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Effective Proportion of Plantain (*Plantago lanceolata* L.) in Mixed Pastures for Botanical Stability and Mitigating Nitrous Oxide Emissions from Cow Urine Patches

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Abstract: Plantain (*Plantago lanceolata* L.) is recognised for its ability to improve summer feed productivity as well as mitigate nitrous oxide (N_2O) emissions from grazed pastoral soils. This study aims to determine the proportion of plantain required in perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.), or RWC, mixed swards to maintain yield stability and to quantify N_2O emission reductions. The botanical composition was monitored when plantain was sown at different rates of 0%, 30%, 50% and 70% in RWC pastures under grazing by dairy cows over 2 grazing years. Urine from cows grazing RWC (6.15 g N L^{-1}), 30% (5.40 g N L^{-1}) and 50% (4.40 g N L^{-1}) plantain mixed pastures was used to measure N_2O emissions (n = 5) from the pastures of their origin and to assess the impact of the plantain rhizosphere on N_2O emissions by applying the RWC urine to the 50% plantain mixed pasture, and vice versa, in late summer–autumn using the static chambers technique. After declining in spring, the plantain content recovered in early autumn and reached a peak of 40% in the 30% plantain mixed pasture and around 50% in the 50% and 70% plantain mixed pastures in winter. A lower N content in urine, and therefore a lower urine N-loading rate from cows grazing in the 50% plantain mixed pasture, resulted in 39% lower total N_2O emissions compared to RWC urine treatment. In conclusion, 30% to 50% plantain in mixed pastures was stable throughout the 2 years, and it not only reduced the urinary N concentration in grazing cattle but also contributed to reduced N_2O EFs.

Keywords: plantain; ryegrass–white clover; summer feed quality; urine N; nitrous oxide

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Citation: Vi, C.; Kemp, P.D.; Saggar, S.; Navarrete, S.; Horne, D.J. Effective Proportion of Plantain (*Plantago lanceolata* L.) in Mixed Pastures for Botanical Stability and Mitigating Nitrous Oxide Emissions from Cow Urine Patches. *Agronomy* **2023**, *13*, 1447. https://doi.org/10.3390/agronomy13061447

Academic Editor: Fujiang Hou

Received: 20 April 2023
Revised: 22 May 2023
Accepted: 22 May 2023
Published: 24 May 2023

1. Introduction

In New Zealand, the main pastoral supply for dairying has traditionally been based on permanent pasture mixtures of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) [1]. However, these pasture species have poor tolerance to drought conditions, which limits their herbage production and nutritive value and, in turn, can negatively affect grazing livestock performance [2]. The establishment of novel pasture species, such as narrow-leaf plantain (*Plantago lanceolata* L.), with high herbage production and tolerance to New Zealand drought and heat as an adaption strategy to climate change has been suggested [3]. 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 [4]. Consequently, the N excreted by dairy cattle in urine is the largest source of nitrous oxide (N_2O) emissions in grazed pastoral systems [5].

In recent years, plantain, a summer active pasture species, incorporated into RWC swards has been adopted by New Zealand farmers due to its drought tolerance and environmental benefits of reducing N losses. Narrow-leaf plantain is a rosette-forming perennial herb occurring naturally in pasture swards throughout temperate regions selected to be used as forage in livestock production systems [6]. Pasture containing plantain has been well documented to have greater herbage dry matter (DM) accumulation and quality than that of RWC in summer and autumn [3,7,8]. Additionally, recent studies have shown 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 has been found to be 28% to 74% lower than that from RWC swards [9,10].

Plantain plays a dual role in reducing N_2O emissions in dairy grazed pastures first by reducing the N content in urine from cows consuming plantain [9,11–13] and second by biologically inhibiting the nitrification of urine N through the production of secondary compounds [14–16]. The quantity of plantain in a pasture can affect its efficacy in reducing N losses to the environment. Minnée et al. [12] 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 results in greater reductions in N_2O emissions [13]. However, the quantity of plantain in mixed RWC swards is likely to change over the year, with the abundance of species varying according to plant competition, grazing impacts and weather conditions [17]. Therefore, focused research is required to provide more quantitative information about the feasibility of maintaining the proportion of plantain needed in mixed swards to obtain its benefits, particularly those related to its effects on N losses (leaching and gaseous), and about the variation in the proportion of plantain in a sward across the seasons.

The aim of this field study was to monitor the plantain content (as a percentage in the DM and plant density) when incorporated in RWC mixed swards across two grazing seasons under dairy conditions and to evaluate N_2O emissions from RWC pastures containing different proportions of plantain. We hypothesized that the incorporation of plantain into RWC mixed swards will increase pasture production during dry periods in summer as well as reduce N_2O emissions via effects on the N-loading rate and N-cycling in the soil and soil microclimate.

2. Materials and Methods

2.1. Experimental Site and Pasture Treatments

A 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 Tokomaru silt loam soil, as termed in the New Zealand soil classification system. Before sowing the treatments, the existing swards were eliminated by spraying with Polaris® 540 (54% glyphosate) at 4 L/ha, Harmony® (50% thifensulfuron-methyl) at 30 g/ha and Pulse® penetrant (organo-silicone penetrant) at 100 mL/ha.

In April 2019, four pasture treatments were established in experimental plots by sowing (direct drilling) mixtures of ryegrass, white clover and plantain seed designed to provide 0% (RWC), 30% (P30), 50% (P50) and 70% (P70) of plantain in the swards. 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	One^{50}	20	15	10	5
White clover	Emerald	3	3	3	3
Plantain	Agritonic	0	4	7	10

Pasture treatments were arranged in a randomised complete design, with five replicates per treatment. In total, the research site consisted of 20 experimental plots of 800 m^2

(40 m × 20 m) each and four adaptation paddocks of 1 ha per paddock, representing each pasture treatment.

Grazing and Pasture Management

The experimental site was first grazed by dairy cows on 18 June 2019 (12 weeks after sowing), a further nine times from September 2019 to July 2020, during the 2019/2020 grazing year (year 1), and eight times from September 2020 to May 2021, in the 2020/2021 grazing year (year 2). In the 2 grazing years, the grazing interval (3–5 weeks) was decided according to pre-grazing masses targeting a range from 3600–3800 kg DM ha^{-1}. For each grazing, 80 dairy cows were 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 with the same pasture type to graze for 2–3 days, with four cows in each replicate plot.

The experimental plots received urea fertilizer at 50 kg N ha^{-1} in October 2019, December 2019, and February 2020 and at 30 kg N ha^{-1} in September 2020, November 2020 and April 2021. The pasture treatments were mown after the January, October and December 2020 grazing to remove seed heads and control post-grazing residue. In March 2020, additional plantain seeds were drilled into the experimental plots to maintain the proportion of plantain, with a sowing rate of 3, 6 and 9 kg ha^{-1} in the P30, P50 and P70 plots, respectively. In May 2021, the P70 pasture treatment was sprayed with herbicide to remove ryegrass and re-establish this pasture treatment.

2.2. Botanical Composition and Plant Density Measurements

The botanical composition and plantain density of the pasture treatments were monitored throughout the 2 grazing years. In the first year, the botanical composition (percentage of total DM) was evaluated pre-grazing in the following periods: September 2019 (early spring), December 2019 (early summer), March 2020 (early autumn) and July 2020 (winter). During the second year, botanical samples from the pasture treatments were collected in September 2020 (early spring), October 2020 (mid-spring), November 2020 (late-spring), February 2021 (late summer) and May 2021 (late autumn). In May 2021, due to the application of herbicide for the re-establishment 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: perennial ryegrass, white clover, plantain (leaf and reproductive stems), other grasses, broadleaf weeds and dead material. Each separated category was individually oven-dried at 70 °C for 48 h and weighed to determine the botanical composition as a 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 within 0.25 m^2 quadrats (n = 5) along a diagonal line in each replicate plot.

2.3. Nitrous Oxide Emission Measurement

2.3.1. Experimental Design

Nitrous oxide emissions from plantain–RWC pastures were undertaken from summer to late autumn 2021 (15 February to 1 June 2021) of the 2020/2021 growing season. According to the results of the experiment on the botanical composition of plantain mixed pastures presented in Section 3.1.1, the proportion of plantain in the 50% plantain and 70% plantain mixed pasture treatments were similar in the 2020/2021 season. Therefore, the 70% plantain mixed plots were excluded in this experiment. This experiment measured N_2O emissions from urine N from dairy cows grazing in RWC mixed with different proportions of plantain in the swards: 0% (RWC), 30% (P30) and 50% (P50). In each of the five replicate plots for each of the three pasture treatments, a representative area (2 m × 5 m) was fenced off and excluded from grazing in December 2020, 9 weeks before the experiment started. The designated areas were cut to 5 cm above ground level, and the herbage was removed before the start of gas emission measurements.

Static chambers (250 mm diameter × 200 mm height) were inserted permanently into the soil (100 mm depth) 24 h prior to the initial sampling. There was an area of 0.25 m^2 (0.5 m × 0.5 m) for soil moisture and mineral N (ammonium and nitrate) measurements adjacent to each gas chamber.

2.3.2. Cow Urine Collection, Analysis and Application

Fresh urine was collected (11–12 February 2021) using vulva stimulation, from individual dairy cows grazing pasture treatments (RWC, P30 and P50), resulting in three respective urine (U) treatments: URWC, U30 and U50. Animal ethics for this study was approved by the Massey University Animal Ethics Committee (application no. 19/54). The urine samples were bulked for each treatment and stored at 4 °C to avoid urea hydrolysis. Sub-samples, 35 mL of each urine type, were taken immediately for total N and urea analysis in the Nutrition Laboratory, Massey University.

On 15 February 2021, urine (URWC, U30 and U50) treatments were applied to the corresponding pasture treatments. The RWC and P50 plots both 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, the eight combined treatments consisted of RWC treated with RWC urine (RWC + URWC), with 50% plantain urine (RWC + U50) and without urine (RWC + NoU); 30% plantain mixed pasture treated with 30% plantain urine (P30 + U30) and without urine (P30 + NoU); and 50% plantain mixed pasture treated with 50% plantain urine (P50 + U50), with RWC 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 an equivalent rate of 10 L m^{-2}, creating a uniformly wetted area. This application rate represented the average wetted surface area (0.24 m^2) and the mean volume of the urine (≈2 L) voided by cattle in a single urination event [18,19]. The control treatments received the equivalent volume of water. The urine types, total urinary N content and application rate expressed as total N in kg ha^{-1} are presented in Table 2.

Table 2. Treatment, urinary N content, urea proportion and application rates.

Treatment [1]	Pasture Type	Urine Type	Urinary N Concentration (g N L^{-1})	Urea Proportion of the Total N (%)	Rate of Urinary N Applied (kg N ha^{-1})
RWC + URWC	RWC	URWC	6.15	92	615
RWC + U50	RWC	U50	4.40	80	440
P30 + U30	P30	U30	5.40	85	540
P50 + U50	P50	U50	4.40	80	440
P50 + URWC	P50	URWC	6.15	92	615
RWC + NoU	RWC	N/A [2]	N/A	N/A	N/A
P30 + NoU	P30	N/A	N/A	N/A	N/A
P50 + NoU	P50	N/A	N/A	N/A	N/A

[1] RWC, ryegrass–white clover; P30, 30% plantain mixed pasture; P50, 50% plantain mixed pasture. NoU, control treatment receiving no urine; URWC, urine collected from cows grazing RWC; U30, urine collected from cows grazing 30% plantain mixed pasture; U50, urine collected from cows grazing 50% plantain mixed pasture. [2] N/A = not applicable.

2.3.3. Nitrous Oxide Measurement

Nitrous oxide emissions were measured using the non-vented closed chamber method, following the N_2O chamber methodology guidelines on gas measurement described by the Global Research Alliance on Agricultural Greenhouse Gases (GRA) [20]. When sampling, chambers were closed with a hermetic PVC lid to collect gas samples in previously evacuated vials of 25 mL capacity. Samples were taken at 0, 40 and 80 min after closing. Background emission samples were taken from each chamber 2 days before the application of treatments to determine the spatial variability of background N_2O flux between the plots and to assist with interpretation of patterns of N_2O flux from individual sampling plots and as a covariate for statistical analysis of post-treatment emissions.

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. During weekly phases of N_2O flux measurements, additional samples were collected as soon as it was practical, following rainfall events greater than 10 mm of rain in the previous 24 h period. 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. [21] and using Equation (1):

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

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 emission factors (EF_3; N_2O–N emitted as a percentage of urine N applied) were calculated using Equation (2):

$$EF_3 = \frac{N_2O\text{–Ntotal (urine)} - N_2O\text{–Ntotal (control)}}{\text{Urine N applied}} \quad (2)$$

where N_2O–N total (urine) and N_2O–N total (control) are 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 is the rate of urine N applied (kg N ha^{-1}).

2.3.4. Soil Sampling and Measurements

Soil samples were collected from the designated soil areas at 1, 4, 9, 16, 23, 38, 52, 84 and 105 days after urine application. At each sampling, two soil cores (25 mm diameter) were taken at two depths, 0–50 mm and 50–100 mm. Two soil cores were then bulked, plant roots were removed and the soil was sieved through a 4 mm sieve for moisture content and mineral N content analysis.

A 5 g sub-sample of field-moist soil was taken and extracted with 30 mL of 2M KCl solution by shaking on an end-over-end shaker for 1 h (1:6 soil:extractant ratio). Next, the extract was filtered through Whatman no. 41 filter paper prior to calorimetric determination for NO_3^- and NH_4^+ content using a Technicon AutoAnalyser [22]. Another 5 g sub-sample of moist soil was oven-dried at 105 °C for 24 h to measure gravimetric soil water content. The gravimetric soil water content (SWC), bulk density and an assumed particle density of 2.65 Mg m^{-3} were used to calculate soil-water-filled pore space (WFPS, %) according to Equations (3) and (4):

$$\text{Total pore space (\%)} = [1 - (\text{bulk density}/\text{particle density})] \quad (3)$$

$$\text{WFPS (\%)} = \text{Volumetric SWC}/\text{total pore space (\%)} \quad (4)$$

2.4. Statistical Analysis

All data were analysed using SAS version 9.4 via mixed models for a completely randomised design. The effect of pasture and urine treatments on cumulative N_2O, EF_3, cumulative DM yield and plant N uptake data were analysed in a mixed model that included the fixed effects of pasture treatments, urine treatments and two-way interaction between pasture and urine treatments and the random effect of replicates. Treatment means were separated using the least significant differences (LSD) test. The significant difference of the means for all analyses was $p < 0.05$.

3. Results

3.1. Botanical Composition of Plantain Mixed Pastures

3.1.1. Botanical Composition

During the summer–autumn period of both years, the proportion of plantain increased to 40%–50%, while the proportion of ryegrass steadily decreased in all pasture treatments (Figure 1). In both years, the proportion of dead materials in all pasture types was highest in late summer and early autumn, ranging from 41%–52% in the first year and from 22%–38% in the second year. The dead materials content in RWC in both years were significantly higher than in pasture treatments including plantain.

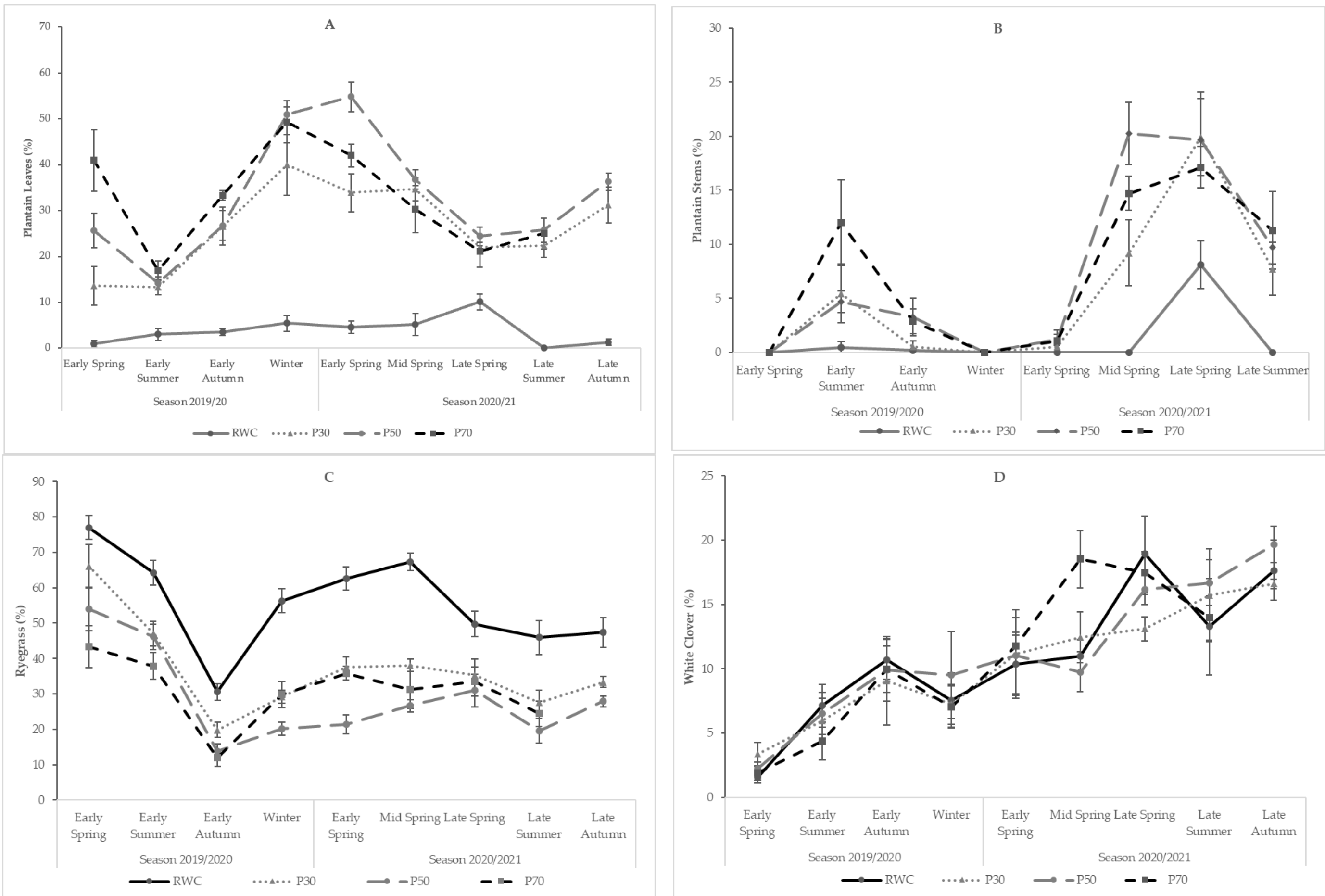

Figure 1. *Cont.*

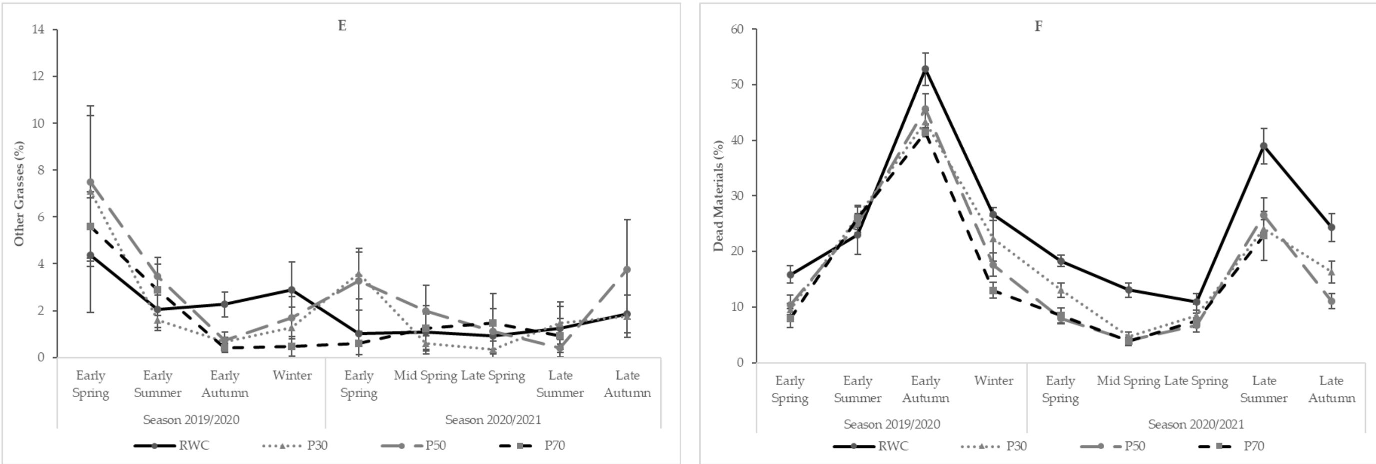

Figure 1. Composition (percentage of DM) of (**A**) plantain leaves, (**B**) plantain stems, (**C**) ryegrass, (**D**) white clover, (**E**) other grasses and (**F**) dead materials in four pasture treatments at each grazing time in the 2019/2020 and 2020/2021 grazing years.

In the first (2019/2020) year, the total initial proportion of plantain (leaf and stem) was 14%, 26% and 41% in the P30, P50 and P70 pasture treatments, respectively. The plantain leaf content in the sward declined to less than 20% in early summer, before increasing in early autumn and winter to 40%, 50% and 51% in the P30, P50 and P70 pasture treatments, respectively.

In the second (2020/2021) year, in early spring, the P30 and P50 pasture swards had 34% and 55% plantain, respectively, which then decreased to around 23% in late spring, along with an increased plantain stem content. After this, the plantain content recovered and reached 31% in P30 and 36% in P50 in late autumn. In the P70 sward, the proportion of plantain decreased over the year, ranging from 25%–30% of plantain in the sward. There was no significant difference in the plantain content between P50 and P70 pasture treatments in the second year.

3.1.2. Plant Density

In year 1, when first measured (early spring 2019), the plantain density in the P70 pasture treatment was significantly greater (140 plants m^{-2}) when compared to the P50 (86 plants m^{-2}) and P30 (64 plants m^{-2}) pasture treatments (Figure 2). From early spring to early summer, the plantain density increased in the three plantain–RWC (P30, P50, P70) treatments. The plantain density remained stable in the P30 and P50 pasture treatments until winter but declined by 20% in the P70 pasture treatment. At the end of the 2019/2020 growing season (winter 2020), there were approximately 40% more plantain plants in the P30 and P50 pasture treatments when compared to the initial plant densities (spring 2019).

In year 2, the plantain density was higher in the P70 and P50 pasture treatments compared to the P30 pasture treatment. The plantain density tended to decline (p = 0.07) in all plantain–RWC (P30, P50 and P70) treatments, with a plant loss ranging from 52% to 62% of the plant density when compared to the plantain density at the beginning of year 2 (early spring 2020). In late autumn of year 2, plantain densities in the three plantain–RWC pasture treatments were similar (p > 0.05).

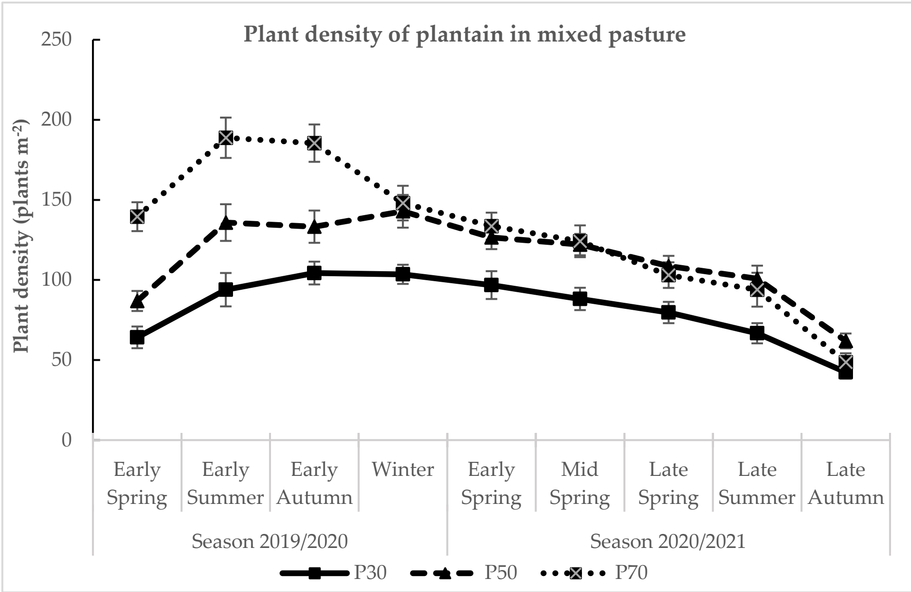

Figure 2. Plant density (plants m^{-2}) of plantain in 30% plantain (P30), 50% plantain (P50) and 70% plantain (P70) mixed pastures throughout the first year (2019/2020) and the second year (2020/2021). Error bars represent plant density standard errors for each treatment (n = 5).

3.2. Nitrous Oxide Emissions

3.2.1. Soil Moisture Conditions

The soil WFPS at the two depths fluctuated between 22% and 75% over the trial period (Figure 3). The lowest WFPS (%) values were observed between 16 and 38 days after urine application (DAA), and these were attributed to low rainfall during this period. After this period, the WFPS (%) values increased to 70% by the end of the trial. The WFPS (%) values at both depths in the P30 and P50 pastures, regardless of applied urine types, were significantly higher than those for soil under standard RWC during the first 4 weeks of the experiment ($p < 0.05$).

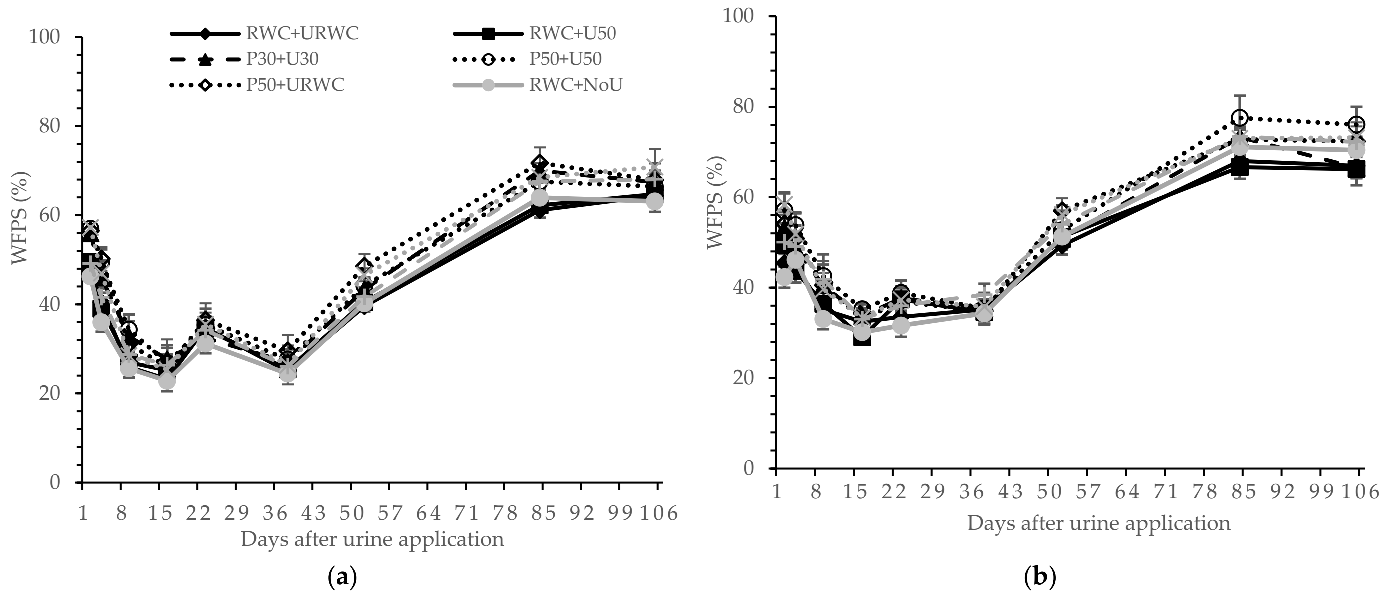

Figure 3. Soil-water-filled pore space (WFPS, %) at (**a**) 0–50 mm and (**b**) 50–10 mm depths on 0% plantain (RWC), 30% plantain (P30) and 50% plantain (P50) pastures, where different urine types from cows grazing RWC (URWC), 30% plantain (U30) and 50% plantain (U50) were applied; water (NoU) was applied as the control. Error bars represent the standard error of the mean for each treatment (n = 5).

3.2.2. Nitrous Oxide Emissions

Nitrous oxide flux from all pasture treatments increased sharply after urine application (Figure 4). At 4 h, P30 + U30, P50 + URWC and P50 + U50 produced the highest peaks 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. Nitrous oxide 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 larger peaks were observed at day 44, with the greatest N_2O fluxes from RWC + URWC (0.51 mg N m^{-2} h^{-1}), followed by P50 + U50 (0.34 mg N m^{-2} h^{-1}), RWC + U50 (0.26 mg N m^{-2} h^{-1}), P30 + U30 (0.2 mg N m^{-2} h^{-1}) and P50 + U50 (0.14 mg N m^{-2} h^{-1}). Nitrous oxide emissions from plantain-containing pastures reached the background level at 88 DAA, while RWC obtained the background emission level at day 102. The fluxes from the control treatments had a similar trend as the urine treatments throughout the duration of the trial.

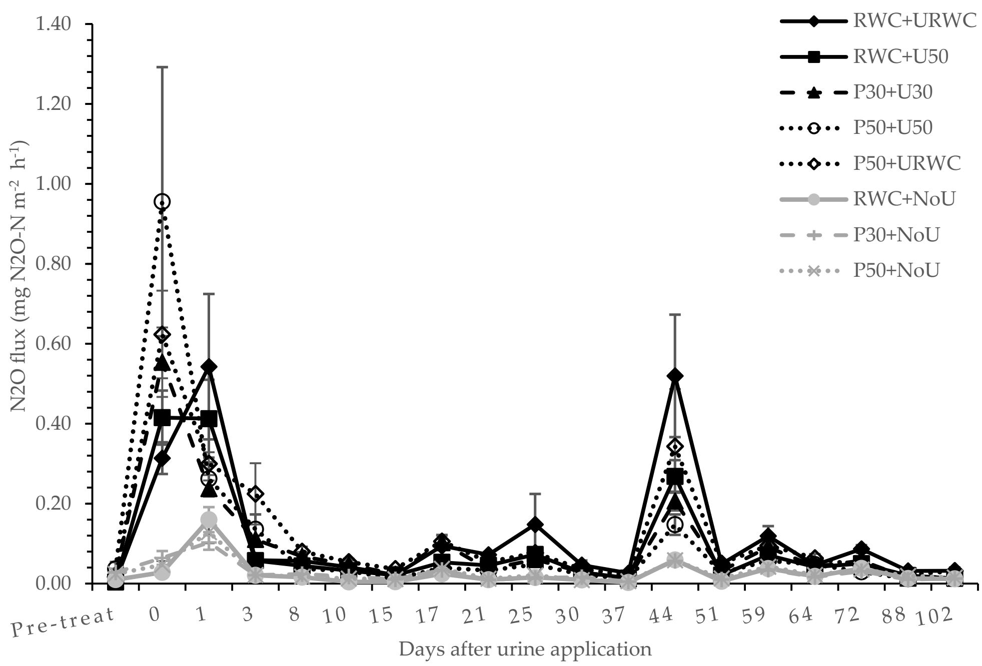

Figure 4. Hourly N_2O fluxes from urine (U) patches on 0% plantain (RWC), 30% plantain (P30) and 50% plantain (P50) pastures, where different urine types from cows grazing RWC (URWC), 30% plantain (U30) and 50% plantain (U50) were applied; water (NoU) was applied as the control. Error bars show the standard error of the mean (n = 5).

Urine types significantly affected ($p < 0.05$) the total cumulative N_2O emissions and the EF_3 (Table 3). The higher urine N-loading rate in the URWC (615 kg N ha^{-1}) significantly increased ($p < 0.01$) the cumulative N_2O–N fluxes from RWC and P50 pasture treatments. The total N_2O emissions from the U50 urine treatment were around 39% less than the emissions from the URWC treatment regardless of pasture type. In contrast, there was no significant difference ($p > 0.05$) in the cumulative N_2O emissions between the N application rate of 540 kg N ha^{-1} and 440 kg 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). No statistical difference ($p > 0.05$) was observed between RWC and P50 pasture treatments treated at the same urine-loading rate. The total N_2O emissions from the control treatments did not significantly differ between pasture types ranging from 0.53 to 0.60 kg N ha^{-1}.

The highest EF_3 values were observed in RWC + URWC (0.31%), followed by P50 + URWC (0.23%) and RWC + U50 (0.22%); see Table 3. 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}. EF_3 values in P30 + U30 and P50 + U50 treatments were 42–51% lower than those in the RWC + URWC treatment, and this difference was significant ($p < 0.05$).

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

Treatments	Urine Rate (kg N ha^{-1})	Change in Urine Rate (%)	Cumulative N_2O Emission (kg N ha^{-1})	Change in N_2O (%)	Emission Factor (EF_3, %)	Change in EF_3 (%)
RWC + URWC	615	-	2.43 (0.26)$_a$	-	0.31 (0.04)$_a$	-
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	-	2.05 (0.25)$_a$	−16%	0.23 (0.03)$_{ab}$	−24%
RWC + NoU	0		0.53 (0.02)$_c$			
P30 + NoU	0		0.53 (0.05)$_c$			
P50 + NoU	0		0.60 (0.04)$_c$			
p-Value						
Pasture type			0.0070		0.0220	
Urine type			<.0001		0.0054	
Pasture × urine			0.3014		0.8731	

RWC, ryegrass–white clover pasture; P30, 30% plantain mixed pasture; P50, 50% plantain mixed pasture; NoU, control treatment receiving no urine; URWC, urine collected from cows grazing RWC; U30, urine collected from cows grazing 30% plantain mixed pasture; U50, urine collected from cows grazing 50% plantain mixed pasture. $_{a,b,c}$ 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 ($n = 5$).

Soil ammonium N and nitrate N concentration (mg N kg^{-1} dry soil) at 0–50 mm depth and 50–100 mm depth during the experimental period (105 days) are presented as Supplementary Materials.

4. Discussion

4.1. Plantain Content in RWC Mixed Pastures

This study highlighted that after declining in the late spring–early summer period, the proportion of plantain leaf in all plantain–RWC mixed pasture treatments recovered in late summer and early autumn, the dry period across the 2 years. This recovery supported earlier work reporting an increase in the plantain content in mixed pastures under water stress conditions [23]. Over 2 grazing years, the highest proportion of plantain among all pasture treatments was on average 50% in the P50 and P70 pasture treatments. These results suggested that plantain was unlikely to contribute more than 50% of the DM in a mixed plantain–RWC pasture despite the high sowing rate of plantain (10 kg/ha) in the P70 pasture treatment. Stewart [24] indicated that plantain naturally represents up to 20% of a productive pasture and typically acts as minor forage in mixed swards. Bryant et al. [25] also observed that plantain contributes less than 30% of DM in established pastures. Therefore, plantain is unlikely to generate the majority of DM yield in mixed pastures.

A decrease in the proportion of plantain in the plantain–RWC pasture treatments was probably associated with plant loss observed at the beginning of the second year. In this study, the plantain density declined during the second growing season (year 2). This was in line with previous findings that plantain persistence reduces under grazing conditions [26,27]. Management techniques that encourage establishment of plantain plants within existing pastures and maintain plantain at the optimum proportion in mixed pastures require further exploration.

Throughout the second year, the proportions and plant density of plantain in the P50 pasture treatment were more stable than in the P70 pasture treatment. The sowing rates of plantain used for the 30% and 50% plantain mixed pastures were effective for 2 years, but the decline observed in the plantain density suggested that broadcasting or direct drilling of additional plantain after 2 years might be required to maintain a proportion of plantain greater than 30% in mixed pastures [25].

4.2. Nitrous Oxide Emissions from Plantain Mixed Pastures

The lower N-loading rate onto soil from plantain-derived urine was the major effect resulting in the reduction in total N_2O emissions observed in this study. The urinary N concentration and urea N content, and the subsequent N-loading rate onto soil, decreased by 12% and 28% (Table 2) when mixed pastures with 30% and 50% of plantain were part of the diet of cows, respectively. This decrease supported earlier studies that demonstrated a lower N content in the urine of cows grazing pastures containing plantain compared to RWC [10–12,28,29].

The effects of plantain on reduced urinary N excretion are most likely associated with increased N partitioning into dung and other sinks rather than urine [7,8,30]. Higher N excreted into dung rather than urine can assist in the reduction of total on-farm N losses, as the emission factor from dung (0.12%) is substantially lower than from dairy cattle urine (0.98%) [31]. Additionally, the reduction in urinary N can also be a consequence of N dilution from a higher urine volume when cows are fed a diet that includes plantain [29,32]. Plantain contains 30% more water content than ryegrass [30,33]; consequently, higher water consumption from the herbage can cause diuretic effects in cows and dilute the N content in urine via increased urination volume and frequency [32,34]. More frequent urination and a higher urination volume may result in a greater surface area being wetted by urine, and to the extent that denitrification is a surface soil phenomenon, this may undermine some of the advantages of plantain in reducing N_2O emissions. However, the total N_2O emissions on the paddock and farm scale are directly linked to total urinary N content excreted [15], which is beyond the scope of this study without information about the excreted urine volume. Therefore, further research is needed to determine total paddock-scale urinary N excreted from plantain-based pastures.

The WFPS values at 0–100 mm soil depth in plantain mixed pastures were significantly higher than those in RWC pastures during the first 16 DAA, when there was no rain. Similarly, Rodríguez-Gelós [10] observed higher WFPS values in plantain mixed pastures than in ryegrass swards in autumn/winter on Tokomaru silt loam soil, the same soil type used in this study. In contrast, in free-draining soil in winter/spring, Luo et al. [9] reported lower WFPS values in plantain mixed pastures, while Podolyan et al. [28] did not observe any significant difference in WFPS in plantain compared to RWC in free-draining soil.

The higher WFPS values in plantain plots in this study might reflect the architecture and growth behaviour of plantain roots. Plantain has a truncated tap root with thick, fibrous side roots, a high root density in the 0–10 0mm depth and an average rooting depth of 0.97 m [27]. In contrast, an estimated 75% of ryegrass roots are present at the 0–70 mm depth, making them unlikely to survive under water stress conditions [35]. Therefore, plantain roots may extract water more uniformly down the soil profile, while ryegrass takes most of its water requirement from the surface soil, particular in early summer. Further systematic research is needed to determine the water extraction patterns and N dynamics under plantain and RWC-based pastures to determine and compare the on-farm water use efficiency in summer under dry soil conditions where plantain appears to have the advantage of its deep root system.

Our study did not account for the impact of the volume and frequency of urinary N excretion, but the observed reduction in the cumulative N_2O emissions appeared to be mainly associated with the decrease in the total N content in plantain-derived urine and subsequently a lower N-loading rate of 440 and 540 kg N ha^{-1} onto the soil. Plantain-based pastures were demonstrated to significantly reduce the urinary N concentration of dairy cows [36]. These lower N-loading rates have been shown to reduce the mineral N content in the soil and, consequently, result in lower N losses to the environment via leaching and gaseous losses of ammonia (NH_3) and N_2O [13,28,29,37]. Carlson et al. [14] did not observe a reduction in the urinary N content affecting N_2O emissions in poorly drained soil in winter, and de Klein et al. [38] indicated that N_2O emissions are not reduced by a lower N-loading rate in poorly draining soils with high N_2O emission rates.

The presence of secondary compounds in the urine of grazing animals is another mechanism that has been proposed to explain decreases in N_2O emissions from plantain urine types [16]. However, when urine from the plantain diet was adjusted to the same N concentration as urine from ryegrass diet, N_2O emissions from the two urine types did not differ at the same N-loading rate [10,13]. Therefore, it appears that the lower N concentrations in urine and the resultant lower N-loading rates are the main factors reducing N_2O emissions from the urine of cows fed plantain.

The summer/autumn EF_3 values in this study ranged from 0.15 to 0.31%, which are significantly lower than the annual EF_3 value of 0.98% for cattle urine in New Zealand [39]. Similarly, lower EF_3 values than the national specific value have been reported under warm and dry conditions and in well-drained soil [14,28,40]. Our results confirm that EF_3 under summer/autumn dry conditions is much lower than the default NZ-specific value.

Plantain secondary compounds may act as biological nitrification inhibitors (BNI) that can potentially reduce the nitrification rates, mineral N content and resultant N_2O emissions from pastoral soils [9,10,41]. In this study, however, we observed that the total N_2O emissions and EF_3 values from P50 + URWC and RWC + URWC were similar, despite the EF_3 in the P50 + UWRC treatment being 24% lower. A reduction in the emission factor can also be influenced by other factors, such as low soil moisture, limited soil carbon content or competition by plants and soil microbes for N rather than the N loading onto soil [15,42,43]. The effect of the proportion of plantain in mixed pastures on N_2O emission was not obvious. Similarly, Pijlman et al. [44] did not observe an effect of increasing the plantain proportion in pastures on a reduction in N_2O emissions in a field experiment. This result could be related to the fluctuation in the plantain composition and insufficient plantain content in mixed pastures over time. According to the results from the botanical composition experiment, the plantain content in the P30 and P50 pasture treatments was 31% and 36% in late autumn, with this difference being insignificant. 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. These findings suggest that at least 30% of plantain in mixed pastures and in the cow's diet reduces N_2O emissions and EF_3 from individual urine patches.

5. Conclusions

This study provided evidence that the highest contribution of plantain to pasture yield is 50% of DM in swards mixed with RWC, even when plantain is sown at a rate expected to attain 70% of the pasture composition. The P30 and P50 plantain mixed pasture treatments maintained more stable plantain compositions and densities during 2 years of field study than the P70 pasture treatment, reflecting the water extraction patterns under plantain–RWC-based pastures.

In this study, the inclusion of 30% and 50% plantain in RWC pastures, in comparison to RWC pastures, reduced the urine N content and consequently N_2O emissions from cow urine patches in summer/late autumn. The nitrous oxide emission factor (EF_3) for urine from cows grazing 30% and 50% plantain in their diets was significantly lower (42 to 51%) compared to an RWC diet.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy13061447/s1, Figure S1: Soil ammonium N concentration (mg N kg^{-1} dry soil) at 0–50 mm depth (a) and 50–100 mm depth (b) during the experimental period (105 days); Figure S2: Soil nitrate N concentration (mg N kg^{-1} dry soil) at 0–50 mm depth (a) and 50–100 mm depth (b) during the experimental period (105 days).

Author Contributions: Conceptualisation, C.V., S.N., S.S., P.D.K. and D.J.H.; methodology, P.D.K., S.S., S.N., C.V. and D.J.H.; formal analysis, C.V.; writing—original draft preparation, C.V.; writing—review and editing, C.V., S.S., P.D.K., S.N. and D.J.H.; supervision, P.D.K. S.S., S.N. and D.J.H.; project administration, S.N.; funding acquisition, S.S., P.D.K., S.N. and D.J.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Dairy NZ-led Sustainable Food and Fibre Futures Plantain Potency & Practice programme (SFFF 19047) funded by DairyNZ, the NZ Ministry for Primary Industries, PGG Wrightson Seeds and Fonterra. Massey University, Manaaki Whenua—Landcare Research and the New Zealand Agricultural Greenhouse Gas Research Centre.

Data Availability Statement: Data are available from the corresponding author on request.

Acknowledgments: The authors thank Thi Truong Nguyen, other staff and students at Massey University, Dairy Farm No. 4 and Manaaki Whenua Landcare Research for their excellent technical support in pasture management, sample collecting and gas analysis over the experimental period.

Conflicts of Interest: The authors declare no conflict of interest.

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