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Incorporating Plantain with Perennial Ryegrass-White Clover in a Dairy Grazing System: Dry Matter Yield, Botanical Composition, and Nutritive Value Response to Sowing Rate, Plantain Content and Season

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Abstract: Incorporating plantain with perennial ryegrass and white clover (RGWC) can improve the quality and quantity of grazing pastures; however, the sowing rate could affect the persistence of plantain, pasture yield, and nutritive value in different seasons. The objective of this study was to evaluate the effect of increasing sowing rates of plantain when established with RGWC on the dry matter (DM) yield, botanical composition, nutritive value, and bioactive compounds of the pasture over the first two years after sowing; and to determine the relationship between plantain content and nutritive characteristics in different seasons. The pasture treatments were RGWC, RGWC + low plantain rate (PLL), RGWC + medium plantain rate (PLM), and RGWC + high plantain rate (PLH). The results showed that annual DM yield was similar between treatments. The average plantain content (including leaves and reproductive stem) was 32, 44, and 48% in PLL, PLM, and PLH, respectively. The plantain composition increased in the first 15 months, then declined rapidly to about 20–30% at day 705 after sowing. Compared with RGWC, the plantain-RGWC pastures (PLL, PLM, PLH) had a higher content of organic matter digestibility (OMD), ash, starch, non-structural carbohydrates (NSC), P, S, Ca, Mg, Na, Cl, Zn, B, Co, aucubin, acteoside, and catalpol, while they contained a lower composition of DM%, acid detergent fibre (ADF), neutral detergent fibre (NDF), crude fat (CF), Fe, and Mn. These differences were linearly associated with the content of plantain leaves in the pasture and were higher in summer and autumn than in spring. In conclusion, incorporating plantain into the RGWC pasture can improve herbage nutritive quality, thus potentially increasing farm productivity and environmental benefits. However, further work is required to investigate management interventions to sustain plantain content beyond two years from sowing.

Keywords: forage yield; mixed swards; pasture quality; percentage of plantain; *Plantago lanceolata* L.

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1. Introduction

Plantain (*Plantago lanceolata* L.) is a forage herb that is highly tolerant of summer heat and drought [1,2]. Since the 2000s, plantain has been increasingly grown in mixed swards in temperate dairy grazing farms [3]. Incorporating plantain into grazing pastures can improve the quality and quantity of feed, especially in late summer and autumn [4,5], thereby potentially increasing farm productivity and profitability [6,7]. Moreover, the inclusion of plantain has been considered an effective forage strategy to reduce nitrate leaching from pastoral dairy systems to meet the strict regulations of local governments [6,8]. While perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) (RGWC) are the mainstays of temperate dairy pastures [9], incorporation of plantain into the RGWC pasture appeals to pastoral dairy farmers because of its advantages in reducing nitrogen (N) losses from the system. Various studies have measured the yield and nutritive value of pure plantain [10,11] or diverse swards containing plantain [4,5]. However, there has been limited understanding of the dry matter (DM) yield and herbage nutritive value of pastures incorporating plantain with RGWC.

Plantain has higher non-structural carbohydrates (NSC) and lower structural carbohydrates than RGWC [12]. Feeding pastures containing plantain can improve the DM intake of dairy cows and provide more readily available energy for microbes to produce microbial nitrogen (N) for milk production [13]. Furthermore, high concentrations of minerals [14], bioactive compounds aucubin and acteoside [15], and water contents [12] in plantain have been implicated in a reduction of ammonium production in cow rumen and N loading in cow urine. Therefore, the inclusion of plantain can lower urinary N excretion by dairy cows [16], contributing to a lower risk of nitrate leaching [17] and nitrous oxide emission [18]. Research has suggested that ryegrass-based pastures may need to contain at least 30% of plantain to have measurable effects on the dairy cow's performance and N use efficacy [19], and an increased plantain content in the diet results in a greater reduction in N losses [17,20]. However, plantain composition in diverse pastures has been reported to decline rapidly beyond two years from sowing [21]. Therefore, it is necessary to determine suitable sowing rates to achieve a high percentage of plantain for an extended period in the ryegrass-based pasture, while maintaining or increasing the herbage DM yield.

Various biological and environmental factors can influence the DM yield and nutritive value of the plantain-RGWC pasture. Our hypothesis is that an increased plantain content is associated with greater effects on the DM yield, herbage nutritive value, and the content of minerals and bioactive compounds. Moreover, the relationships between plantain content and nutritive value traits vary in different seasons. Specifically, as a drought and heat-tolerant herb, plantain may produce a higher quantity and quality of forage than RGWC during summer and autumn [5,12,21]. On the other hand, the contents of minerals and bioactive compounds in the plantain-RGWC pasture are higher than in the RGWC pasture, especially in drier seasons [15,22,23].

The objective of the current study was to compare the herbage DM yield, botanical composition, nutritive value, and the contents of minerals and bioactive compounds of the plantain-RGWC pasture sown with increased plantain seed rates and grazed with dairy cows. Furthermore, the study aimed to illustrate the change in plantain content of the pasture throughout two years from sowing, and to estimate the relationship between plantain content and herbage nutritive value traits of the RGWC-plantain pasture in different seasons.

2. Materials and Methods

2.1. Study Site, Pasture Treatment, and Establishment

The study was conducted at Massey University's No 4 Dairy Farm (40°23′26.9′′ S, 175°36′43.5′′ E) in Palmerston North, New Zealand, between April 2019 and March 2021. The soil at the experimental site is Tokomaru silt loam soil. The analysis of soil to 7.5 mm reported a content of minerals (parts per million) of 30 Olsen-P, 3.7 Sulfate-S, 5.9 K, 9.0 Ca, 31 Mg, 4.8 Na, and a pH of 5.8. The experimental site has a temperate climate, with an average annual precipitation of 962 ± 129 mm, and a mean yearly temperature of 14.1 ± 0.2 °C over the study period.

Pasture treatments were four mixed swards sown with an increased plantain seed rate and a concomitant decrease in the ryegrass seed rate, resulting in perennial ryegrass (*Lolium perenne*, cv. ONE^{50}) and white clover (*Trifolium repens*, cv. Tribute) RGWC, RGWC + 4 kg plantain (*Plantago lanceolata*, cv. Agritonic) (PLL), RGWC + 7 kg plantain (PLM), and RGWC + 10 kg plantain (PLH). Agritonic is a recent cultivar of plantain that was developed in a breeding program for use within grazing pasture mixes and with the aim to reduce nitrate leaching from pastoral systems [24]. The experimental pasture was established on 5 April 2019. The seed was sown by direct drilling with the rates presented in Table 1. The existing swards were eliminated before sowing 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. The experiment was a completely randomised design with five replicate plots. Twenty experimental plots of 800 m^2 (40 m × 20 m) and four adaptation areas (one ha area per pasture treatment) were split from an approximately seven ha paddock. No fertiliser was applied at the sowing time. In March 2020, plantain seeds were direct drilled at 3, 6, and 9 kg/ha in the plots of PLL, PLM and PLH, respectively. The pasture was mowed after the grazing in October 2019, January 2020, October 2020, and December 2020 to control the pasture seed head and to equilibrate the post-grazing residual height between plots. The mowing was conducted using a Tiger forage harvester and defoliating to seven cm height. Urea fertiliser (46% N) was applied at 50 kg N/ha in October 2019, December 2019, and February 2020, and 30 kg N/ha in September 2020, and November 2020.

Table 1. Cultivars and sowing rates of perennial ryegrass-white clover (RGWC), RGWC + low plantain rate (PLL), RGWC + medium plantain rate (PLM), and RGWC + high plantain rate (PLH).

Species/Cultivar	Sowing Rate (kg seed/ha)			
	RGWC	**PLL**	**PLM**	**PLH**
Plantain (Agritonic)	0	4	7	10
White clover (Tribute)	3	3	3	3
Perennial ryegrass (ONE^{50})	20	15	10	5

2.2. Grazing Management

The experimental pasture was rotationally grazed by dairy cows over two lactation years, from September 2019 to March 2021. The grazing rotation was at 3–6-week intervals, equating to nine grazings in the lactation year 2019/2020 and seven grazings in the lactation year 2020/2021 (Table 2). In addition, a short grazing event was conducted in four hours with 100 dry cows in week 12 after sowing to graze the top of the ryegrass pasture to promote plantain establishment. Pasture cover was managed at under 4000 kg DM/ha for pre-grazing mass and above 1300 kg DM/ha for post-grazing mass. In each grazing, either three or four cows were allocated for grazing from 1.5–3 days in each plot after having six days of grazing in the adaptation areas with the same pasture type.

Table 2. Season, grazing period, number of cows and days grazing in each plot, and pasture measurements for grazing events from September 2019 to March 2021.

	2019/2020			2020/2021		
Season	**Grazing Period**	**Cow × Day/Plot**	**Measurement**	**Grazing Period**	**Cow × Day/Plot**	**Measurement**
Spring	18–20 September	4 × 2	DM, BC, NV	2–5 September	3 × 3	DM, BC, NV
	22–24 Octorber	4 × 2	DM, BC	12–15 Octorber	4 × 3	DM, BC, NV
	19–21 November	4 × 2	DM, BC	16–19 November	4 × 3	DM, BC, NV
Summer	10–12 December	4 × 2	DM, BC, NV, BA	8–10 December	3 × 2	DM, BC, NV, BA
	14–17 January	4 × 3	DM, BC	11–14 January	4 × 3	DM, BC
	11–12 February	3 × 1.5	DM, BC, NV, BA	8–10 February	3 × 2	DM, BC, NV, BA
Autumn	17–19 March	3 × 2	DM, BC, NV, BA	29–31 March	3 × 2	DM, BC, NV, BA
	12–15 May	3 × 3	DM			
Winter	6–10 July	3 × 4	DM, BC			

DM = dry matter growth; BC = botanical composition; NV = nutritive value and the content of minerals; BA = bioactive compounds.

2.3. Herbage Measurement

The period of measurements for herbage DM yield, botanical composition, nutritive value along with the content of minerals, and bioactive compounds of the pastures are presented in Table 2. Herbage yield and botanical composition were measured for every grazing event over the experimental period. Nutritive value, along with the content of minerals, was measured for four grazings in the first lactation year (September 2019, December 2019, February 2020, and March 2020) and seven grazings in the second lactation year (September 2020, October 2020, November 2020, December 2020, February 2020, March 2020, May 2020). In addition, the concentration of bioactive compounds was measured for all grazing events with the measurement of nutritive value, except grazing in September 2019, September 2020, October 2020, and November 2020.

Pre- and post-grazing herbage DM mass was measured for individual plots in every grazing. In each grazing, three herbage samples (0.1 m^2 quadrat) were randomly cut to ground level from each plot a day before and after the grazing, using an electric shearing handpiece. Herbage samples were cleaned by removing soil and faecal debris and dried in an oven at 75 °C until a constant weight was achieved. Herbage DM production between two consecutive grazing events was estimated by deducting the average post-grazing DM mass of the previous grazings from the average pre-grazing DM mass of the current grazings.

Botanical composition and herbage nutritive value were measured from hand-plucked samples collected a day before the grazing in individual experimental plots. A hand-plucked sample (approximately 400 g) was collected from each plot in every grazing with 15–20 random grabs to the grazing height. Each sample was mixed thoroughly and divided into two sub-samples for botanical composition separation and for herbage nutritive value analysis. The botanical composition samples (approx. 100 g fresh weight) were manually separated into perennial ryegrass, white clover, plantain leaves, plantain reproductive stem, weeds, and dead materials. These components were dried in an oven at 75 °C until a constant weight was achieved to calculate the botanical composition. The herbage nutritive value sub-samples were weighed to record the fresh weight and then oven-dried at 60 °C until a constant weight was achieved to determine DM content. Then, the dried samples were ground through a 1 mm sieve for chemical analyses.

Analytical testing for nutritive value was conducted by a commercial laboratory using near-infrared (NIR) spectroscopy [25]. The samples were analysed for organic matter digestibility (OMD) (in-vivo using the Australian Fodder Industry Association method), crude protein (CP) (N multiplied by 6.25), acid detergent fibre (ADF), neutral detergent fibre (NDF), lignin, ash, metabolisable energy (ME) (calculated from OMD), soluble sugars, starch, crude fat (CF), and NSC (100 − CP − ash − CF − NDF). In addition, the samples were analysed for N, P, K, S, Ca, Mg, Na, Cl, Fe, Mn, Zn, Cu, B, Mo, Co, Se, ryegrass staggers index, and dietary cation-anion difference (DCAD). The analysis for aucubin, acteoside, and catalpol was conducted by high-performance liquid chromatography (HPLC) at Massey University. The detail of this method was described in [15].

2.4. Statistical Analysis

Annual DM yield was obtained by accumulating the DM growth between grazing events. Data on the DM yield, botanical compositions, nutritive value, and content of minerals and bioactive compounds over the experimental period were analysed using PROC mixed procedure in SAS [26]. Pasture treatment, season, and the interaction between treatment and season were fixed effects. Replicate plot, grazing, and year were random effects. Means among treatments were compared using the Fisher's Least Significant Difference test. A significant difference between means was declared at $p < 0.05$.

The change in plantain content (percentage of pastures composed of plantain) of the pasture treatments from the first grazing to 705 days after sowing (between September 2019 and March 2021) was analysed according to a modified Gaussian functional model: $y = p \times \exp(-0.5 \times ((\text{day-d})/s)^2$ [27]. Where p is the peak of plantain content, d is the day of the peak; and s is the coefficient controlling the peak's width (slope). The significant difference in p, d, and s between treatments was declared when 83.4% confidence intervals (CI) did not overlap [28].

The relationships between the content of plantain leaves and parameters related to nutritive values, the content of minerals and bioactive compounds were estimated for different seasons using a PROC GLM procedure in SAS [26]. The model was $y = \alpha + \beta \times P_j + S_i + e_{ij}$. This model included an intercept (α), a slope of plantain effect (β), the content of plantain leaves of a plot in a grazing event (P_j), and the season (spring, early summer, late summer, and autumn). The model was presented for all parameters with a significant effect of the plantain leaves (%) in the pasture ($p < 0.05$). The effects of plantain leaves (%) and season (p-value), the coefficient of determination (R^2), and the root mean square of error (r.m.s.e) were presented for each generated model. A significant effect of plantain content and season was declared at $p < 0.05$.

3. Results

3.1. Herbage Yield

There was no significant difference in the annual DM yield between the pasture treatments over the experimental period (Table 3). Under a dairy grazing system, the annual yield across treatments was approximately 14,500 kg DM/ha. Mean pre- and post-grazing DM mass was managed at about 3400 and 1700 kg DM/ha, respectively. Pre-grazing DM mass was similar between treatments; however, post-grazing DM mass of PLL, PLM and PLH was lower than that of RGWC ($p < 0.05$).

Table 3. Mean, standard error of the mean (SEM), and p-values for parameters of pasture production, botanical composition, nutritive value, the content of minerals and bioactive compounds in RGWC, PLL, PLM, PLH.

Parameter	RGWC	PLL	PLM	PLH	SEM	P_{treat}	P_{sea}	$P_{treat \times sea}$
Pasture production								
Pre-grazing mass (kg DM/ha)	3466	3418	3255	3356	424	0.263	0.189	0.420
Post graze mass (kg DM/ha)	1875 ^{a}	1761 ^{b}	1571 ^{c}	1661 ^{bc}	451	0.006	<0.001	0.056
Annual DM yield (kg DM/ha)	14,303	14,361	14,407	14,999	1981	0.495	NA	NA
Botanical composition								
Plantain leaves (%)	3.3 ^{a}	23.8 ^{b}	34.5 ^{c}	37.6 ^{c}	3.0	<0.001	0.599	0.090
Plantain stem (%)	1.2 ^{a}	7.9 ^{b}	9.5 ^{b}	10.2 ^{b}	2.1	<0.001	0.345	<0.001
Perennial ryegrass (%)	62.7 ^{a}	37.7 ^{b}	27.6 ^{c}	25.2 ^{c}	7.4	<0.001	0.024	0.145
White clover (%)	16.1	14.0	15.3	14.5	5.8	0.842	0.002	0.516
Weeds (%)	1.1	1.3	0.8	1.1	0.6	0.759	0.144	0.814
Dead material (%)	16.3 ^{a}	13.7 ^{b}	10.8 ^{c}	10.0 ^{c}	1.4	<0.001	<0.001	0.014
Nutritive value								
DM (%)	27.0 ^{a}	22.8 ^{b}	20.8 ^{c}	20.5 ^{c}	2.1	<0.001	<0.001	<0.001
OMD (g/kg DM)	701 ^{a}	713 ^{a}	734 ^{b}	726 ^{ab}	14	<0.001	0.027	0.007
CP (g/kg DM)	186	188	194	192	23	0.333	0.924	0.750
ADF (g/kg DM)	269 ^{a}	265 ^{a}	254 ^{b}	255 ^{b}	6.5	0.003	0.027	0.427
NDF (g/kg DM)	465 ^{a}	440 ^{b}	403 ^{c}	405 ^{c}	10	<0.001	0.002	0.987
Lignin (g/kg DM)	77	81	86	90	5.9	0.103	0.018	0.342
Ash (g/kg DM)	109 ^{a}	111 ^{ab}	118 ^{b}	115 ^{b}	9.5	<0.001	0.678	0.297
Soluble sugars (g/kg DM)	72	68	70	67	11	0.671	0.018	0.003
Starch (g/kg DM)	7.7 ^{a}	10 ^{ab}	11 ^{b}	13 ^{b}	1.7	0.009	0.673	0.709
CF (g/kg DM)	28 ^{a}	27 ^{ab}	26 ^{bc}	25 ^{c}	0.5	<0.001	<0.001	0.317
NSC (g/kg DM)	211 ^{a}	232 ^{b}	258 ^{c}	253 ^{c}	19	<0.001	0.055	0.988
ME (MJ/kg DM)	10.0	10.2	10.4	11.1	0.9	0.683	0.171	0.894

Table 3. *Cont.*

Parameter	RGWC	PLL	PLM	PLH	SEM	P_{treat}	P_{sea}	$P_{treat \times sea}$
Mineral content								
P (g/kg DM)	3.5 ^{a}	3.8 ^{b}	3.9 ^{c}	3.9 ^{c}	0.2	<0.001	<0.001	0.038
K (g/kg DM)	23.5	23.7	24.2	24.6	1.8	0.699	<0.001	0.470
S (g/kg DM)	2.8 ^{a}	3.1 ^{b}	3.3 ^{c}	3.3 ^{c}	0.1	<0.001	0.492	0.094
Ca (g/kg DM)	8.4 ^{a}	11.9 ^{b}	14.6 ^{c}	14.6 ^{c}	0.7	<0.001	0.277	0.546
Mg (g/kg DM)	2.6 ^{a}	2.7 ^{ab}	2.8 ^{b}	2.8 ^{b}	0.1	<0.001	0.001	0.287
Na (g/kg DM)	3.6 ^{a}	5.0 ^{b}	5.9 ^{b}	5.7 ^{b}	0.5	<0.001	0.154	0.072
Cl (g/kg DM)	12.8 ^{a}	15.7 ^{b}	17.2 ^{b}	17.0 ^{b}	1.9	<0.001	0.711	0.052
Fe (mg/kg DM)	242 ^{a}	184 ^{b}	162 ^{b}	162 ^{b}	39	<0.001	0.032	0.181
Mn (mg/kg DM)	125 ^{a}	115 ^{ab}	109 ^{ab}	95 ^{b}	14	0.025	0.125	0.297
Zn (mg/kg DM)	36 ^{a}	42 ^{b}	46 ^{c}	46 ^{c}	2.5	<0.001	0.071	0.553
Cu (mg/kg DM)	11 ^{a}	13 ^{b}	14 ^{b}	14 ^{b}	2.5	<0.001	0.143	0.002
B (mg/kg DM)	12 ^{a}	16 ^{b}	19 ^{c}	19 ^{c}	2.4	<0.001	0.468	0.676
Mo (mg/kg DM)	0.48	0.51	0.51	0.51	0.07	0.877	<0.001	0.535
Co (mg/kg DM)	0.43 ^{a}	0.44 ^{a}	0.43 ^{a}	0.59 ^{b}	0.16	<0.001	0.266	<0.001
Se (mg/kg DM)	0.03	0.03	0.04	0.03	0.00	0.058	0.197	0.213
Ryegrass staggers index (meq)	1.01	0.80	0.69	1.10	0.38	0.804	0.257	0.912
DCAD (meq/kg DM)	228	204	191	195	41	0.129	0.209	0.008
Bioactive compound								
Aucubin (g/kg DM)	0.69 ^{a}	3.78 ^{b}	5.76 ^{c}	6.58 ^{c}	2.00	<0.001	0.249	0.136
Acteoside (g/kg DM)	0.68 ^{a}	4.79 ^{b}	8.01 ^{c}	8.89 ^{c}	0.79	<0.001	0.090	0.131
Catalpol (g/kg DM)	0.38 ^{a}	0.69 ^{b}	0.80 ^{b}	0.80 ^{b}	0.31	0.002	0.158	0.087

$^{a, b, c, d}$ Means within a row with different superscripts differ at $p < 0.05$. ADF = acid detergent fibre; CF = crude fat; CP = crude protein; DCAD = dietary cation-anion difference; DM = dry matter; ME = metabolisable energy; NA = not applicable; NDF = neutral detergent fibre; NSC = non-structural carbohydrate; OMD = organic matter digestibility; treat = treatment; sea = season.

3.2. Botanical Composition

The composition of plantain leaves, plantain reproductive stem, perennial ryegrass, and dead materials differed between treatments over the experimental period ($p < 0.05$, Table 3). The contents of plantain leaves in PLH (37.6%) and in PLM (34.5%) were higher than in PLL (23.8%) ($p < 0.05$). In addition, the plantain-RGWC pastures (PLL, PLM, PLH) had between 7.9 and 10.2% plantain reproductive stem. The greater plantain content resulted in a lower composition of perennial ryegrass and dead materials in the swards. Perennial ryegrass and dead material accounted for 25.2% and 10% in PLH, 27.6% and 10.8% in PLM, and 37.7% and 13.7% in PLL, respectively. Those figures were lower than that of RGWC (62.2% and 16.3%) ($p < 0.05$). No significant difference in the composition of white clover and weeds was observed among treatments over the experimental period.

The Gaussian models estimated that the total plantain content (including leaves and reproductive stem) increased over the first year to reach a peak of 46.5% in PLL on day 442, 63.0% in PLM on day 455, and 64.2% in PLH on day 421 after sowing. Then, the proportion of plantain declined rapidly to approximately 20% in PLL and 30% in PLM and PLH on day 705 after establishment (Table 4 and Figure 1). The peaks of plantain content in PLM and PLH were similar, and those figures were significantly higher than in PLL. The time for PLH to achieve the highest plantain content (day 421) was statistically earlier than for PLM (day 455). No difference in the slope of the models was found in our analysis.

Table 4. Mean, standard error (SE), and 83.4% confidence interval (CI) of Gaussian models to estimate plantain percentage from day 150 to 705 after sowing for PLL, PLM, and PLH.

Parameter	PLL			PLM			PLH		
	Mean	**SE**	**83.4% CI**	**Mean**	**SE**	**83.4% CI**	**Mean**	**SE**	**83.4% CI**
Peak	46.5	2.8	42.7–50.4	63.0	2.1	60.1–65.9	64.2	2.7	60.5–67.9
Day	442	11	427–458	455	7	445–465	421	9	408–433
Slope	200	16	177–222	211	10	196–225	235	16	212–258

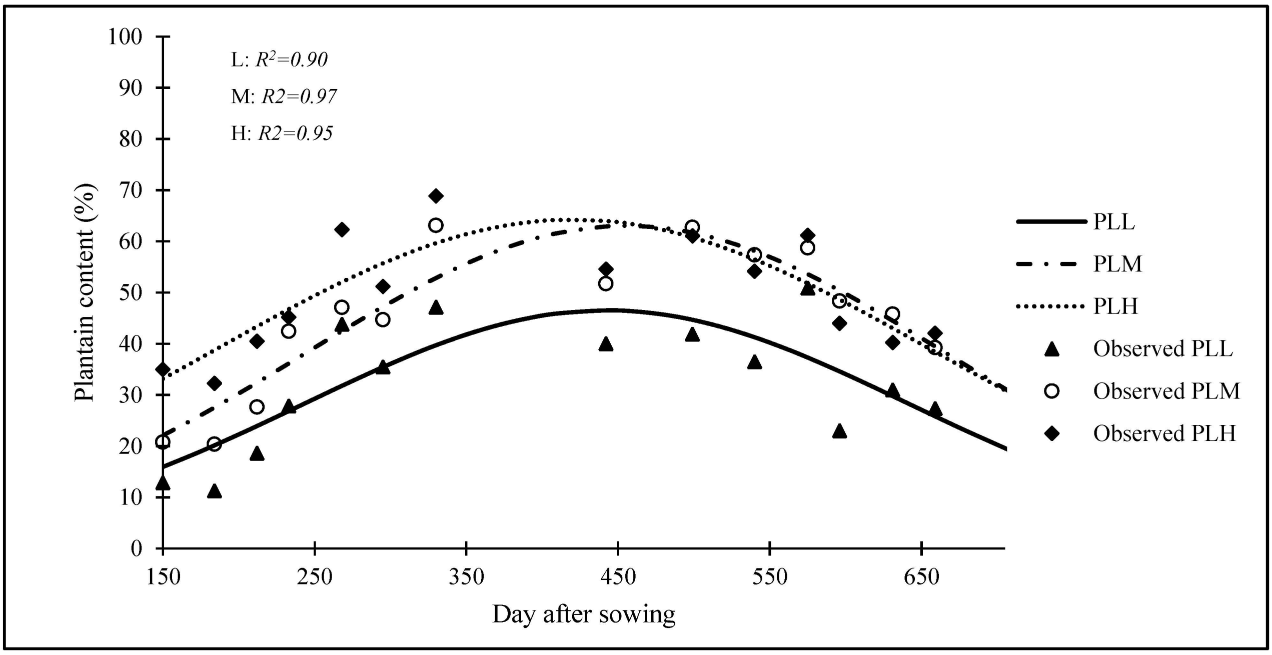

Figure 1. The modelled and observed percentage of plantain (leaves and reproductive stem) in PLL, PLM, and PLH from day 105 to 705 after sowing.

3.3. Herbage Nutritive Value and Contents of Minerals and Bioactive Compounds

Most parameters for nutritive value in Table 3 were significantly affected by treatment, except for CP, lignin, soluble sugars, and ME, K, Mo, Se, ryegrass staggers index, and DCAD. In detail, PLH and PLM were higher than RGWC in OMD, ash, starch, and NSC, but these plantain-RGWC treatments (PLH and PLM) were lower in DM content, ADF, NDF, and CF ($p < 0.05$). In addition, PLM and PLH had a higher concentration of catalpol, aucubin, and acteoside, and most minerals (P, S, Ca, Mg, Na, Cl, Zn, Cu, B, Co) than RGWC, except for Fe and Mn, which were greater in RGWC ($p < 0.05$). The same trends were observed between PLL and RGWC, with a lower difference. A significant difference between PLL and RGWC was found for DM%, NDF, NSC, P, S, Ca, Na, Cl, Fe, Zn, Cu, B, catalpol, aucubin, and acteoside.

3.4. Effect of Treatment in Different Seasons

The interaction between treatment and season was significant on plantain reproductive stem, dead materials, DM content, OMD, P, Cu, and Co ($p < 0.05$). At the same time, this interaction effect was a tendency for post-grazing DM mass, plantain leaves, S, Na, Cl, and catalpol ($p < 0.1$) (Table 3). The effect of pasture treatment on the main measured experimental parameters in different seasons are presented in Figure 2. Overall, the trend of treatment effect was consistent over seasons; however, the level of treatment effect on those variables was different between seasons. Specifically, the treatment effect on plantain reproductive stem in early summer was greater than in spring, late summer, and autumn.

The higher composition of plantain stem resulted in a lower content of plantain leaves in early summer compared with other seasons. In addition, the stronger effect of pasture treatment on dead materials, DM%, OMD, and Na was observed in late summer and autumn than in spring and early summer.

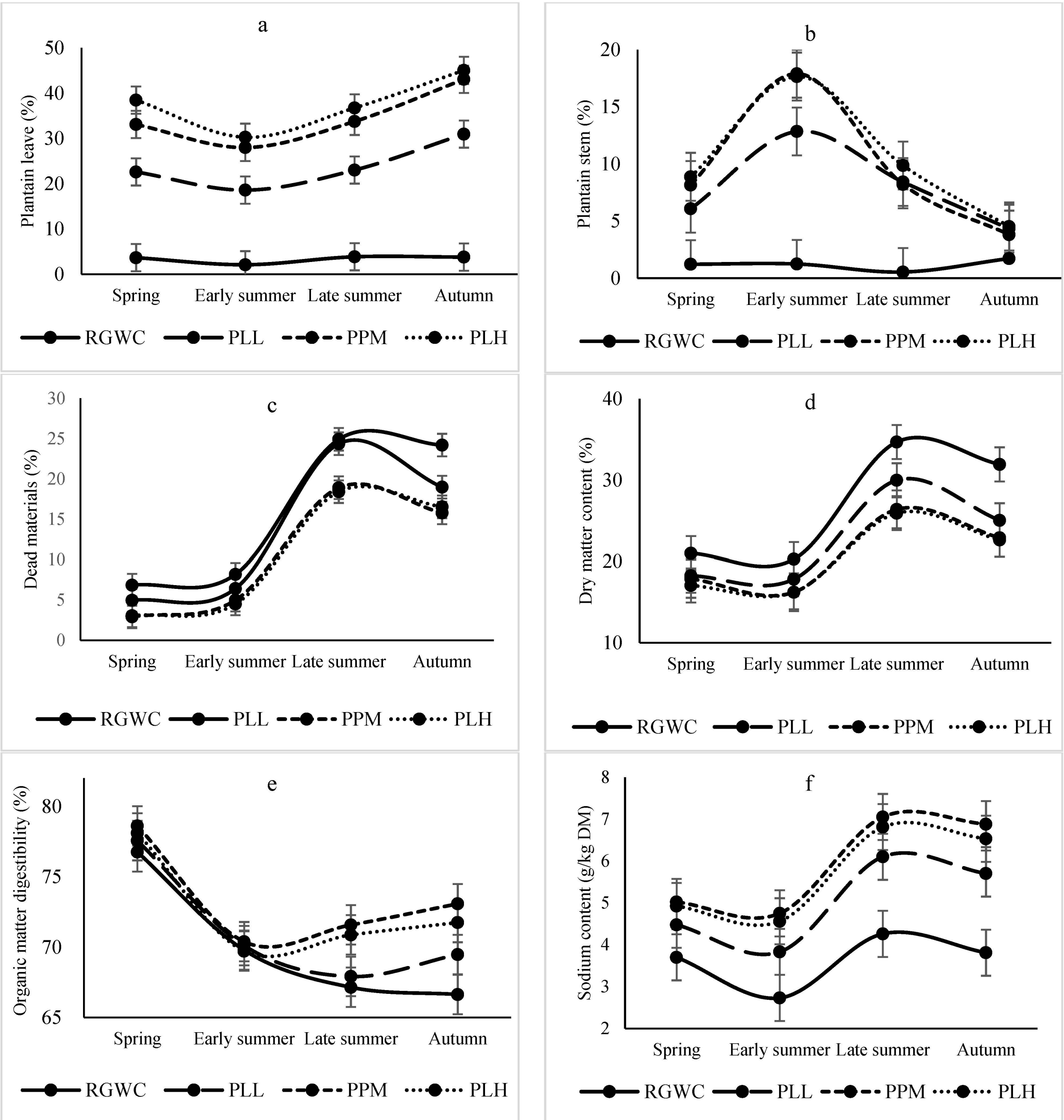

Figure 2. Composition of plantain leaves (**a**), plantain reproductive stem (**b**), dead materials (**c**), dry matter (**d**), organic matter digestibility (**e**), and sodium (**f**) of RGWC, PLL, PLM, and PLH in spring, early summer, late summer, and autumn between September 2019 and March 2021.

3.5. Effect of Plantain Content and Season

The relationships between the content of plantain leaves in the pasture and herbage nutritive value traits in different seasons are presented with general linear regression models covariate with the season in Table 5. Plantain leaves percentage significantly influenced all the given parameters in Table 5. Specifically, the proportion of plantain leaves was negatively associated with the composition of perennial ryegrass and dead materials, DM content, ADF, NDF, Fe, and Mn ($p < 0.05$). In contrast, it was positively associated with OMD, ash, CF, NSC, P, S, Ca, Mg, Na, Cl, Zn, Cu, B, catalpol, aucubin, and acteoside ($p < 0.05$). In addition, the effect of season was significant on all given parameters in Table 5, except for ash and aucubin ($p < 0.05$). In particular, the content of perennial ryegrass, OMD, CF, and NSC was higher in spring than other seasons, while the composition of dead materials, DM%, ADF, NDF, and most minerals (S, Ca, Mg, Na, Zn, Cu, B) was greater in late summer and autumn than spring ($p < 0.05$). The R^2 of the model was higher than 0.50 for the content of perennial ryegrass, dead materials, DM%, OMD, NDF, P, Ca, Mg, Na, Cl, Zn, and aucubin, was between 0.3 and 0.5 for ADF, CF, S, B, and acteoside, and was lower than 0.3 for ash, NSC, Fe, Mn, Cu, and catalpol.

Table 5. Relationship between the content of plantain leaves (%) and parameters related to botanical composition, nutritive value, mineral content, and bioactive compound in spring, early summer, late summer, and autumn between September 2019 and March 2021.

Parameter	α	β	Season				*p*-Value		R^2	r.m.s.e
			Spring	Early Summer	Late Summer	Autumn	Plantain	Season		
Ryegrass (%)	81	−1.03	0 ^{a}	−18.2 ^{b}	−31.3 ^{c}	−17.1 ^{a}	<0.001	<0.001	0.75	12.2
Dead (%)	6.9	−0.10	0 ^{a}	1.1 ^{a}	17.2 ^{b}	15.1 ^{b}	<0.001	<0.001	0.75	4.2
DM (%)	21	−0.11	0 ^{a}	−1.4 ^{a}	10.7 ^{b}	7.7 ^{c}	<0.001	<0.001	0.60	3.9
OMD (g/kg DM)	757	0.73	0 ^{a}	−71 ^{b}	−81 ^{b}	−77 ^{b}	<0.001	<0.001	0.51	41
ADF (g/kg DM)	255	−0.46	0 ^{a}	32 ^{b}	22 ^{c}	16 ^{c}	<0.001	<0.001	0.42	19
NDF (g/kg DM)	436	−1.61	0 ^{a}	59 ^{b}	38 ^{c}	36 ^{c}	<0.001	<0.001	0.59	34
Ash (g/kg DM)	108	0.28	0	−3.0	−3.7	2.6	<0.001	0.100	0.20	13
CF (g/kg DM)	31	0.06	0 ^{a}	−4.9 ^{b}	−3.4 ^{c}	−3.2 ^{c}	<0.001	<0.001	0.36	2.9
NSC (g/kg DM)	235	0.93	0 ^{a}	−49 ^{b}	−12 ^{b}	−29 ^{b}	<0.001	<0.001	0.28	44
P (g/kg DM)	4.0	0.01	0 ^{a}	−0.02 ^{a}	−0.42 ^{b}	−1.08 ^{c}	<0.001	<0.001	0.53	0.4
S (g/kg DM)	2.4	0.02	0 ^{a}	0.43 ^{b}	0.53 ^{b}	0.38 ^{b}	<0.001	<0.001	0.49	0.4
Ca (g/kg DM)	7.3	0.15	0 ^{a}	1.0 ^{a}	2.7 ^{b}	1.3 ^{a}	<0.001	<0.001	0.64	2.2
Mg (g/kg DM)	2.1	0.01	0 ^{a}	0.12 ^{b}	0.81 ^{c}	0.98 ^{d}	<0.001	<0.001	0.73	0.28
Na (g/kg DM)	3.0	0.06	0 ^{a}	−0.14 ^{a}	1.67 ^{b}	0.98 ^{c}	<0.001	<0.001	0.51	1.3
Cl (g/kg DM)	11.2	0.21	0 ^{a}	−3.0 ^{b}	−0.80 ^{a}	0.88 ^{a}	<0.001	<0.001	0.51	4.3
Fe (mg/kg DM)	198	−1.22	0 ^{a}	62 ^{b}	−14 ^{a}	54 ^{b}	<0.001	<0.001	0.16	92
Mn (mg/kg DM)	110	−0.45	0 ^{a}	16 ^{b}	0.4 ^{a}	27 ^{c}	<0.001	<0.001	0.22	26
Zn (mg/kg DM)	26	0.29	0 ^{a}	10 ^{b}	12 ^{bc}	14 ^{c}	<0.001	<0.001	0.61	6.3
Cu (mg/kg DM)	10.3	0.06	0 ^{a}	1.9 ^{b}	2.6 ^{b}	1.2 ^{b}	<0.001	<0.001	0.18	2.0
B (mg/kg DM)	11.6	0.18	0 ^{a}	0.2 ^{a}	4.0 ^{b}	−1.4 ^{a}	<0.001	<0.001	0.37	3.8
Aucubin (mg/g DM)	−0.68	0.18	NA	0	0.79	0.10	<0.001	0.186	0.70	2.1
Acteoside (mg/g DM)	1.27	0.16	NA	0 ^{a}	1.68 ^{b}	−1.0 ^{a}	<0.001	0.001	0.47	3.1
Catalpol (mg/g DM)	0.51	0.01	NA	0 ^{a}	−0.12 ^{a}	−0.47 ^{b}	<0.001	<0.001	0.28	0.43

$^{a, b, c, d}$ Values within a row with different superscripts differ at $p < 0.05$ between seasons; R^2 = the coefficient of determination; r.m.s.e = root mean square error. NA = not applicable (variables were not measured in spring). The model: y = α + β × plantain (%) + season; where, y = independent variable, α = intercept, β = slope of plantain effect (% plantain leaves), season = spring (September–November), early summer (December), late summer (January–February), or Autumn (March–May).

4. Discussion

The current study confirmed that under a dairy grazing system, the plantain-RGWC pasture has a similar annual yield to RGWC [6]. Our additional analyses found no difference in the DM yield between the plantain-RGWC and RGWC in any seasons and no statistical relationship between seasonal yield with sowing rate, and climate factors such as rainfall, water deficit, and soil temperature. A previous study conducted on a grazing dairy farm indicated that annual and seasonal DM yields are maintained at the farm scale with the plantain-RGWC compared to the RGWC pasture [6]. Other studies reported a higher DM yield of mixed pastures containing plantain than RGWC pasture. However, those mixtures included more pasture species such as chicory (*Cichorium intybus*), prairie grass (*Sporobolus cryptandrus*), lucerne (*Medicago sativa*), and red clover (*T. pratense*) [4,5,29]. Perhaps, the variation in the pasture DM yield of the paddock-scale measurement is large [30]; therefore, a big difference between treatments may be required to demonstrate significance. Our results suggested that under a rain-fed grazing dairy system, plantain-RGWC pastures containing an average of 32–48% plantain, achieved through adding between 4 and 10 kg plantain seed with a concomitant decrease in the perennial ryegrass component in the establishment, maintains the annual DM yield of the pasture in the first two years from the establishment.

The biggest challenge for farmers is to maintain a high proportion of plantain in the RGWC-based pasture, especially under the grazing conditions [31]. The plantain content in our study was greater than in newly established mixed pastures with multiple forage species [16,29,32] or when plantain seed was added into an existing RGWC sward [33], but lower than in mixed pastures with plantain and clover [6,34]. The low plantain composition in PLH indicated that incorporation of plantain into the RGWC pasture is unlikely to achieve 70% or more plantain for an extended period under grazing conditions. Plantain seeds added by direct drilling at the end of the first year were not successful in increasing the percentage of plantain in the pastures. Our observations did not record young plantain establishment as they could not compete well with the existing pastures. A similar result has been indicated in a study by Bryant, Dodd, Moorhead, Edwards, and Pinxterhuis [33]. The failure of the addition of plantain seed suggested that the proportion and persistence of plantain in our study was obtained from the original establishment. In addition, our results agree with Dodd, Moss, and Pinxterhuis [31] and Bryant, Dodd, Moorhead, Edwards, and Pinxterhuis [33] that plantain percentage in grass-based pastures increases in the first year and then declines in the second year from establishment. The time to reach the highest plantain content in the current study (421–455 days) is relatively similar to Bryant, Dodd, Moorhead, Edwards, and Pinxterhuis [33], who indicated the highest plantain content was achieved between 432 and 470 days after sowing. In the present study, the pasture sown with a high plantain seed rate (PLH) reached the highest plantain content earlier than those with the lower rate (PLM), but then it declined faster to have a similar plantain composition as PLM in the second year. Plantain has been reported to have a high DM yield early after the autumn sowing [35]. The treatments with a higher density of plantain can produce a larger amount of its DM biomass in the first 15 months before perennial ryegrass is increasingly dominant. Over time, plantain continually disappears due to its short lifespan and the competition from stronger perennial ryegrass and white clover in the mixed pasture [31,36]. This loss of plantain may occur faster in the pasture with a higher density of ryegrass; for example, amongst treatments, PLM and PLH were better than PLL in achieving and maintaining a high plantain content over grazing events. A survey of 21 New Zealand farms [31] showed that most ryegrass and clover-based mixed paddocks had less than 20% plantain after three years after sowing. This low plantain content is not expected to significantly improve farm productivity and environmental benefit [32]. Therefore, further studies on interventions and managements are required to maintain plantain content in plantain-RGWC beyond two years from the establishment.

The main advantages of the plantain-RGWC pasture include an improvement in herbage nutritive value, potentially increasing animal performance and reducing N losses from the dairy system [37]. The current study is consistent with previous studies that included plantain in the RGWC pasture resulting in increased NSC [12], the content of minerals [38], and bioactive compounds [39], but decreased fibre content [40]. Previous studies have reported that pure plantain had a lower content of DM by 30%, NDF by

33%, but contained a higher concentration of NSC by 30%, and total mineral content by 26%, compared to ryegrass [12]. Plantain was also reported to contain 0.44–6.87 g/kg DM of aucubin and 0.5–41.7 g/kg DM of acteoside [15]. Those inherent differences cause changes in herbage nutritive characteristics when plantain is incorporated into the RGWC pasture in our study. Additionally, the comprehensive and large data set in the current study found significant difference between plantain-RGWC and RGWC in parameters with a smaller effect such as lignin, OMD, starch, CF, S, Mg, Fe, Mn, Zn, Cu, B, Co. These parameters have either not been reported or were inconsistently or not significantly affected by plantain inclusion in previous studies [15,38,39]. Those changes in nutritional value have been reported to improve animal performance and to provide environmental benefits. Specifically, the lower fibre content of plantain can increase DMI because animals can consume and digest more plantain-based pastures than the RGWC in the same period, thus increasing milk production [41]. In addition, the greater content of moisture and minerals can result in an increase in water intake and urine volume to dilute urinary nitrogen of animals [42]. Moreover, bioactive compounds, aucubin, and acteoside in plantain can reduce ammonia production in the rumen to decrease urinary nitrogen excretion of grazing animals, and the extraction of these compounds from roots and leaves can inhibit nitrification processes in soils to decrease nitrate leaching and nitrous oxide emission from pastoral systems.

Regarding the effect of pasture treatment, there is a clear trend that the pastures sown with more plantain seed had a greater difference in herbage nutritive characteristics than RGWC. A certain amount of plantain content is required to have a measurable improvement in the value of several nutritive parameters. For example, the concentration of OMD, ADF, lignin, ash, starch, CF, Mg, Mn, and Co of plantain-RGWC significantly differed from RGWC when containing 44% plantain or more (PLM and PLH), but were not statistically different when containing 32% plantain (PLL). In support of this, previous studies [19,20] concluded that the RGWC-based pasture require more than 30% plantain to cause a measurable effect on reducing urinary N excretion, while improving herbage nutritive value and, hence, milk production of dairy cows. The greater effect of plantain-RGWC pasture in late summer and autumn on key parameters (DM%, OMD, S, Na, Cu, and Co) aligns with our hypothesis that plantain improves herbage quality during drier seasons [15,22,23]. The greater improvement in the pasture quality in late summer and autumn is expected to expand the effect of plantain-RGWC on animal performance and environmental benefits during the drier months when the nutritive value of ryegrass is lower [9]. Our results suggested that under a grazing dairy system, the plantain-RGWC pastures sown with 7 kg (PLM) and 10 kg (PLH) plantain seed are better than those sown with 4 kg (PLL) plantain seed in improving nutritive value in the first two years from sowing.

Various factors may affect the quality of the pasture containing plantain, including plantain composition and season. The present study generated four models to estimate the relationship between each nutritive parameter and the content of plantain leaves in spring, early summer, late summer, and autumn. The high coefficient of determination of the estimated models for DM content, OMD, NDF, P, Ca, Mg, Na, Cl, Zn, and aucubin ($R^2 > 0.5$) resulted from a large difference between plantain and RGWC in these nutritive characteristics [12,15,38]. For the seasonal effect, our result was consistent with other studies that the plantain-RGWC pasture contains a higher DM content, ADF, NDF, and most minerals, but it has a lower concentration of OMD, CF, and NSC in late summer and autumn, compared to spring [2,12,15,22]. The concentration of minerals has been suggested to be affected by precipitation and is usually higher in dry summer and autumn [43,44]. Our analysis has not considered other factors potentially influencing herbage nutritive value, such as grazing intervals, leaf age, water stress, and temperature [2,22,43]. These may be the reason for a low R^2 ($R^2 < 0.3$) of the estimated models for several parameters (lignin, ash, NSC, Fe, Mn, Cu, and catalpol). Further studies are needed to investigate the effect of these associated factors on the quality of the pasture containing plantain.

The role of plantain on dairy farms to reduce N losses is currently highly topical. Plantain can either be sown as a monoculture crop, incorporated with conventional pastures at sowing to form a mixed sward, or added into existing pastures by broadcasting or direct drilling [31,33]. However, there is limited understanding of the effectiveness of these establishment methods on plantain content, DM yield, and herbage nutritive value. Bryant et al. 2019 indicated that direct drilling could achieve a higher plantain rate than broadcasting for adding plantain into an existing RGWC pasture; however, these two methods rarely reach more than 30% of plantain in the sward [33]. Therefore, the new establishment of the plantain-RGWC pasture could be the option for farmers to obtain a higher plantain content in the cow's diet in the first two years. From the current study, 30% of plantain in a dairy farm might be achieved if the whole farm was sown with plantain equivalent to the PLL treatment rate (see sowing rates in Table 1), or if about 60% of the farm area was sown with plantain equivalent to the PLM or PLH treatment rate. However, we would expect that plantain would only remain at the 30% level for the first 24 months from establishment. Pure plantain or plantain-clover has been reported to achieve higher plantain content than plantain-RGWC pasture [31]; therefore, a smaller area is needed to obtain 30% plantain in the cow's diet. However, the pure plantain pasture has been reported to have various disadvantages such as poor winter growth, sensitivity to treading damage, weed ingression, risk of bloat to the animal, and low palatability in the seeding season [2]. Further studies are required to investigate suitable methods to get plantain into the dairy system and to sustain plantain content in the plantain-RGWC pasture beyond two years after the establishment.

5. Conclusions

Our study indicated that incorporating plantain with RGWC with the sowing rate between 4 and 10 kg plantain seed per hectare maintains annual DM yield close to the RGWC pasture in the first two years after the establishment. Over two years, the average composition of plantain across plantain-RGWC treatments was 41%. The percentage of plantain in the plantain-RGWC pasture increased in the first year to reach a peak of 46–64% at between 421 and 455 days, then declined to 20–30% plantain at day 705 after sowing. We conclude that incorporation of plantain into the RGWC pasture is unlikely to achieve 70% or more plantain under grazing conditions over an extended period. Moreover, maintaining more than 30% plantain in the RGWC pasture after two years will likely require a management intervention such as sowing in more plantain.

In addition, incorporating plantain into the RGWC pasture resulted in a lower concentration of DM, ADF, NDF, CF, Fe, and Mn, but a higher concentration of OMD, ash, starch, NSC, P, S, Ca, Mg, Na, Cl, Zn, Cu, B, Co, aucubin, acteoside, and catalpol in comparison to the standard RGWC pasture. The effect of pasture treatment on dead materials, DM content, OMD, and Na were greater in late summer and autumn than in spring and early summer. Moreover, the proportion of plantain leaves was strongly associated with decreased concentration of dead materials, DM, OMD, NDF, and increased concentration of P, Ca, Mg, Na, Cl, Zn, and aucubin in the pastures.

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