet with plantain (*Plantago lanceolata*) reduces nitrogen excreted in urine from dairy cows in late lactation. Livest Sci. <https://doi.org/10.1016/j.livsci.2020.104093>
- Moorhead AJE, Piggot GJ (2009) The performance of pasture mixes containing ‘Ceres Tonic’ plantain (*Plantago lanceolata*) in Northland. New Zeal Grassland Assoc, 71.
- Navarrete S, Kemp PD, Pain SJ, Back PJ (2016) Bioactive compounds, aucubin and acteoside, in plantain (*Plantago lanceolata* L.) and their effect on in vitro rumen fermentation. Anim Feed Sci Technol 222:158–167. <https://doi.org/10.1016/j.anifeedsci.2016.10.008>
- NIWA. (2018) Climate and weather of New Zealand.
- Nyameasem JK, Malisch CS, Loges R, Taube F, Kluß C, Vogeler I, Reinsch T (2021) Nitrous oxide emission from grazing is low across a gradient of plant functional diversity and soil conditions. Atmosphere 12:223. <https://doi.org/10.3390/atmos12020223>
- O’Connell C, Judson HG, Barrell GK (2016) Sustained diuretic effect of plantain when ingested by sheep. Proc New Zeal Soc Anim Prod 76:14–17.
- Pausch J, Kuzyakov Y (2018) Carbon input by roots into the soil: quantification of rhizodeposition from root to ecosystem scale. Glob Change Biol 24(1):1–12. <https://doi.org/10.1111/gcb.13850>
- Pijlman J, Berger SJ, Lexmond F, Bloem J, van Groenigen JW, Visser EJW, Erisman JW, van Eekeren N (2020) Can the presence of plantain (*Plantago lanceolata* L.) improve nitrogen cycling of dairy grassland systems on peat soils? N Z J Agric Res. <https://doi.org/10.1080/00288233.2019.1698620>
- R Core, T (2022) R: A language and environment for statistical computing. In R Foundation for Statistical Computing. <https://www.R-project.org/>.
- Reinsch T, Loges R, Kluß C, Taube F (2018) Renovation and conversion of permanent grass-clover swards to pasture or crops: effects on annual N₂O emissions in the year after ploughing. Soil Tillage Res 175:119–129. <https://doi.org/10.1016/j.still.2017.08.009>
- Rodriguez MJ, Navarrete S, Horne DJ, Hanly JA, Kemp PB, Kemp PD (2021) Can secondary metabolites of plantain reduce N losses from urine patches? N Z J Agric Res 66(1):83–100. <https://doi.org/10.1080/00288233.2021.1991388>
- Seidel K, Kayser M, Müller J, Isselstein J (2009) The effect of grassland renovation on soil mineral nitrogen and on nitrate leaching during winter. J Plant Nutr Soil Sci 172(4):512–519
- Simon PL, de Klein CAM, Worth W, Rutherford AJ, Dieckow J (2019) The efficacy of *Plantago lanceolata* for mitigating nitrous oxide emissions from cattle urine patches. Sci Total Environ 691:430–441. <https://doi.org/10.1016/j.scitotenv.2019.07.141>
- Simon P, de Klein C, Gerard E, Shi S (2020) Ammonia-oxidizing bacteria dynamics affected by plantain under synthetic cattle urine patches. J N Z Grassl. <https://doi.org/10.33584/jnzs.2020.82.433>
- Soil Survey Staff (2022) Keys to soil taxonomy, 13th edition. USDA Natural Resources Conservation Service
- Sturite I, Rivedal S, Dörsch P (2021) Clover increases N₂O emissions in boreal leys during winter. Soil Biol Biochem 2021:108459. <https://doi.org/10.1016/j.soilbio.2021.108459>. (v.163, pp.)
- Subbarao GV, Nakahara K, Hurtado MP, Ono H, Moreta DE, Salcedo AF, Yoshihashi AT, Ishikawa T, Ishitani M, Ohnishi-Kameyama M, Yoshida M, Rondon M, Rao IM, Lascano CE, Berry WL, Ito O (2009) Evidence for biological nitrification inhibition in *Brachiaria* pastures. Proc Natl Acad Sci U S A 106(41):17302–17307. <https://doi.org/10.1073/pnas.0903694106>
- Subbarao GV, Sahrawat KL, Nakahara K, Ishikawa T, Kishii M, Rao IM, Hash CT, George TS, Srinivasa Rao P, Nardi P, Bonnett D, Berry W, Suenaga K, Lata JC (2012) Biological nitrification inhibition—a novel strategy to regulate nitrification in agricultural systems. Adv Agron 114:249–302. <https://doi.org/10.1016/B978-0-12-394275-3.00001-8>
- Thomas SM, Beare MH, Francis GS, Barlow HE, Hedderley DI (2008) Effects of tillage, simulated cattle grazing and soil moisture on N₂O emissions from a winter forage crop. Plant Soil 309(1):131–145. <https://doi.org/10.1007/s11104-008-9586-4>
- van der Weerden TJ, Clough TJ, Styles TM (2013) Using near-continuous measurements of N₂O emission from urine-affected soil to guide manual gas sampling regimes. N Z J Agric Res 56(1):60–76. <https://doi.org/10.1080/00288233.2012.747548>
- van der Weerden TJ, Styles TM, Rutherford AJ, de Klein CAM, Dynes R (2017) Nitrous oxide emissions from cattle urine deposited onto soil supporting a winter forage kale crop.

- N Z J Agric Res 60(2):119–130. <https://doi.org/10.1080/00288233.2016.1273838>
- van't Hull J, van Middelkoop J, van Schooten H, Ros M, van Groenigen JW, Velthof G (2025) Potential measures to reduce nitrate and nitrous oxide losses from renovated grasslands. Agric Ecosyst Environ 384:109549. <https://doi.org/10.1016/j.agee.2025.109549>
- Wall AM, Wecking AR, Goodrich JP, Pronger J, Campbell DI, Morcom CP, Schipper LA (2023) Paddock-scale carbon and greenhouse gas budgets in the first year following the renewal of an intensively grazed perennial pasture. Soil Tillage Res 234:105814. <https://doi.org/10.1016/j.still.2023.105814>
- Warembourg FR, Estelrich HD (2001) Plant phenology and soil fertility effects on below-ground carbon allocation for an annual (*Bromus madritensis*) and a perennial (*Bromus erectus*) grass species. Soil Biol Biochem 33(10):1291–1303. [https://doi.org/10.1016/S0038-0717\(01\)00033-5](https://doi.org/10.1016/S0038-0717(01)00033-5)
- Wichtl M (2004) Herbal drugs and phytopharmaceuticals: a handbook for practice on a scientific basis. Medpharm GmbH Scientific Publishers, Stuttgart

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.