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Animal Supplementation and Legume Pastures Enhance Nitrogen Balance and Efficiency in Integrated Crop-Livestock Systems

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Abstract

Improving sustainability in agricultural systems depends on increasing the efficiency of nitrogen (N) use and recycling. This study evaluated whether animal supplementation and legume-based pastures can enhance N balance and residual N availability in an integrated crop-livestock system (ICLS). The experiment was conducted in two phases—livestock and cropping—using three treatments: a control pasture (oat + ryegrass), a legume mixture (oat + ryegrass + arrowleaf clover), and a supplementation treatment (oat + ryegrass with concentrate supplementation at 1% of live weight), each replicated three times. Soybeans were grown during the cropping phase. Supplementation increased the stocking rate by 21%, while both supplementation and legumes led to a 30% increase in residual N returned via feces and urine, without negatively affecting soybean yield (~4.1 Mg ha^{-1}). N off-take by soybean grain was approximately 9% higher in these treatments, while N exported via cattle carcasses remained unchanged across treatments, averaging 8.2 kg ha^{-1}. Overall, soybeans accounted for 96–97% of total N export, and animals for only 3–4%. These results demonstrate that animal supplementation and legume integration enhance N use efficiency and contribute to nutrient recycling in ICLS, offering a viable strategy to reduce dependence on synthetic fertilizers. The findings support the development of more sustainable livestock and crop systems by maximizing nutrient retention, maintaining yield, and improving soil fertility. Furthermore, the implications for soybean yield and the sustainability of livestock systems indicate a potential positive economic and environmental impact for producers and policymakers.

Keywords: urinary nitrogen excretion; forage mixture; sustainable intensification; grazing supplementation; livestock system

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1. Introduction

Nitrogen (N) is an essential macronutrient in agriculture, required in large amounts to boost yield [1]. Integrated crop-livestock systems (ICLS) benefit from the synergistic relationship between animals and pasture, enhancing nutrient balance and potentially

reducing the reliance on chemical fertilizers [2]. In the crop phase, soybean is a demanding crop that off-take high amounts of N for grain production; only a small portion is returned to the system through residues, even though soybean can fix atmospheric N. The reduced need for mineral N in ICLS therefore depends on how much N is actually recycled within the system, which varies with the production strategy and still needs to be quantified.

Given that temperate pastures cover roughly 9 million km^2 globally, their N dynamics are well-studied [3,4]. One of the key elements in achieving a positive N balance that favors ICLS begins in the livestock phase, particularly in the forage provided to cattle. About 90% of the nutrients ingested by animals while grazing is returned to the soil through feces and urine [3]. Therefore, the choice of forage significantly influences nutrient cycling, enhancing soil organic matter and benefiting subsequent crops [5]. Incorporating legumes into pastures elevates N cycling via biological fixation and augmented N excretion by animals, since legumes have the potential to increase animal stocking rates (SR) when compared to single-species pastures [6]. This process contributes N to following crops and minimizes the need for synthetic N fertilizers, promoting environmentally friendlier farming practices [7].

Energy supplementation on pasture is also used to intensify beef production, because it allows higher stocking pressure without degrading the pasture [8–11]. As stocking rate increases, excreta deposition per hectare increases, enhancing N return to the soil. The higher SR, combined with nutrient supplementation from feed and legumes, can increase residual N through feces and urine, benefiting the subsequent crop. Therefore, animal supplementation and legumes in ICLS can reduce N fertilization in the production system, as the use of integrated systems has been shown to decrease fertilizer use [12]. Research indicates that grass-legume mixtures in pastures can improve phytomass, forage quality, and, subsequently, soybean yield, with an observed 8% yield increase in mixed pastures compared to monoculture [13]. However, most ICLS studies report pasture and crop responses only, and do not quantify how much N is exported in animal products and how much is effectively returned via feces, urine, and post-grazing residues. This represents a practical gap, because recommendations on reducing N fertilizer depend on knowing these animal-driven N flows. Our study addresses this point by measuring N intake, N excretion, and N off-take by soybean within the same production cycle.

Given this context, we hypothesized that legume inclusion and animal supplementation positively affects the internal N balance of ICLS by enhancing N return via excreta and improving soybean N uptake (Figure 1). It aims to investigate the influence of animal supplementation (1% of live weight [LW]) and legume integration on residual N from feces, urine, and post-grazing residue, N export by cattle, N off-take by soybean crop, and grain yield.

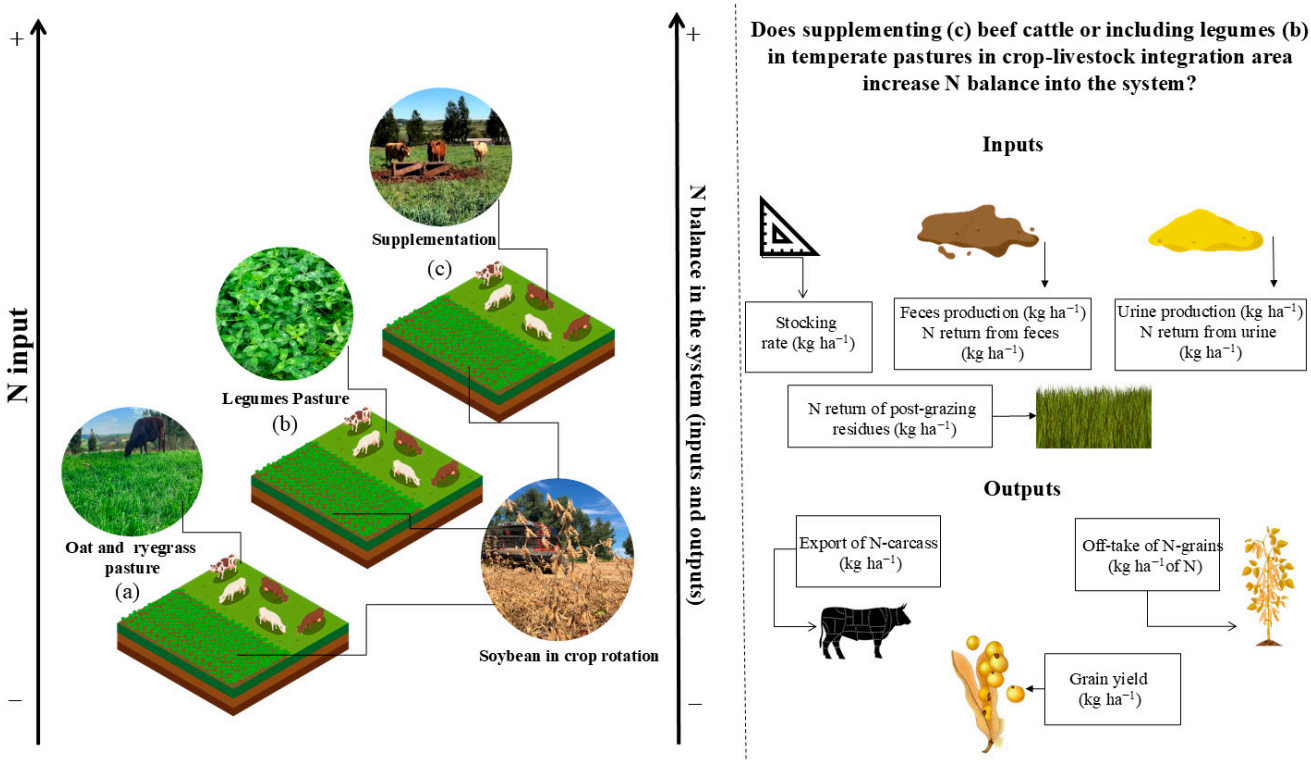

Figure 1. Conceptual framework illustrating the effects of animal feeding on N balance in integrated crop-livestock systems.

2. Materials and Methods

2.1. Experimental Site

The study was conducted from May 2020 to March 2021 at the Federal Technological University of Paraná in Paraná station, southern Brazil (25°33′ S and 51°29′ W, ~520 m); the region is classified as a humid subtropical climate (Cfa) according to the Köppen–Geiger classification [14]. The research area encompassed 7 ha of ICLS, initiated in 2017, and included a winter pasture phase (oat [*Avena strigosa* Schreb. 'Iapar 61'], ryegrass [*Lolium multiflorum* Lam. 'Ponteio'], and arrowleaf clover [*Trifolium vesiculosum* Savi 'BRS Piquete']) for beef cattle grazing, followed by an annual summer rotation of maize (*Zea mays* L.) or soybeans (*Glycine max* L.). This study focused on both the livestock and crop phases starting in 2020.

2.2. Experimental Design and Treatments

The study was divided into two phases: a 90-day livestock evaluation followed by a 137-day soybean evaluation. A randomized block design was employed to assess animal performance, as well as the production and quality of pastures and soybean, featuring three treatments and three replications each. A 3 × 3 double Latin square design (treatments x evaluation periods) was implemented to measure dry matter (DM) intake, fecal and urinary outputs, and pasture digestibility repeatedly over time. The research area was segmented into ten experimental units (paddocks), averaging 0.7 ha each, accommodating three treatments with three repetitions, and one paddock reserved for regulator animals. The treatments included the control (oat + ryegrass), legume (oat + ryegrass + arrowleaf clover), and supplementation (oat + ryegrass with daily animal supplementation of 1% LW of distillery dry grain at 11:30 a.m.). The 1% LW supplementation level was used because it is commonly associated with improved intake and performance in grazing cattle without markedly reducing forage intake. Arrowleaf clover was chosen because it is well adapted to

the region, commonly used by farmers, and has high biological N fixation, contributing to N inputs and cycling. Animal weights were monitored every 21 days to adjust supplement amount, and steers had ad libitum access to water and mineral salt (130 g Ca, 85 g P, 12 g Mg, 10 g S, 170 g Na, 2.5 g Zn, 0.5 g Cu, and 0.02 g Se per kg of product).

2.3. Pasture Phase

The livestock phase began with winter pasture sowing on 6 May 2020. Following the maize harvest for the 2019/2020 crop year, forage species were sown in rows, while the legume was broadcast sown without soil tillage. The sowing densities were 55 kg ha^{-1} for oat (N input of 1.04 kg ha^{-1}), 25 kg ha^{-1} for ryegrass (N input of 0.75 kg ha^{-1}), and 10 kg ha^{-1} for arrowleaf clover (N input of 0.40 kg ha^{-1}). The arrowleaf clover's dormancy was broken to enhance germination, and the seeds were inoculated with a bacteria strain for N fixation. A uniform fertilization rate of 250 kg ha^{-1} of NPK (8-20-15) was applied across all experimental plots at sowing. Additionally, two topdressing applications of urea (45% N) were made during the pasture's tillering phase and again 50 days later, totaling 75 kg ha^{-1} of N.

Grazing commenced in July 2020 when the forage mass reached approximately 1500 kg ha^{-1} DM and concluded in October 2020, spanning 90 days. A continuous grazing system with variable SR was employed, adjusting SR using the "put-and-take" technique [15]. The SR was estimated every 21 days based on a forage allowance of 9 kg DM 100 kg^{-1} LW, maintaining two tester animals $plot^{-1}$. The average SR for the period was calculated by combining the average weights of tester and non-tester animals, multiplied by the number of grazing days, and divided by the total grazing days.

Forage allowance was estimated as outlined by Sollenberg et al. [16], which consisted of the direct relation of forage mass per unit of SR. The double sampling technique, as proposed by Wilm et al. [17], was employed to determine forage kg ha^{-1} of DM. This involved cutting pasture close to the ground over a 0.25 m^2 area at five points in each plot, followed by 20 visual evaluations every 21 days. After double sampling at the end of each period, samples were homogenized. A portion was then used for botanical separation to identify the percentage of arrowleaf clover and the structural components of oat and ryegrass (stem and leaf). These samples were dried in a forced air oven at 60 °C for 72 h to measure DM.

Forage allowance, expressed as kg DM kg^{-1} LW^{-1}, was calculated by dividing the average forage mass for each experimental period by the average SR (kg ha^{-1}). The pasture accumulation rate was determined using two grazing exclusion cages $plot^{-1}$, following Campbell [18] formula. These cages were placed at similar sites to those used for mass cutting. Cuts were made close to the ground every 21 days, with the daily forage accumulation rate (kg ha^{-1} d^{-1} of DM) calculated by subtracting the DM of the previous cuts from the current one and dividing by the number of days between cuts. Total forage production (kg DM ha^{-1}) was calculated by summing the daily accumulation rates for each period (kg ha^{-1} d^{-1} of DM) and multiplying by the number of days in each period plus the initial forage mass before grazing began.

To assess the nutritional value of pasture, we employed the grazing simulation method described by Euclides et al. [19]. Every 21 days, samples mimicking the forage consumed by animals grazing were manually collected. A subsample from each $plot^{-1}$ was dried in a forced-air oven at 60 °C for 72 h to measure DM, then ground using a Willey knife mill with a 1 mm sieve for N content analysis. Another portion was ground to 2 mm for in vitro DM digestibility testing using a commercial incubator (Daisy II 200, Ankom Technology, Macedon, NY, USA), as outlined by Wiseman [20]. After the grazing period, residual forage mass (kg ha^{-1} of DM) was estimated using the same method as the initial forage mass

assessment. These samples were dried at 60 °C for 72 h and stored in litter bags to study decomposition post-soybean sowing.

2.4. Animal Evaluations

Twenty-one commercial crossbred steers predominated by Angus breed, averaging 25 ± 3 months old and 444.5 kg LW, were used. Three baseline animals were slaughtered at the experiment's start, and the remaining 18 were utilized as testers. Following a 15-day adaptation period to the new feed and management conditions, weights were recorded at the experiment's start, end, and every 21 days following a 14–16 h fast. This study was approved by the research ethics committee of the Federal Technological University of Paraná, Dois Vizinhos campus (protocol no. 2020-11).

2.5. Nutrient Intake and N Excretion

Titanium dioxide (TiO_2) was utilized to estimate the animals' fecal production (FP). For this purpose, animals were administered 10 g of TiO_2, encapsulated in tracing paper cartridges, directly into the esophagus for 12 days, divided into three intervals of 21 days each. The initial seven days were aimed at stabilizing the TiO_2 content within the feces. Subsequently, fecal samples were collected manually from the rectum of the animals twice daily over the last five days. At the end of each period, a composite fecal sample was compiled for each animal, representing the five days of collection, and then stored at −10 °C. Total forage intake was determined as described by Astigarraga [21].

2.6. Feces and Urine Analysis

Fecal samples were collected directly from the animals' rectum, dried in a forced-air oven at 60 °C to determine dry matter, and then ground in a knife mill with a 1 mm screen for N analysis. Following this, chemical analyses were performed to determine the TiO_2 content in the samples, utilizing atomic absorption spectrophotometry. The FP (kg DM d^{-1}) was calculated using the Formula (1) adapted from Myers et al. [22]:

$$FP = (OF/[TiO_2]) \times 1000/LW \quad (1)$$

where FP_i is the individual daily fecal production expressed per unit of live weight (g kg^{-1} LW day^{-1}), OF: TiO_2 offered is the daily marker intake (g day^{-1}), TiO_2 in feces is the marker concentration in feces (g kg^{-1}), and LW is the animal's average live weight (kg).

To estimate pasture-level fecal production (FP_p), individual FP_i (kg DM $animal^{-1}$ day^{-1}) was multiplied by the average stocking rate (animals ha^{-1}), yielding FP_p in kg DM ha^{-1} day^{-1}.

Five instances of urine and feces sampling were performed per period, and each animal provided approximately 50 mL of instant urine through manual stimulation in the field. The urine was stored in individual polyethylene bottles for each animal. Each day, a portion of the urine was preserved while another was acidified with 40 mL of sulfuric acid (0.036 N) to achieve a 10 mL aliquot, also stored in polyethylene bottles. The samples were kept at −10 °C and later thawed, filtered, and grouped by animal and experimental period. N contents were determined using the acidified samples and the Kjeldahl method (method 984.13; [23]). Creatinine contents were measured from non-acidified samples using a commercial kit (Labtest, Diagnóstica S.A, Lagoa Santa, Brazil). This data was used to calculate daily urine production (mL kg^{-1} LW d^{-1}) based on the method proposed by Chizzotti et al. [24].

2.7. N Retention by Beef Cattle

Three baseline animals were slaughtered at the experiment's start and 18 tester animals at the end to assess N exportation. Each organ, including blood post-total bleeding, was weighed at the slaughterhouse, and subsamples were collected. The organ samples were freeze-dried and ground in a mixer before chemical analysis. The carcasses were then halved, cooled, and sampled between the 12th and 13th ribs of the left half, following Hankins and Howe's [25] technique as adapted by Müller et al. [26]. This enabled the separation and weighing of bone, muscle, and fat to determine their proportions in the carcass using Hankins and Howe [25].

After determining N using the Kjeldahl (method 984.13; [23]) in all collected animal samples, it was possible to determine the amount of N present in the animals' bodies. By comparing the N levels in animals at the start of the trial (baseline animals) to those in animals slaughtered at the end of the experiment (90 days), we determined the total N retention over the experimental period.

2.8. Crop Performance

Soybeans 'TMG 7062' were sown using no-till on a pasture underlaid with straw on 12 November 2020. The planting density was 10 plants m^{-1} (equivalent to 222,222 plants ha^{-1} with N input of 1.70 kg ha^{-1}). A uniform fertilizer application of 350 kg ha^{-1} of NPK 2-20-18 was applied along the sowing line. Grains were harvested mechanically on 30 March 2021, from two subareas $plot^{-1}$. The grain yield (Mg ha^{-1}) was calculated after adjusting the moisture content to 13%. During the harvest, grain subsamples were collected and ground using a Wiley knife mill with a 1 mm sieve to assess N content.

2.9. Decomposition and N Release from Animal Feces and Post-Grazing Residue

Samples of fecal boluses ejected in the pasture were manually collected from each animal immediately after defecation to assess their decomposition and N release. These samples were collected at the end of all four periods (every 21 days), and at the experiment's conclusion, they were composited by the $plot^{-1}$. To investigate decomposition and N release from the forage, samples of the residual biomass left on the last day of the experiment after the animals had been removed were also analyzed.

Both sample types (post-grazing residue and feces) were dried in a forced-air oven at 60 °C for 72 h for DM determination. About 20 g of the dried samples were placed in permeable bags (litterbags) with a 400 cm^2 area (20 × 20 cm) and laid on the soil surface within the soybean cultivation area to simulate natural decomposition conditions. Litterbags containing residual forage and fecal samples were retrieved on days 0, 11, 22, 90, 121, and 160, dried, and then weighed on a semi-analytical scale to calculate the percentage of decomposed DM. The samples were ground post final weighing for N content analysis.

Nitrogen content in the decomposed material was assessed on the specified days. The decomposition rates of the residues and N release were calculated based on the changes in DM and N content over time, using nonlinear regression models as proposed by Wider and Lang [27]. The decomposition model (2) used was:

$$f = ae^{(bx)} + ce^{(dx)} \quad (2)$$

where: f = remaining material (%); a = initial fraction of easily decomposable material; b = decomposition rate of fraction a; c = initial fraction of more resistant material; d = decomposition rate of fraction c; e = exponential function; x = time (days).

2.10. Statistical Analyses

Statistical analyses were conducted using the Statgraphics Plus v. 4.1 (Statistical Graphics Corp., Rockville, MD, USA). Data normality was confirmed by the Shapiro-Wilk test. Analysis of variance (ANOVA) was performed ($p \leq 0.05$), and when significant differences were detected, means were compared using Tukey's test ($p \leq 0.05$).

The mathematical model for data from a Latin square design (covering animal intake, digestibility, and feces and urine production) was (3):

$$\text{Yijkl} = \mu + \text{Ti} + \text{Rj(Ti)} + \text{Mk} + \text{Ti x Mk} + \text{eijkl} \quad (3)$$

where μ is the overall mean, Ti is the impact of treatment i, Rj(Ti) is the effect of replication j within treatment i, Mk is the period effect for period k, Ti x Mk is the interaction between treatment i and period k, and eijkl is the experimental error.

Pasture, soybean, and animal variables, data were analyzed in a randomized complete block design. The statistical model was:

$$Y_{ij} = \mu + T_i + B^j + \varepsilon ij \quad (4)$$

where Y_{ij} is the observation in treatment i and block j, μ is the overall mean, T_i is the fixed effect of treatment, B_j is the random effect of block (field position), and ε_{ij} is the residual error. Blocking was adopted to account for spatial variation in soil fertility and microrelief across the experimental area.

To analyze the DM decomposition and nutrient release curves from litterbags, nonlinear regression analysis was performed and the curves were graphically represented using the SigmaPlot v. 10.0 (Systat Software Inc., San Jose, CA, USA).

3. Results

3.1. Nitrogen in the Livestock Phase

No significant differences were observed in the composition of oat leaf, ryegrass leaf, grass stems, or leaf-to-stem ratio of the pasture. Similarly, the average LW of the animals, forage availability, and post-grazing residues were uniform across all treatments, indicating similar grazing conditions for the cattle. Supplementation led to a 21% increase in SR compared to the control. N content in both the grazing simulation and post-grazing residue did not differ significantly among treatments (Table 1).

Table 1. Pasture composition, forage allowance, live weight, stocking rate, and N content in post-grazing residue in an integrated crop-livestock system.

Variables	Control	Legume	Supplementation	*p*-Value
Oat leaf (g kg^{-1} DM)	219.13 ± 29.6 ns	234.60 ± 31.7	242.18 ± 32.7	0.5265
Ryegrass leaf (g kg^{-1} DM)	182.03 ± 10.5 ns	187.80 ± 10.8	194.67 ± 11.2	0.2103
Grass stems (g kg^{-1} DM)	354.80 ± 33.5 ns	364.50 ± 34.4	369.63 ± 34.9	0.9328
Leaf: stem ratio (g kg^{-1} DM)	1.15 ± 0.07 ns	1.17 ± 0.07	1.20 ± 0.07	0.9337
Forage allowance (kg DM kg^{-1} BW)	8.60 ± 1.29 ns	7.90 ± 1.18	7.50 ± 1.12	0.3579
Legume (g kg^{-1} DM)	-	212.64	-	
Live weight (kg)	482.8 ± 10.4 ns	510.0 ± 12.1	506.8 ± 5.0	0.0685
Stocking rate (kg LW ha^{-1} d^{-1})	1370.2 ± 189.1 b	1472.3 ± 40.1 ab	1666.1 ± 82.1 a	0.0320
N supplementation (g kg^{-1} DM)	-	-	31.6	-
N grazing simulation (g kg^{-1} DM)	26.9 ± 1.21 ns	29.4 ± 1.32	28.0 ± 1.26	0.3508
N post-grazing residue (g kg^{-1} DM)	21.7 ± 2.2 ns	24.2 ± 2.5	22.0 ± 2.3	0.4100

DM: dry matter; BW: body weight; control: oat + ryegrass; legume: oat + ryegrass + legume; supplementation: oat + ryegrass + animal supplementation. Means followed by the same letter are not significantly different by Tukey's test ($p < 0.05$); ns = not significant by the F-test ($p < 0.05$).

Supplementation resulted in a lower daily intake of forage DM (15.79 g kg^{-1} LW d^{-1}) compared to the legume and control treatments, which averaged 26.12 g kg^{-1} LW d^{-1}. N intake from forage was higher in the legume compared to the control and supplementation (Table 2).

Table 2. Daily dry matter and N intake per live weight and during winter/spring in an integrated crop-livestock system.

Variables	Control	Legume	Supplementation	*p*-Value
Daily intake of forage DM (g kg^{-1} LW d^{-1})	24.99 ± 0.10 a	27.25 ± 0.73 a	15.79 ± 0.42 b	0.0001
Daily intake of supplementation DM (g kg^{-1} LW d^{-1})	--	--	10.00	--
Daily intake of total DM (g kg^{-1} LW d^{-1})	24.99 ± 1.16 ns	27.25 ± 1.27	25.79 ± 1.21	0.1141
N intake of forage (g kg^{-1} LW d^{-1})	0.67 ± 0.15 b	0.80 ± 0.19 a	0.50 ± 0.12 c	0.0010
N intake of supplementation (g kg^{-1} LW d^{-1})	--	--	0.32	--
Total daily N intake (g kg^{-1} LW d^{-1})	0.67 ± 0.07 b	0.80 ± 0.09 a	0.82 ± 0.09 a	0.0002
Total daily N intake per hectare (kg LW ha^{-1} d^{-1})	0.92 ± 0.13 b	1.18 ± 0.03 a	1.36 ± 0.07 a	0.0257
Total N intake 90-day (kg ha^{-1})	82.51 ± 11.3 b	106.2 ± 2.9 a	122.4 ± 6.0 a	0.0234

DM: Dry matter; LW: live weight; control: oat + ryegrass; legume: oat + ryegrass + legume; supplementation: oat + ryegrass + animal supplementation. Means followed by the same letter are not significantly different by Tukey's test ($p < 0.05$); ns = not significant by the F-test ($p < 0.05$).

Total daily N intake was higher in the legume and supplementation treatments than in the control, primarily due to the additional 0.10 g N kg^{-1} LW d^{-1} provided by the supplement. Differences were also noted in daily and total N intake ha^{-1} over the 90-day period, which were higher in the legume and supplementation compared to control. N content in feces (~34 g kg^{-1} DM) did not vary among treatments. However, N contents in urine were higher in the legume and supplementation treatments. The daily N residue through feces was consistent across treatments, averaging 0.24 g kg^{-1} LW. The N residue ha^{-1} was larger with supplementation, which was attributed to the higher SR. Over 90 days, the daily and total N residue via urine was elevated in the legume and supplementation (Table 3).

Table 3. Fecal and urinary N excretion and pasture residue N per live weight and during winter/spring in an integrated crop-livestock system.

Variables	Control	Legume	Supplementation	*p*-Value
N Feces (g kg^{-1} DM)	34.6 ± 0.69 ns	34.3 ± 0.69	33.3 ± 0.67	0.5000
N Urine (g L^{-1})	5.8 ± 1.11 b	8.5 ± 1.61 a	7.9 ± 1.50 a	0.0010
Daily urine residual N (g kg LW^{-1})	0.36 ± 0.05 b	0.53 ± 0.03 a	0.49 ± 0.04 a	0.0001
Daily residual N from feces (g kg LW^{-1})	0.25 ± 0.02 a	0.22 ± 0.02 a	0.27 ± 0.03 a	0.0091
Total residual N from urine (kg ha^{-1} day)	0.50 ± 0.07 b	0.78 ± 0.02 a	0.82 ± 0.04 a	0.0078
Total residual N from feces (kg ha^{-1} day)	0.34 ± 0.05 b	0.33 ± 0.01 b	0.44 ± 0.02 a	0.0490
Daily of total residual N (U + F) excreted ha^{-1}	0.84 ± 0.12 b	1.11 ± 0.03 a	1.27 ± 0.06 a	0.0180
Total residual N urine (kg ha^{-1} 90 days)	44.95 ± 6.2 b	70.15 ± 1.9 a	74.03 ± 3.6 a	0.0071
Total residual N feces (kg ha^{-1} 90 days)	30.70 ± 4.2 b	29.54 ± 0.8 b	39.83 ± 6.2 a	0.0500
Total residual N of excreted (kg ha^{-1} 90 days)	75.65 ± 10.4 b	100.04 ± 2.7 a	113.86 ± 5.6 a	0.0199
Total residual N of post-grazing residues (kg ha^{-1})	39.77 ± 1.25 ns	40.10 ± 1.26	39.92 ± 0.72	0.5012
Total residual N during winter (kg ha^{-1})	115.42 ± 5.7 b	140.14 ± 7.0 a	153.78 ± 7.7 a	0.0120

DM: Dry matter; LW: live weight; control: oat + ryegrass; legume: oat + ryegrass + legume; supplementation: oat + ryegrass + animal supplementation. U + F: urine + feces. Means followed by the same letter are not significantly different by Tukey's test ($p < 0.05$); ns = not significant by the F-test ($p < 0.05$).

The total residual N and over 90 days from animal excretions did not differ between the legume and supplementation treatments but was 24.4% and 33.5%, respectively, higher than the control. Calculating the residual N from post-grazing by multiplying residue production by N contents revealed no significant differences. However, the combination

of residual N from animal excretion with that from post-grazing residue indicated an average of 146 kg ha^{-1} of residual N in the legume and supplementation treatments versus 115 kg ha^{-1} in the control.

3.2. Nitrogen Retained in the Beef Cattle Carcass

As shown in Table 4, the N retention in various organs was consistent across treatments, with muscles retaining the most N on average 12.1 g kg^{-1} LW. The total N content in the body of beef cattle slaughtered at 25 months showed no significant difference between treatments, averaging 14.9 kg of N per animal. The N retention over 90 days of the experiment was likewise similar among treatments, averaging 2.7 kg. N export by animals did not differ significantly across treatments, averaging 8.2 kg ha^{-1}, though the supplementation treatment showed a trend towards higher exports due to the increased SR.

Table 4. N content in various tissues per live weight in beef cattle.

Variables	Control	Legume	Supplementation
N Blood (g kg^{-1} LW)	2.31 ± 1.2 ns	0.98 ± 0.51	1.04 ± 0.54
N Muscle + diaphragm (g kg^{-1} LW)	11.91 ± 0.24 ns	12.13 ± 0.25	12.4 ± 0.25
N Carcass fat (g kg^{-1} LW)	1.04 ± 0.2 ns	0.96 ± 0.18	1.36 ± 0.26
N GIT fat (g kg^{-1} LW)	0.13 ± 0.07 ns	0.29 ± 0.16	0.11 ± 0.06
N Lung + trachea (g kg^{-1} LW)	0.16 ± 0.01 ns	0.16 ± 0.01	0.18 ± 0.01
N Tail (g kg^{-1} LW)	0.09 ± 0.01 ns	0.09 ± 0.01	0.11 ± 0.01
N GIT (g kg^{-1} LW)	1.71 ± 0.42 ns	2.82 ± 0.69	2.58 ± 0.64
N Vitals (g kg^{-1} LW)	0.69 ± 0.01 ns	0.71 ± 0.01	0.71 ± 0.01
N Bone + foot + hide (g kg^{-1} LW)	7.58 ± 0.38 ns	7.24 ± 0.36	8 ± 0.4
N Leather + ear (g kg^{-1} LW)	4.07 ± 0.21 ns	3.8 ± 0.19	4.2 ± 0.21
N Total organs (g kg^{-1} LW)	29.73 ± 0.75 ns	29.2 ± 0.74	30.69 ± 0.78
N Total organ input (kg animal^{-1})	11.83 ± 0.33 ns	12.26 ± 0.34	12.49 ± 0.34
N Total organ output (kg animal^{-1})	14.35 ± 0.58 ns	14.89 ± 0.6	15.56 ± 0.63
Average live weight output (kg)	482.8 ± 14.36 ns	510 ± 15.17	506.8 ± 15.07
N Total retained: animal output (kg)	2.52 ± 0.3 ns	2.51 ± 0.3	3.07 ± 0.36
N Animal retention in 90 days of grazing (g kg^{-1} LW)	5.22 ± 0.56 ns	4.93 ± 0.53	6.04 ± 0.64
N Exported animal LW (kg ha^{-1})	7.15 ± 1.47 ns	7.27 ± 1.5	10.13 ± 2.09

DM: Dry matter; LW: live weight; GIT: gastrointestinal tract fat; vitals: heart, liver, kidney, and spleen; control: oat + ryegrass; legume: oat + ryegrass + legume; supplementation: oat + ryegrass + animal supplementation. ns: not significant by the F-test ($p < 0.05$).

3.3. Nitrogen in Soybean Yield and Grain Off-Take

Soybean grain yield did not differ significantly among treatments, with an average of 4.15 Mg ha^{-1}. N contents in grains were similar between the control and legume treatments but were 1.4% higher with supplementation. The N off-take via soybean grains varied among treatments, with the control exporting the least (220 kg ha^{-1}) and increases of 4.5% and 9.6% for the legume and supplementation, respectively (Table 5).

Regarding N uptake by soybean grains, the supplemented exhibited the highest N uptake rates, while the control showed the lowest, as shown in Table 5. The amount of residual N in soybean straw was statistically similar across all treatments, with an average value of 79 kg ha^{-1} (Table 5). N uptake in legume and supplemented (~227 kg ha^{-1}) was 19.3% higher than in the control (191 kg ha^{-1}). In addition, in the control, soybean crops returned 39% of the residual N (75 kg ha^{-1}), post-grazing residues contributed 21% (39 kg ha^{-1}), and animal excreta accounted for 40% (16% from feces and 24% from urine).

Table 5. Grain yield, N uptake, off-take, return in straw in soybeans in an integrated crop-livestock system.

Variables	Control	Legume	Supplementation	*p*-Value
Grain yield (Mg ha^{-1})	3.97 ± 0.27 a	4.19 ± 0.28 a	4.30 ± 0.29 a	0.4134
N uptake (kg ha^{-1})	296.25 ± 6.8 c	310.46 ± 7.1 b	323.88 ± 7.4 a	0.0001
N content in grains (g kg^{-1})	5.56 ± 0.13 b	5.51 ± 0.09 b	5.62 ± 0.07 a	0.0696
N off-take in grains (kg ha^{-1})	220.77 ± 5.10 c	230.87 ± 3.59 b	242.11 ± 3.04 a	0.0001
N straw return (kg ha^{-1})	75.47 ± 4.1 ns	79.59 ± 4.3	81.77 ± 4.5	0.4510

Control: oat + ryegrass; Legume: oat + ryegrass + legume; Supplementation: oat + ryegrass + animal supplementation. Means followed by the same letter are not significantly different by Tukey's test ($p < 0.05$). ns: not significant by the F-test ($p < 0.05$).

The minimal residual N from feces and urine significantly affected this result, as animal excreta constituted over half of the N return (Figure 2A). Conversely, the legume and supplementation treatments saw an average soybean crop contribution of 35% to residual N, with 40 kg ha^{-1} from post-grazed residue and 47% from animal excreta (15% from feces and 31% from urine), indicating higher amounts of residual N returned through feces and urine compared to the control. The total N export in the system was calculated by summing the N off-take by grains and the N exported by animals. The treatments had no significant difference, with an average N export of 239 kg ha^{-1} (Figure 2B). However, animal excreta had the most influence on N cycling in the system, exhibiting the highest values of recycled N in the legume and supplementation treatments (Figure 2B). It was observed that the N measured in the production system, which includes both livestock and crop phases, reached up to 487 kg ha^{-1} (Figure 2B).

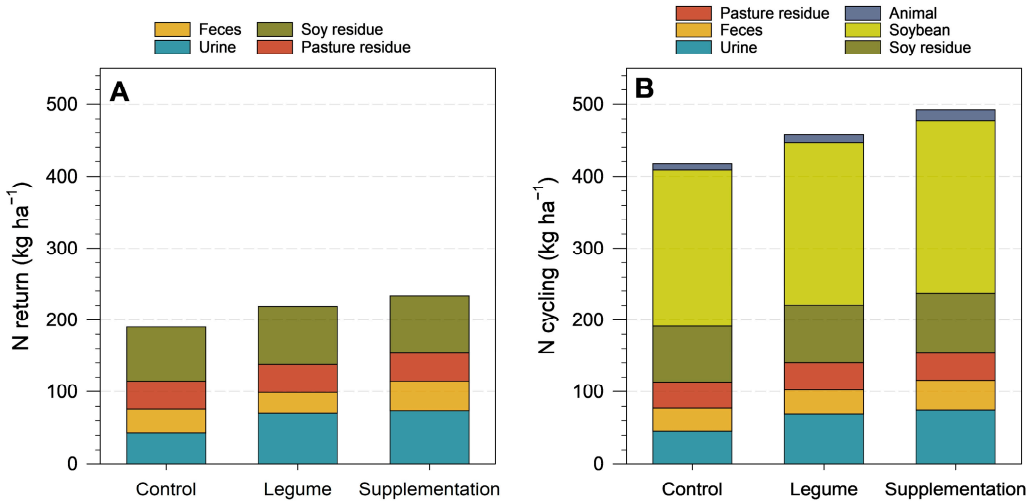

Figure 2. Total N return (**A**) and cycling (**B**) in an integrated crop-livestock system. Control: oats + ryegrass; Legume = oats + ryegrass + legume; Supplementation = oats + ryegrass + animal supplement.

3.4. Release of N by Post-Grazing Residue and Animal Feces

Nitrogen release from post-grazing residue was similar across treatments until day 22. From day 90 onwards, the legume treatment showed higher cumulative N release rates, followed by the control and, lastly, the supplementation, which had the lowest release rate (Figure 3A). By day 160, the legume treatment had released 38 kg ha^{-1} of N, the control 35 kg ha^{-1}, and the supplementation 31 kg ha^{-1}. A similar pattern was observed for N release from feces, with significant differences emerging after day 90 (Figure 3B). Contrary to residue release patterns, the supplementation treatment exhibited the highest

fecal N release rate, peaking at 22 kg ha^{-1} by day 160, followed by the legume treatment at 16 kg ha^{-1} and the control at 12 kg ha^{-1}. The decomposition rate of both materials decreased over time until reaching a stable state.

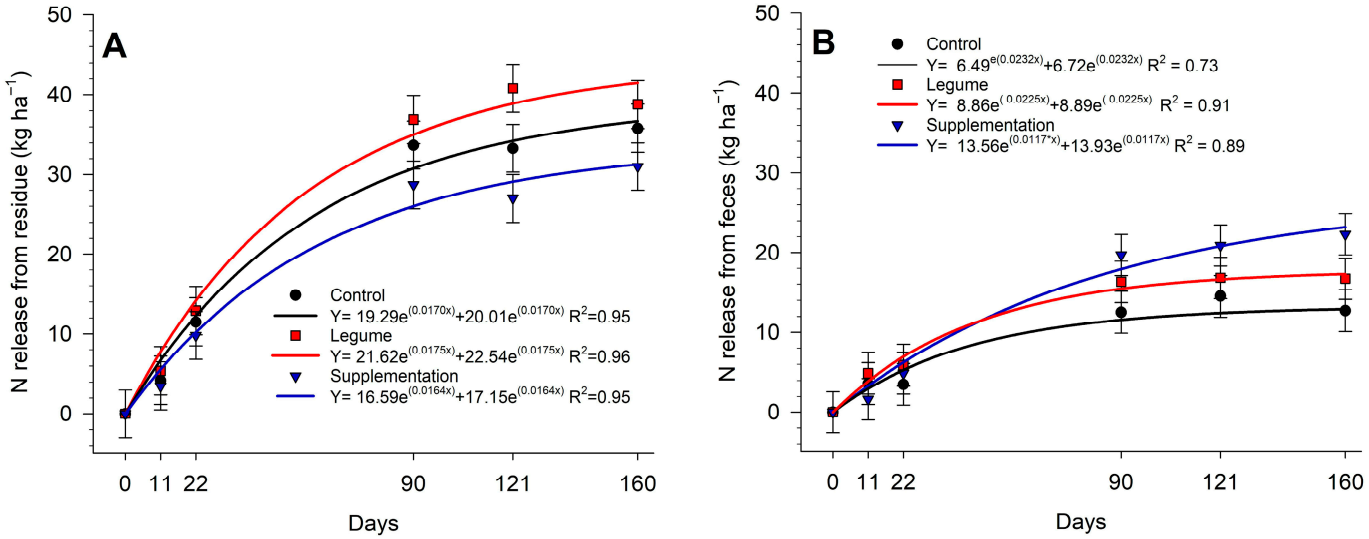

Figure 3. N release from post-grazing residue (**A**) and feces (**B**) in a 160 days evaluation after distributing the litter bags in the area on the soil surface during soybean cultivation. Control: oats + ryegrass; Legume = oats + ryegrass + legume; Supplementation = oats + ryegrass + animal supplement. N release is modeled by the equation: $y = ae^{(bx)} + ce^{(dx)}$ where: y = remaining material (%); a = initial fraction of easily decomposable material; b = decomposition rate of fraction a; c = initial fraction of more resistant material; d = decomposition rate of fraction c; e = exponential function; x = time (days).

4. Discussion

4.1. Nitrogen in the Livestock Phase

The 21% increase in stocking rate (SR) observed in the supplementation treatment was consistent with expectations and aligned with previous studies [28,29]. This increase is attributed to the substitution effect, where animals reduce forage intake when supplemented, increasing the pasture's carrying capacity without compromising animal performance.

As anticipated, supplemented animals exhibited lower N intake from forage, reflecting the replacement of grazed biomass by the supplement. In contrast, animals in the legume treatment consumed 19.4% more N than those grazing exclusively on oat and ryegrass, despite similar total DM and N contents. This increase likely reflects a behavioral preference for legumes during spring, which are more palatable, less structurally resistant, and more digestible than grass stems and sheaths [30]. Such selective grazing patterns may be strategic for enhancing voluntary intake and nutrient use efficiency. Total N intake was also substantially higher in the supplementation (47.8%) and legume (28.2%) treatments compared to the control, the former due mainly to increased SR. These findings confirm that animal diet composition and stocking rate are major drivers of N intake and excretion.

Higher daily N intake led to increased urinary N content, especially in the supplementation and legume treatments. This aligns with previous findings that excess dietary N, particularly from crude protein-rich sources, is excreted primarily via urine [31]. The high degradability and digestibility of N in legumes, along with the 31.6 g kg^{-1} DM N content in the supplement, contributed to this elevated urinary N excretion [32]. Importantly, the grass-legume mixture enhances selective grazing and extends forage availability without requiring synthetic N inputs, promoting a more balanced forage distribution

across seasons [33]. This positions the grazing animal as a biological vector of N cycling, redistributing biologically fixed N through its excreta.

Although fecal N concentrations were similar among treatments (34 g kg^{-1} DM, above the 24 g kg^{-1} DM reported by Bellows [34]), residual N returned via feces was higher in the supplementation treatment. This suggests that stocking rate exerts greater influence on N deposition via feces than diet composition alone. While fecal N mineralizes more slowly than urinary N, its effects on soil fertility persist for longer periods—up to two years [35,36]. Thus, management strategies that increase grazing intensity may enhance long-term nutrient recycling, supporting subsequent crop yield.

Over the 90-day grazing period, the combined N returned via feces and urine was higher in both the legume and supplementation treatments, confirming the role of diet quality and stocking rate in determining excretory N flows. As noted by Menezes et al. [37], crude protein content in the diet directly influences both fecal and urinary N excretion. Ultimately, both legumes and supplementation act as significant N inputs into the system. While supplementation provides a direct nutritional boost, legumes contribute indirectly through biological N fixation, offering a sustainable pathway to reduce reliance on inorganic fertilizers. Diversified practices such as these enhance N-use efficiency, system resilience, and environmental sustainability [38,39].

4.2. Nitrogen Retained in the Beef Cattle Carcass

At the start of the experiment, all animals had similar levels of N retention in their bodies, averaging 12 kg N $animal^{-1}$. By the end of the experiment, post-slaughter measurements showed no significant difference in N levels, with an average of 14 kg N $animal^{-1}$. This indicates a 3–4% export of N by the animals. Consequently, the study reveals that the proportion of N ingested from pasture and retained in the animal's body is lower than the previously estimated 10% [3].

The amount of N in animal organs may vary between treatments if there were weight differences in each organ. However, this did not occur in the present study. During the fattening/finishing phase, mature animals already possess well-defined organs and tend to deposit more fat relative to muscle. Macitelli [40] observed that providing different protein diets to beef cattle did not influence the weights and biometric measurements of internal organs.

Muscle tissue retained the highest amount of N, averaging 9.8% N, which is lower than the 13.2% N (equating to 82.4% crude protein) observed by Rodrigues and Andrade [41] in Nellore cattle. This discrepancy highlights breed-specific differences in carcass N content. N levels in organs and blood were consistent with previous studies [42–44], although these components account for a minor portion of carcass weight and thus contribute less to overall N retention.

Understanding N extraction and retention in animals enhances our knowledge of nutrient cycling. The study underscores that a small proportion of nutrients ingested from the pasture are retained in the animal's body, with the majority returned to the soil as excreta [45]. In ICLS, nutrient off-take varies between grain yield, which has high nutrient off-take, and meat production, which exhibits low nutrient off-take [46].

4.3. Nitrogen in Soybean Yield and Grain Off-Take

Increasing N contents in grains by 1.4% through supplementation suggests animal supplementation enhances nutrient cycling and the nutritional value of grains without affecting soybean yield negatively. This approach allows for a higher N off-take in grains and supplies additional nutrients like P and K.

Similar to supplementation, the introduction of legumes also resulted in an increased N off-take in grains (9.6% and 4.5%, respectively, compared to the control). Due to their protein richness and ability to fix atmospheric N, legumes could potentially reduce the need for high-protein feedstuffs in animal diets and lessen the requirement for N fertilization in subsequent crops [47]. Soybeans can form symbiotic relationships with bacteria to assimilate atmospheric N. This N-cycling is beneficial in ICLS, where studies have shown that soybean yields increase, utilizing three times less fertilizer than traditional cultivation methods [38]. This indicates that supplementation at high N off-take for the grains in the supplementation treatment can be explained by the high total yield in the plot^{-1} where there were supplemented animals, even though they did not differ statistically.

The greater residual N through SR does not detriment grain yield in the present study. The study also found that N from the feces of supplemented animals significantly contributed to increased N off-take by soybeans in ICLS. Since inorganic N release from feces is slow, it has a low volatilization loss, making it better utilized by subsequent crops than N from urine [35,48].

Our findings highlight that residual N in winter, through animal diet and excretion, significantly impacts N cycling across a complete production season. This underlines the importance of optimizing winter areas with animal production to boost nutrient supply. The study observed that soybean plants contribute significantly to N recycling by returning N to the system through animal and crop (straw) residues. This characteristic, along with soybeans' ability to off-take high amounts of N, enhances integration within crop-pasture rotations.

Such rotations are identified as effective strategies for reducing reliance on intensive input use, thereby promoting sustainability over time [49,50]. When examining the N balance within the system, it was noted that residual N from animals in the supplementation and legume treatments surpassed the N applied via mineral fertilizers (Figure 4). This suggests that N atoms are utilized multiple times within the system, improving nutrient efficiency in ICLS. The interaction of N also highlights the reduced necessity for additional N, especially when grass is used after grazing, for example, in maize cultivation. Additionally, soybean crops play a crucial role by making N available for subsequent oat and ryegrass cycles.

Analysis of the production system, encompassing both livestock and crop phases, revealed N levels up to 487 kg ha^{-1}. With inputs from fertilizers at 90 kg ha^{-1} and supplements up to 47 kg ha^{-1}, it is evident that a significant portion of N undergoes recycling, predominantly through biological fixation by legumes, particularly soybeans. This underscores the importance of focusing on production systems that prioritize biological N fixation, recycling, and nutrient balance. In contrast, 3 to 4% of the total exports were through animals, and 96–97% were through soybean grains (Figure 4). While N is highly dynamic within the soil-plant-animal-atmosphere system, it is possible to identify recycling patterns applicable in production environments.

The amount of N cycled in the system was similar to that exported through grains (Figure 4). However, what was recycled allowed high animal gains. Scheeren et al. [9] evaluated animal production in the present study and found gains of 97 kg ha^{-1} of LW more in the supplemented animals, when compared to the control, and 29 kg ha^{-1} of LW more in the legume treatment compared to the control. Therefore, these gains happened at a much lower cost of N. The soybean crop could also use the recycled N, even though it is a legume.

The sustainability of livestock farming systems differs with different degrees of intensification [51]. Adapting to adverse climate changes poses a global challenge, requiring sustainable practices that enhance animal production [52]. Hence, the importance of plan-

ning pasture management and exploring how different management practices influence an integrated production system is underscored [2].

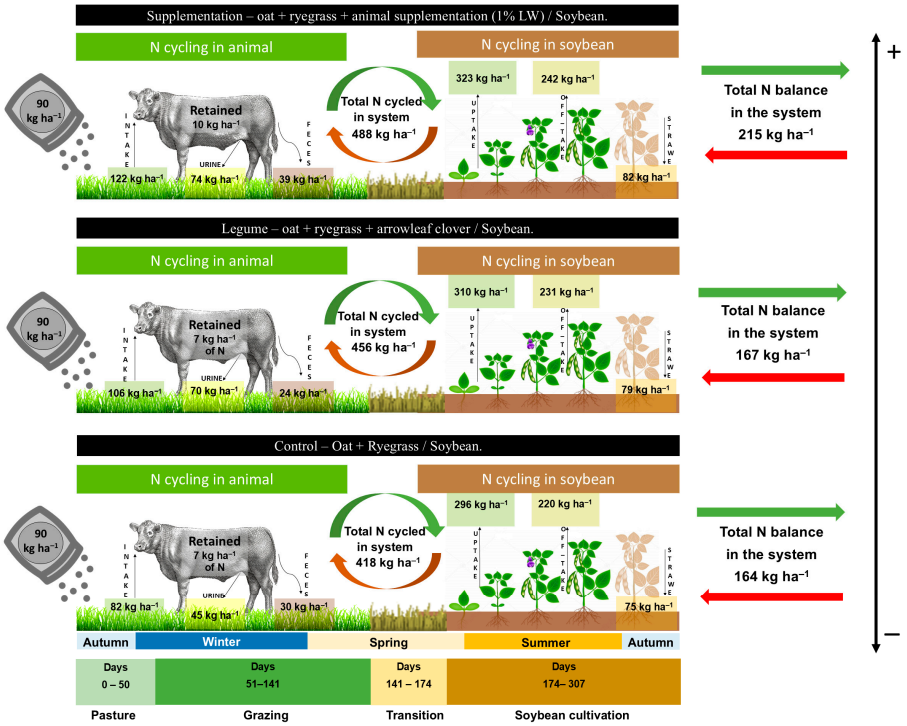

Figure 4. Schematic representation of N balance in control, legume, and supplementation. The figure illustrates the N flow, including the amount consumed by animals, export in the carcass, and residual N through feces and urine. For soybeans, it shows the off-take, uptake, and residual N via soybean residue.

4.4. Release of N by Post-Grazing Residue and Animal Excreta

Understanding the rate of nutrient release from residues is crucial to synchronize their availability with the needs of the succeeding crop [53]. However, supplementing animals can alter the chemical composition of these materials, and there is limited information on the dynamics of decomposition and N release from excreta [54].

In this study, an initial phase of N immobilization was observed for up to 90 days, which then tended to stabilize. This pattern is typical of residues with higher structural C and moderate C:N, in which microorganisms first immobilize N to decompose the material and only later, as the labile fraction is exhausted, net mineralization occurs. The rate of nutrient release is affected by various factors, including the activity of decomposers, the chemical characteristics of the material, management practices, and edaphoclimatic conditions (temperature, humidity, soil pH, and nutrients) [55]. Legume-derived residues and feces usually have narrower C:N and slightly higher pH, which favor faster N mineralization compared with grass-only residues.

The lower N release from supplementation may be attributed to the lower leaf:stem ratio, as indicated in Table 1. This resulted in a higher proportion of stems due to a potential preference by animals receiving supplementation. During selective grazing, animals prefer the greener, more nutritious parts of forage [56].

Supplementation leads to animals replacing pasture with supplements, thereby becoming more selective during grazing. Over time, this decreases the leaf:stem ratio and reduces the quality of the available forage. Lang et al. [57] observed a low mineralization rate of oat and ryegrass organic matter with high stem:leaf ratios. This phenomenon occurs because stems contain more lignin than leaves, making them harder to decompose [58].

However, faster decomposition of crop residues releases more nutrients. Decomposition is slower when lignin content and the C:N ratio are high [59]. Heinrichs et al. [60] suggested that combining grasses and legumes can lower the C:N ratio of crop residues. In our study, the legume treatment showed increased N accumulation both due to the N introduced with arrowleaf clover seed and to the higher N release from residues after 90 days, improving overall N cycling.

Among the treatments, supplementation resulted in the highest N release from fecal residues. This increase can be attributed to the dietary shift from pasture to supplement and the selective grazing by animals, which enhances fecal N release. Additionally, concentrated supplements in cattle diets improve total digestibility, making N in feces more readily available [61]. Fecal N release, besides being influenced by physical factors, relates to the nutrient content of the feces [3]. Thus, feces from animals fed with legumes showed higher N release than the control due to improved dietary N intake.

When comparing N release from post-grazing residues and fecal matter, plant residues released greater amounts of N, indicating that nutrients in feces contribute more gradually to soil fertility over time. This is explained by the low digestibility of fecal components rich in cellulose, hemicellulose, and lignin, which decompose more slowly [54]. Notably, the timing of N release from both sources aligns with key developmental stages of the soybean crop. Oliveira-Junior et al. [62] observed that soybean N uptake intensifies around 80 days, coinciding with the onset of grain filling (R5.1), and peaks by stage R5.5. In our case, the higher N release observed in the legume and supplementation treatments is consistent with this window of crop demand, but we did not perform time-aligned measurements (residue $\times$ plant) to demonstrate a strict synchronization; therefore, this interpretation should be viewed as mechanistic rather than conclusive. According to Hungria et al. [63], up to 84% of the plant's N requirement is directed to grain production. These patterns support the interpretation that the elevated N release observed in the legume and supplementation treatments contributed meaningfully to grain N accumulation around day 90 (Table 5).

While these findings offer valuable insight into the timing and contribution of organic N sources to crop nutrition, some limitations should be acknowledged. First, the study did not quantify total N losses to the environment, including leaching, runoff, denitrification, and volatilization. However, it is noteworthy that this work uniquely assessed N exported via animal carcass, a component often overlooked in integrated system research. Second, the experiment was limited to one winter and one summer season (May 2020 to March 2021), and the performance of grazing systems—particularly with legumes—can vary widely under different climatic conditions.

Therefore, we encourage future studies to adopt more comprehensive approaches, such as long-term monitoring and the use of stable isotope techniques like ^{15}N labeling, to precisely trace N flows through plants, animals, soil, and losses to the environment. Future studies using time-resolved sampling or ^{15}N tracing are needed to confirm whether residue and fecal N are effectively synchronized with soybean uptake. Despite its constraints, this study provides a meaningful baseline for future research on N cycling in ICLS.

5. Conclusions

Integrating animal supplementation and forage legumes into crop-livestock systems is an effective strategy to enhance N balance and use efficiency without compromising crop yield. The inclusion of these components increased stocking rates and led to greater N deposition via animal excreta—particularly feces and urine—thereby enriching the system with N biologically recycled. Importantly, these gains occurred without any reduction in soybean yield and were accompanied by an approximate 9% increase in N off-take via grain harvest.

The results also confirm that N off-take in the system is predominantly driven by the soybean crop (96–97%), while animals account for a much smaller share (3–4%) through carcass retention. Treatments had no significant effect on N export via cattle but played a critical role in determining N return to the soil through excretions, especially under higher stocking densities and diets enriched with legumes or supplements.

Collectively, these findings highlight the potential of integrated practices to improve the internal N economy of agricultural systems. By promoting biologically fixed or recycled N flows, supplementation and legume-based strategies enhance nitrogen-use efficiency, which could, over time, reduce dependence on synthetic N inputs. Future research incorporating long-term monitoring and ^{15}N isotopic labeling is encouraged to further elucidate N pathways and optimize nutrient management in integrated crop-livestock systems.

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Institutional Review Board Statement: This study was approved by the research ethics committee of the Federal Technological University of Paraná, Dois Vizinhos campus (protocol no. 2020-11).

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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