

Can silvopastoral systems with legume trees affect the spatial distribution of cattle dung in grazing lands?

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ABSTRACT - Animal excreta represent one of the primary pathways for nutrient return to pastures. We investigated the spatial distribution pattern of dung pads during seven grazing cycles over two years and the nutrient concentration in forage and cattle dung in different systems in a subhumid tropical region. The treatments were: (I) brachiaria grass (*Brachiaria decumbens* syn. *Urochloa decumbens* Stapf R. D. Webster) + gliricidia (*Gliricidia sepium* (Jacq.) Kunth ex. Walp.; B+G); (II) brachiaria grass + sabia (*Mimosa caesalpinifolia* Benth.; B+S); (III) brachiaria grass + N fertilization (B+N); and (IV) brachiaria grass without fertilization (B), allocated in a randomized complete block design with four replications. The spatial distribution of cattle dung was assessed using geostatistical approaches. The N concentration in the forage grass was significantly greater ($P<0.05$) in B+S (31 g kg^{-1}) compared with B (19.3 g kg^{-1}) during the first evaluation (January), with no statistical difference among the other treatments or evaluations ($P>0.05$). Within treatments, N concentration was higher in B+G and B+S in January than in the other evaluations ($P<0.05$). The nutrient concentrations in cattle dung were similar across all systems ($P>0.05$), varying only among evaluations ($P<0.05$). Similarly, there were no differences in the spatial distribution of cattle dung among systems ($P>0.05$), which followed an aggregated distribution pattern throughout the study. The inclusion of leguminous trees did not affect nutrient excretion or the spatial distribution of cattle dung within the tree arrangement and conditions evaluated. These findings suggest that effective nutrient redistribution depends on tree development and appropriate management scale, supporting improved nutrient cycling in grazing systems.

Keywords: cattle excreta, *Gliricidia sepium*, *Mimosa caesalpinifolia*, nutrient cycling, silvopastoral systems

1. Introduction

Animal excreta represent one of the primary pathways for nutrient replenishment in grazing lands. Excreta deposition positively contributes to nutrient cycling (Sollenberger et al., 2019) and can increase soil carbon (C), nitrogen (N), and phosphorus (P) stocks (Koch et al., 2018; Rayne and Aula,

2020; Fan et al., 2021; Brummerloh and Kuka, 2023), improving soil aggregation and benefiting the soil microbial community (Dubeux Jr. and Sollenberger, 2020; Li et al., 2021; Segura et al., 2023). These factors are crucial for the sustainability of grassland ecosystems.

Despite the high relevance of cattle manure to nutrient cycling process in grassland ecosystems, its spatial distribution is typically heterogeneous, with most dung pads often found in the hotspots of animal lounging, such as water troughs, gates, and shaded areas (Dubeux Jr. et al., 2006; Dubeux Jr., 2007; Carpinelli et al., 2020; Bretas et al., 2023). The uneven distribution of excreta creates heterogeneous nutrient availability in the soil, influencing forage biomass distribution (Dubeux Jr. et al., 2014; Draganova et al., 2016; Silva et al., 2020). Moreover, this can lead to the translocation of nutrients to areas of little or no importance for animal production since these lounging areas are typically not grazed. Furthermore, spatially concentrated cattle excreta can increase nitrogen losses through nitrate leaching, ammonia volatilization, and nitrous oxide emissions, leading to negative environmental impacts (Dubeux Jr. et al., 2007; Saggar et al., 2022). However, uniform excreta distribution in pastures may also occur in some systems, evenly returning nutrients to plants and thereby reducing the need for fertilizer application (Apolinário et al., 2015; Cubillos et al., 2016; Lira Junior et al., 2020).

The volume, frequency, and distribution of cattle dung deposition in grazing lands can be influenced by several factors, such as stocking rate, grazing management, animal behavior, animal category (e.g., age, sex, breed, etc.), climatic and environmental conditions, and pasture characteristics such as the positioning of water troughs and shade (Carpinelli et al., 2020; Silva et al., 2020; Hassan-Vásquez et al., 2022). Regarding climatic conditions, seasonal variation plays a central role in grazing ecosystems by modifying both animal behavior and forage characteristics. Seasonal changes in temperature, rainfall, and solar radiation affect forage growth dynamics, biomass accumulation, and plant nutrient concentrations, particularly crude protein, fiber fractions, and mineral availability. These shifts might influence forage selection, grazing patterns, intake behavior, and animal movement across paddocks, thereby altering dung deposition patterns.

Another factor that may affect dung distribution is the presence of trees in silvopastoral systems. This type of system has been increasingly adopted in livestock systems because it provides a range of ecosystem services (Carpinelli et al., 2020). The presence of trees in pastures alters the microclimate by reducing air and soil temperatures, improving thermal comfort, and modifying radiation and moisture dynamics, which can influence animal behavior and grazing patterns, affecting animal spatial distribution (Karvatte et al., 2016), pasture structure (Rodrigues et al., 2016), and forage nutritive value (Santos et al., 2018). Trees can also enhance nutrient cycling by redistributing nutrients from deeper soil layers through litter deposition and root turnover, while also improving soil fertility and biological activity (Carvalho et al., 2024). When legume trees are included, biological N fixation and the production of nutrient-rich litter may increase nutrient availability and improve forage nutritive value, potentially influencing animal intake and the nutrient composition of excreta returned to the soil (Apolinário et al., 2016). Legume trees often alter litter quality and dung characteristics by reducing C:N, C:P, lignin:N, and lignin:P ratios (Dubeux Jr et al., 2014; Lima et al., 2016), thereby accelerating residue decomposition and enhancing nutrient cycling (Pessoa et al., 2024). Additionally, the presence of distributed shade may modify animal movement patterns, potentially affecting the spatial redistribution of nutrients through dung and urine deposition. Despite these potential benefits, the effects of trees on cattle dung spatial distribution remain inconsistent across silvopastoral systems, likely reflecting differences in tree density, system design, and grazing management reported in the literature.

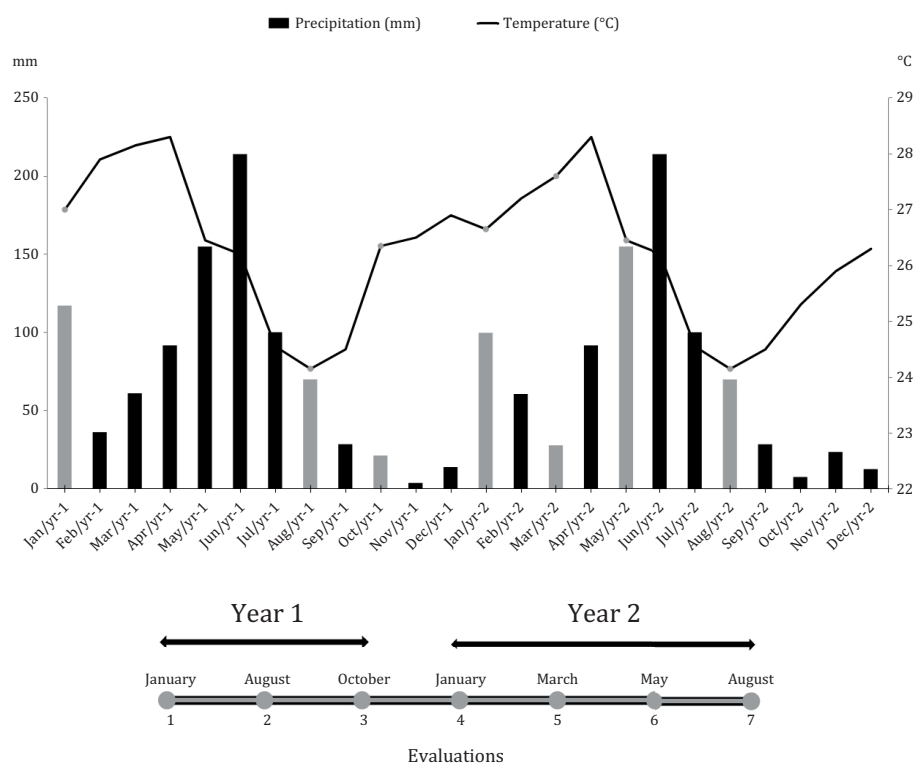
We hypothesized that silvopastoral systems with a high tree density would promote a more uniform dung distribution compared with conventional monoculture pastures, as the spatially distributed tree shade reduces animal aggregation in specific resting areas. Conversely, we believe uneven dung distribution will likely occur in monoculture systems. In many conventional grazing systems, shade is restricted to localized areas such as isolated trees or artificial shade structures, which can promote animal aggregation and concentrated dung deposition. In addition, we expected that silvopastoral systems, including legume trees, may increase the nutrient concentrations of dung pads deposited in the soil. Furthermore, we hypothesized that seasonal environmental variation would affect forage

nutrient concentrations and animal behavior, thereby modifying both the spatial distribution and nutrient composition of dung deposition across grazing periods. Thus, the objective of this study was to evaluate the spatial distribution pattern of cattle dung and the nutrient composition of forage and cattle dung in brachiaria grass (*Brachiaria decumbens* syn. *Urochloa decumbens* Stapf R. D. Webster) pastures under monoculture with and without nitrogen fertilization, or in a silvopastoral system with legume trees in a subhumid tropical region.

2. Material and methods

2.1. Site description and experimental design

The study was conducted over 2 years at the Itambé Experimental Station (07°25'S, 35°06'W, and ~180 m a.s.l.), located at the Instituto Agrônômico de Pernambuco (IPA), Pernambuco, Brazil. The average annual precipitation is 1200 mm, and the average yearly temperature is 25 °C (RIMA, 2014). According to the Köppen climate classification, the climate is hot and humid tropical. Figure 1 depicts the precipitation and temperature during the experimental years and evaluation periods.



Grey bars and dots highlight periods with field evaluations over the two study years.

Figure 1 - Precipitation and temperature (monthly averages) during the experimental years.

The soil in the experimental area is classified as a Red-Yellow Dystrophic Argisol according to the Brazilian Soil Classification System (SiBCS), corresponding to an Ultisol in the USDA Soil Taxonomy and an Acrisol in the World Reference Base for Soil Resources (WRB). The initial chemical analysis of the soil in the 0- to 10-cm layer revealed: pH (water 1:2.5) = 5.4; plant-available phosphorus (P; Mehlich-1) = 3.4 mg dm⁻³; potassium (K⁺) = 0.9 mmolc dm⁻³; calcium (Ca²⁺) = 26.8 mmolc dm⁻³; magnesium (Mg²⁺) = 16.3 mmolc dm⁻³; exchangeable aluminum (Al³⁺) = 2.8 mmolc dm⁻³, and potential acidity (H+Al³⁺) = 61.8 mmolc dm⁻³, following standard soil fertility assessment procedures.

The treatments evaluated were: (I) brachiaria grass + gliricidia (*Gliricidia sepium* (Jacq.) Kunth ex. Walp.) in a silvopastoral system (B+G); (II) brachiaria grass + sabia (*Mimosa caesalpinifolia* Benth.) in a silvopastoral system (B+S); (III) brachiaria grass monoculture + N fertilization ($60 \text{ kg N ha}^{-1} \text{ year}^{-1}$; B+N); and (IV) brachiaria grass monoculture without fertilization (B). The treatments were allocated in a randomized complete block design with four replications, totaling 16 experimental units (plots). Each plot measured 660 m^2 ($33 \text{ m} \times 20 \text{ m}$) and contained a water trough and mineral supplementation. Silvopastoral systems (B+G and B+S) provided natural shading distributed evenly across the plot, whereas monoculture plots relied solely on artificial shade installed at fixed spots.

The brachiaria grass was established in 1980 over the experimental area. In the silvopastoral systems (B+G and B+S), the legume trees were planted in double rows with a spacing of $10 \text{ m} \times 1.0 \text{ m} \times 0.5 \text{ m}$ and a total population of approximately $2500 \text{ trees ha}^{-1}$ in a north-south direction two years before the field trial. The adopted tree density characterizes a relatively high density, which differs substantially from low-density temperate systems, such as dehesa-type systems, where isolated trees often promote animal aggregation beneath canopies. However, the silvopastoral component consisted of young trees in active growth, with incomplete canopy development at the time of data collection. Tree height and canopy architecture produced partial shading, resulting in higher incident radiation than typically observed in mature silvopastoral systems. Thus, the experimental conditions represent an early-stage tree–pasture interaction, which may influence the magnitude of shade-driven plant responses. In the B+N treatment, the N application was split into two doses using urea, with the first (30 kg N ha^{-1}) applied in March of both study years and the second dose (30 kg N ha^{-1}) applied 56 days after the first application, totaling $60 \text{ kg N ha}^{-1} \text{ year}^{-1}$. Crossbred Holstein $\times$ Zebu heifers with an average body weight of $385 \pm 53.5 \text{ kg}$ were used as grazing animals. Seven grazing cycles of 35 days each (three days of grazing and 32 days of regrowth) were conducted with a fixed stocking rate. Stocking rate was adjusted to 0.9 animal units (AU) per experimental plot, considering $1 \text{ AU} = 450 \text{ kg}$ of live weight.

2.2. Forage and dung analysis

Forage samples were collected using the hand-plucking method (Cook, 1964) in four grazing cycles (January, March, May, and August) of the second experimental year to analyze the chemical composition and characterize the animal diet and nutrient cycling via residues (litter and excreta) among systems. In the silvopastoral systems, grass and legume samples were collected separately to compare the chemical composition of both legume species. Leaves and twigs up to 1.5 m were collected from the legume trees. A composite grass sample was taken from each plot, comprising 10 single samples. Only the brachiaria grass composition was compared across systems, as we did not evaluate the proportions of grass and legumes in the diet. After collection, the samples were dried in an oven at 55°C until constant weight to estimate dry matter concentration before processing for chemical composition analysis.

Similarly, dung samplings were conducted throughout the same grazing cycles (January, March, May, and August) of year 2 to analyze the chemical composition. Sampling was conducted on the second day of grazing, with three dung pads collected per plot, using only the superficial portion of the fecal bolus to avoid soil contamination. Additionally, measurements of the major (D) and minor (d) diameters of the dung pads were taken and used for calculating the dung pad area ($A = (Dd \cdot \pi)/2$), assuming an elliptical shape for the dung pads. After collection, the dung was weighed and placed in an oven at 55°C until constant weight to estimate the dry matter concentration, then processed for chemical composition analysis.

After drying, forage and dung samples were ground to a 1-mm sieve to determine phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) concentrations using the methods described by AOAC (2007). The C and N concentrations were analyzed by the Dumas dry combustion method using a CHNS analyzer (Vario Micro Cube; Elementar, Hanau, Germany) coupled with an isotopic ratio mass spectrometer (IsoPrime 100; IsoPrime, Manchester, UK).

Forage and dung samples were analyzed only during the evaluated grazing cycles of the second experimental year, as the dung spatial distribution was the main focus of the study.

2.3. Spatial distribution of cattle dung

The spatial distribution of dung pads was analyzed in seven grazing cycles over two years using the methodology proposed by Dubeux Jr. et al. (2014). The evaluated grazing cycles were selected to capture rainfall and temperature fluctuations over the years. This approach was adopted because the evaluations were laborious and time-consuming, making it unfeasible to assess the distribution across all grazing cycles. Dung pads existing from the previous grazing cycle were marked with spray paint. The evaluations were conducted 48 hours after the animals had been in the experimental plots, using three 50-m measuring tapes. Two tapes were fixed at the shorter ends of the plot, and the third was stretched horizontally by two observers who recorded the locations of unmarked dung pads, yielding “x” (moving tape) and “y” (fixed tape) coordinates.

The spatial analysis of dung pad distribution in the plots was performed by dividing each plot into 100 quadrants of 6.6 m² using Microsoft Excel. Four individual coordinates were allocated per quadrant, and the number of dung pads per area unit (m²) was determined to calculate the semivariance $\gamma(h)$ and the semivariogram. The semivariogram is defined by the nugget effect (C_0), which is the intercept of the semivariogram with the y-axis and indicates how much variability is present at very short distances; range ($C_0 + C$), which is the distance over which the data points have a significant spatial correlation; and sill (S), which represents the total variance of the data and is essentially the plateau value of the semivariogram after the range. The Gaussian, spherical, and exponential models were evaluated to select the best fit for the experimental data. The symmetry and normality of the spatial distribution data were verified using the skewness (Cs) and kurtosis (Ck) coefficients. The more the skewness coefficient approaches zero, the more symmetrical the distribution is, while a kurtosis coefficient close to zero indicates normality.

The spatial dependence pattern was evaluated through geostatistical analysis, where $\gamma(h)$ was calculated using the GS+ software® version 7.0 (Gamma Design Software, Plainwell, MI, USA; Robertson, 2008) according to Vieira et al. (1983) (Equation 1):

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [Z(xi) - Z(xi + h)]^2 \quad (1)$$

in which $\gamma(h)$ is the semivariance, $N(h)$ is the number of pairs of measured values, and Z represents the number of dung pads/m² at each coordinate of the study site (xi), separated by a distance vector (h). $Z(xi)$ and $Z(xi + h)$ were defined based on the geographic locations of the field samples. The spatial dependence index (SDI) was then calculated by Equation 2 (Cambardella et al., 1994) to assess the degree of spatial dependence.

$$SDI = [C_0 / (C_0 + C)] \times 100 \quad (2)$$

This classification categorizes attributes based on the relationship between the (C_0) and $C_0 + C$ of their adjusted semivariogram, identifying whether the spatial dependence is strong, moderate, or weak. Spatial dependence indices below 25% indicate strong spatial dependence, values between 25% and 75% indicate moderate spatial dependence, and values above 75% indicate weak spatial dependence (Carpinelli et al., 2020; Silva et al., 2020). Based on the models derived from the semivariograms, estimates of dung pad concentration were extrapolated to unsampled locations using the Kriging interpolation method. Contour maps were constructed with the estimated values using Surfer 8.0 software® (Golden Software, 2002) to represent the spatial variability of dung deposition in the systems.

From the records of fecal distribution in each quadrant, the mean and variance were calculated following the 48-hour stocking period. These calculations enabled the determination of the dung dispersion index (DI). To statistically test the DI, the chi-square test (χ^2) was utilized following the equation described by Ferreira et al. (2004) (Equation 3):

$$\chi^2_{obs} = DI \times (n - 1) \quad (3)$$

in which χ^2_{obs} represents the observed chi-square value, DI denotes the dispersion index, and n represents the number of quadrants counted (100). Dispersion indices evaluate whether observed frequencies can be adequately fitted to Poisson or negative binomial models using a two-sided chi-square test. Additionally, the exponent K of the negative binomial distribution and the Standardized Morisita Index (SMI) proposed by Smith-Gill (1975) and adapted by Krebs (1999) were calculated to characterize the spatial distribution of the dung pads. The SMI was calculated to appropriately characterize the distribution model since it is independent of density and sample size (Malhado and Petrere Jr., 2004). The SMI, ranging from -1.0 to $+1.0$, with a 95% confidence interval at $+0.5$ and -0.5 , was obtained through the Morisita Dispersion Index (MDI; Morisita, 1972; Equation 4), with the adjustments described by Krebs (1999).

$$MDI = q \Sigma \frac{n_i(n_i - 1)}{N(N - 1)} \quad (4)$$

in which q is the number of subquadrats, n_i is the number of dung pads in the ' i 'th subquadrat, and N is the total number of dung pads in all subquadrats. The random distribution model is characterized by $SMI = 0$, the aggregated model by $SMI > 0$, and the uniform model by $SMI < 0$ (Krebs, 1999). The random, aggregated, and uniform spatial distribution models used to characterize the spatial distribution of cattle dung, were adapted from Krebs (1999; Figure 2).

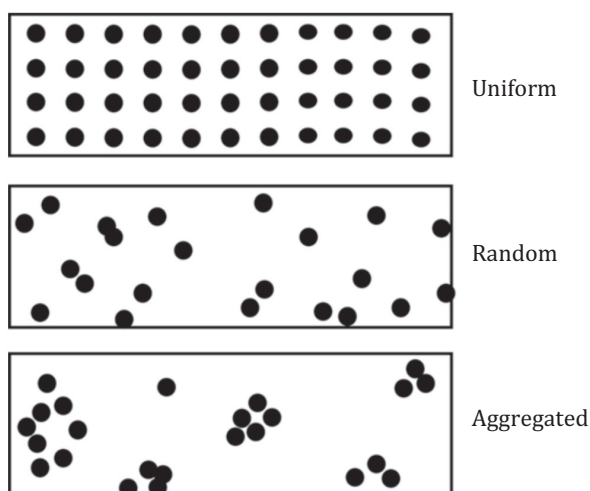


Figure 2 - Representation of uniform, random, and aggregated spatial distribution models. Adapted from Krebs (1999).

The density of dung pads deposited in the pasture (DD) was calculated using the equation described by Silva et al. (2020) (Equation 5).

$$DD = \frac{N \times a}{A} \quad (5)$$

in which DD is the density of dung pads in the pasture, N is the total number of dung pads at the end of the stocking period, a is the average area of each dung pad, and A is the total area of the pasture.

The DD was then used to calculate the pasture area covered by dung pads (PC) according to equation 6.

$$PC = 100 \times DD \quad (6)$$

in which PC is the percentage of the pasture area covered by dung and DD is the dung density (pads m^{-2}).

2.4. Statistical analysis

The chemical composition of the forage and dung, along with the spatial distribution parameters for each treatment, were analyzed as repeated measures over time. Treatments were considered fixed effects, blocks as random effects, and evaluations as repeated measures. Treatment means were compared by the Tukey test at a significance level of 5% using the MIXED procedure of SAS On Demand® for the statistical analysis.

The statistical model was:

$$y_{ijk} = \mu + \alpha_i + b_j + \tau_k + e_{ijk}$$
(7)

in which y_{ijk} is the response variable measured in the j -th block (b_j) that received the i -th treatment (α_i) at the k -th evaluation (τ_k); μ is the general constant; α_i is the fixed effect of treatment; b_j is the random effect of block; τ_k is the repeated effect of evaluation; and e_{ijk} is the random error term.

3. Results

3.1. Forage nutrients

The average N concentration in the forage samples from leguminous trees across the evaluations was greater ($P<0.05$) in gliricidia (~41 g kg⁻¹) than in sabia (~31 g kg⁻¹; Table 1). Consequently, gliricidia had a lower C:N ratio (10.6) than sabia (14.7; Table 1). The leguminous N concentration was greater in the third evaluation (May) compared with the other evaluations ($P<0.05$), resulting in a lower C:N in that evaluation compared with the first, with no differences among the others (Table 2).

Regarding the grass component of the systems (brachiaria grass), there was an interaction treatment × evaluation for N concentration and C:N ratio ($P<0.05$). The N concentration was significantly greater in B+S (31 g kg⁻¹) compared with B (19.3 g kg⁻¹) during the first evaluation (January), with no statistical difference among the other treatments (Figure 3A). There was no statistical difference in N concentration among treatments over the other evaluations ($P>0.05$). Within treatments, there was a greater N concentration in B+G and B+S during evaluation 1 (Jan) than in the other evaluations ($P<0.05$). Also, B+N had a lower N concentration in evaluation 2 (Mar) compared with the other evaluations ($P<0.05$), while no differences among evaluations were observed in B ($P>0.05$; Figure 3A).

Table 1 - Carbon and nitrogen composition of leguminous trees integrating different silvopastoral systems

Item	Gliricidia	Sabia	SEM	P-value		
				Treat	Evaluation	Treat × Evaluation
C (g kg ⁻¹)	429.4	439.9	6.293	0.251	0.119	0.886
N (g kg ⁻¹)	40.9a	30.8b	0.920	<0.0001	0.001	0.639
C:N ratio	10.6b	14.7a	0.516	<0.0001	0.016	0.544

C: carbon; N: nitrogen; SEM: standard error of the mean; Treat: treatment.
Means followed by different letters within the row are significantly different from each other according to the Tukey test at a 5% significance level.

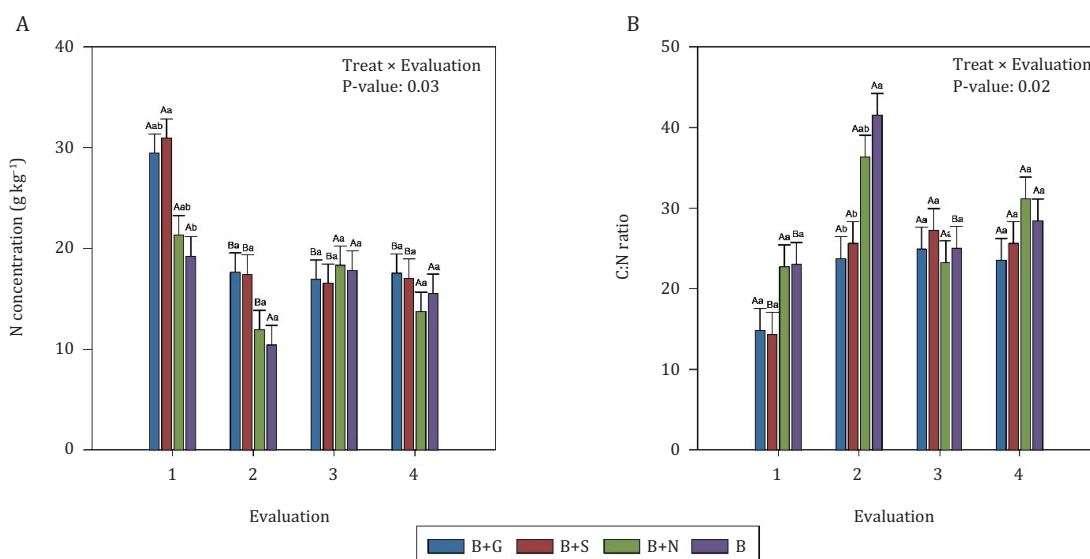
Table 2 - Average nitrogen concentration and C:N ratio of leguminous trees over the evaluations

Item	Evaluation				SEM	P-value
	1	2	3	4		
N (g kg ⁻¹)	33.6b	34.5b	41.5a	33.8b	1.289	0.001
C:N ratio	14.0a	13.3ab	10.6b	12.8ab	1.010	0.016

C: carbon; N: nitrogen; SEM: standard error of the mean; Treat: treatment.
Means followed by different letters within the row are significantly different from each other according to the Tukey test at a 5% significance level.

There was no statistical difference among evaluations in the forage C:N ratio for B+G and B+N ($P>0.05$; Figure 3B). However, the C:N ratio in B+S was lower during the first evaluation compared with all the other evaluations ($P<0.05$), following the period of greater N concentration (Figure 3B). Overall, the greatest forage C:N ratio was observed in the second evaluation for treatment B (41.6), with the lowest values observed in the silvopastoral systems (B+G and B+S) during the first evaluation (14.6; Figure 3B).

The forage K concentration was greater in the silvopastoral systems (B+G and B+N) compared with unfertilized Brachiaria (B), with no statistical differences from fertilized brachiaria (B+N) to the others ($P<0.05$; Table 3). Forage Mg concentration was greater in B+G compared with all the other treatments ($P<0.05$), while the P concentration was lower in B+G compared with B (Table 3). There was no statistical difference in the Ca concentration among the treatments. There was no evaluation effect or treatment $\times$ evaluation interaction ($P>0.05$) for the forage macronutrient concentrations, except N (Table 3).



Means followed by the same uppercase letter within systems and lowercase letters within evaluations do not differ significantly by the Tukey test at a 5% significance level.

Figure 3 - Nitrogen concentration and C:N ratio of brachiaria grass pastures under four different systems and evaluations.

Table 3 - Nutrient concentration of brachiaria grass under four different systems

Item	B+G	B+S	B+N	B	SEM	P-value		
						Treat	Evaluation	Treat $\times$ Evaluation
K (g kg ⁻¹)	35.8a	35.3a	30.5ab	28.1b	1.635	0.003	0.591	0.66
Mg (g kg ⁻¹)	4.4a	2.8b	2.8b	3.1b	0.245	<0.0001	0.127	0.161
P (g kg ⁻¹)	2.1b	1.7ab	1.7ab	2.3a	0.189	0.039	0.050	0.209
Ca (g kg ⁻¹)	3.5	2.1	1.9	2.7	0.644	0.291	0.544	0.100

B+G: brachiaria grass + gliricidia in a silvopastoral system; B+S: brachiaria grass + sabia in a silvopastoral system; B+N: fertilized brachiaria grass in monoculture; B: unfertilized brachiaria grass in monoculture; K: potassium; Mg: magnesium; P: phosphorus; Ca: calcium; SEM: standard error of the means; Treat: treatment.

Means followed by different letters within the row are significantly different from each other by the Tukey test at a 5% significance level.

3.2. Dung nutrients

There was no statistical difference among treatments in cattle dung nutrients ($P>0.05$); however, the concentration of nutrients varied across the evaluations ($P<0.05$; Table 4). The dung C and N concentrations were lower in the first evaluation (Jan) compared with all the other evaluations. Overall, the lowest K, Mg, and P concentrations in the dung were during the first evaluation, peaking in evaluation 3, with statistical differences between both seasons ($P<0.05$). The Ca concentration was greater in evaluation 4 compared with all the other seasons ($P<0.05$; Table 5).

Table 4 - Nutrient concentration of cattle dung in four different systems

Item	B+G	B+S	B+N	B	SEM	P-value		
						Treat	Season	Treat × Season
C (g kg ⁻¹)	431.6	429.0	429.8	424.9	10.478	0.970	<0.0001	0.189
N (g kg ⁻¹)	18.1	21.2	19.5	19.2	1.238	0.146	0.001	0.740
C:N ratio	24.1	20.9	22.5	23.0	1.501	0.321	0.269	0.943
K (g kg ⁻¹)	17.9	14.6	18.3	18.8	1.304	0.139	0.001	0.409
Mg (g kg ⁻¹)	4.7	4.2	4.1	4.5	0.267	0.456	0.017	0.395
P (g kg ⁻¹)	7.0	4.3	7.1	6.0	0.896	0.139	0.011	0.687
Ca (g kg ⁻¹)	7.4	5.8	6.6	6.7	0.571	0.280	0.002	0.377

B+G: brachiaria grass + gliricidia in a silvopastoral system; B+S: brachiaria grass + sabia in a silvopastoral system; B+N: fertilized brachiaria grass in monoculture; B: unfertilized brachiaria grass in monoculture; C: carbon; N: nitrogen; K: potassium; Mg: magnesium; P: phosphorus; Ca: calcium; SEM: standard error of the means; Treat: treatment.

Table 5 - Nutrient concentration of cattle dung across evaluations

Item	Evaluation				SEM	P-value
	1	2	3	4		
C (g kg ⁻¹)	376.5c	431.3b	480.8a	426.4b	10.478	<0.0001
N (g kg ⁻¹)	15.7b	20.1a	21.9a	20.3a	1.238	0.001
K (g kg ⁻¹)	13.8b	18.1ab	22.5a	15.2b	1.304	0.001
Mg (g kg ⁻¹)	3.6b	4.7a	4.5a	4.8a	0.267	0.017
P (g kg ⁻¹)	4.3b	4.5b	8.5a	7.1ab	0.895	0.011
Ca (g kg ⁻¹)	6.0b	6.0b	5.5b	9.0a	0.571	0.002

C: carbon; N: nitrogen; K: potassium; Mg: magnesium; P: phosphorus; Ca: calcium; SEM: standard error of the mean.

Means are averaged across four different systems.

Means followed by different letters within the row are significantly different from each other by the Tukey test at a 5% significance level.

3.3. Dung spatial distribution

Assessing seven 48-hour periods of grazing, totaling 14 days of dung deposition, there was no significant difference in the spatial distribution parameters of dung pads among the systems ($P>0.05$). Descriptive statistics of the semivariogram are presented in Table 6. The spatial distribution followed an aggregated pattern ($DI>1$ and $SMI>0.5$) in all treatments (Table 7). On average, 22 dung pads were observed per treatment ($DD = 0.035$ pads/m²) with an individual dung pad area of 0.06 m², covering approximately 0.2% of the pasture area over a 48-hour monitoring period (Table 7).

Regarding the evaluations, there was greater DD and, consequently, PC during evaluation 2 (August/yr-1), followed by evaluation 1 (January/yr-1), with no statistical differences among the other evaluations. The distribution also followed an aggregated pattern across different evaluations, with the highest DI observed in evaluations 3 (October/yr-1) and 7 (August/yr-2; Table 7).

Table 6 - Descriptive statistics of the semivariogram of cattle dung spatial distribution in four different grazing systems

Item	B+G	B+S	B+N	B	SEM
C_0	0.001	0.001	0.001	0.001	0.0002
$C_0 + C$	23.80	30.10	23.40	32.20	4.964
S	0.005	0.005	0.004	0.005	0.001

B+G: brachiaria grass + gliricidia in a silvopastoral system; B+S: brachiaria grass + sabia in a silvopastoral system; B+N: fertilized brachiaria grass in monoculture; B: unfertilized brachiaria grass in monoculture; C_0 : nugget effect; $C_0 + C$: range; S : sill; SEM: standard error of the mean.

Table 7 - Geostatistics of the spatial distribution of cattle dung in different grazing systems

Item	B+G	B+S	B+N	B	SEM	P-value		
						Treat	Evaluation	Treat × Evaluation
DD (pads/m ²)	0.04	0.04	0.03	0.03	0.004	0.924	<0.0001	0.972
PC (%)	0.21	0.19	0.19	0.22	0.023	0.605	<0.0001	0.999
DI	1.37	1.29	1.29	1.38	0.065	0.622	0.003	0.780
SMI	0.53	0.53	0.53	0.54	0.005	0.859	<0.0001	0.729
SDI	23.50	19.50	18.00	18.50	3.821	0.705	0.002	0.22
C_s	1.42	2.11	1.75	2.13	0.183	0.392	0.001	0.462
C_k	4.89	5.73	3.39	5.68	0.866	0.199	0.001	0.523

B+G: brachiaria grass + gliricidia in a silvopastoral system; B+S: brachiaria grass + sabia in a silvopastoral system; B+N: fertilized brachiaria grass in monoculture; B: unfertilized brachiaria grass in monoculture; SEM: standard error of the means; DD: dung density; PC: percentage of the pasture area covered by dung; DI: dispersion index; SMI: standardized Morisita index; SDI: spatial dependence index; C_s : skewness coefficient; C_k : kurtosis coefficient.

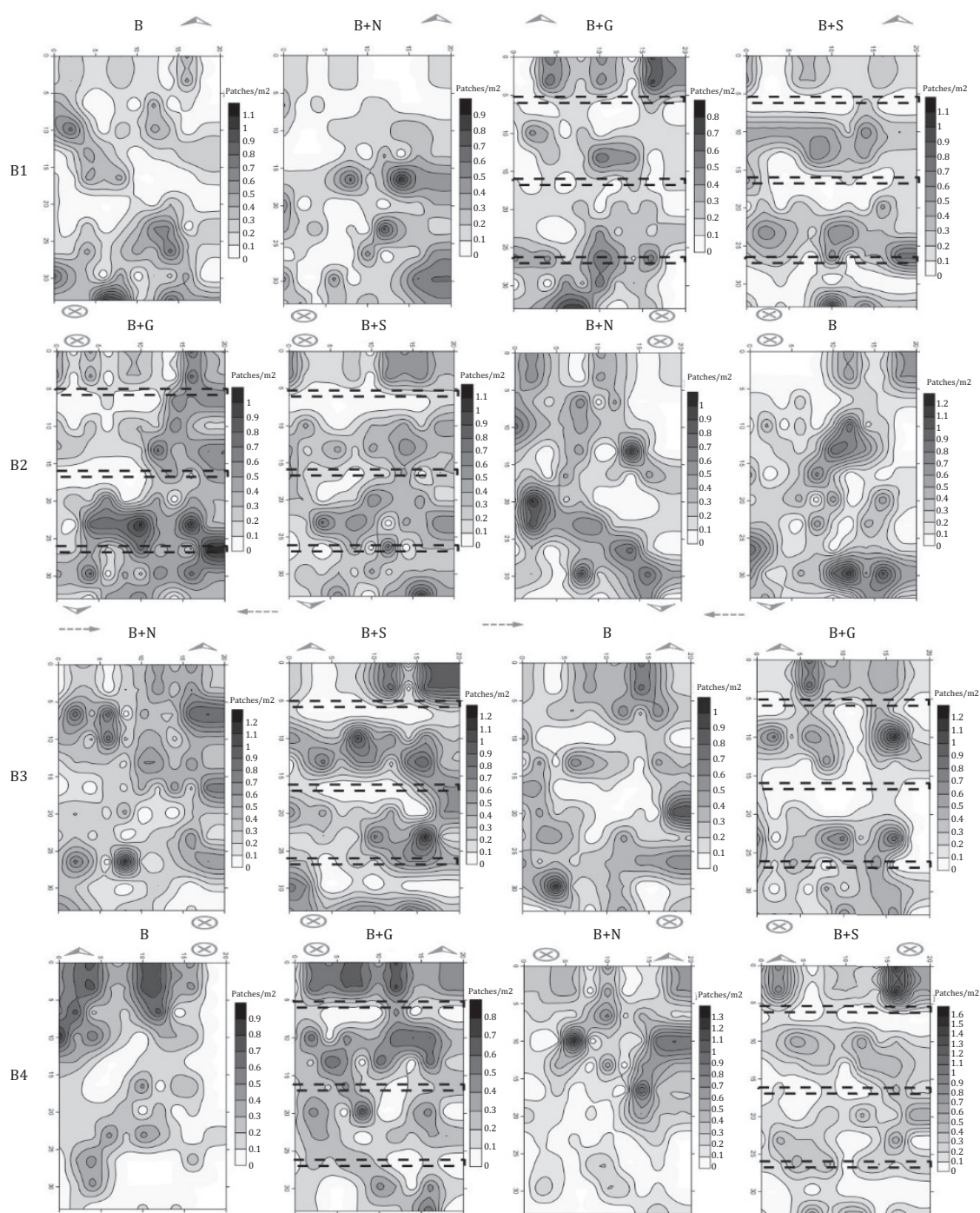
Means were compared by the Tukey test at 5% of significance.

Evaluations 3, 6, and 7 (October/yr-1, May/yr-2, and August/yr-2, respectively) showed an increased aggregation of dung pads (SMI) compared with the other evaluations. In evaluation 1 (January/yr-1) and 6 (May/yr-2), the spatial dependence between the dung pads was considered moderate (SDI between 25% and 75%). For the other evaluation periods, the spatial dependence was considered strong ($SDI \leq 25\%$). The C_s was lower in evaluation 2 (August/yr-1) compared with all the other evaluations, except evaluation 5 (March/yr-2). The greatest C_k values were observed in evaluations 3 (October/yr-1) and 7 (August/yr-2). For the evaluations, the B+S system was randomly selected for mapping representation since there was no significant difference among treatments (Figures 4 and 5).

4. Discussion

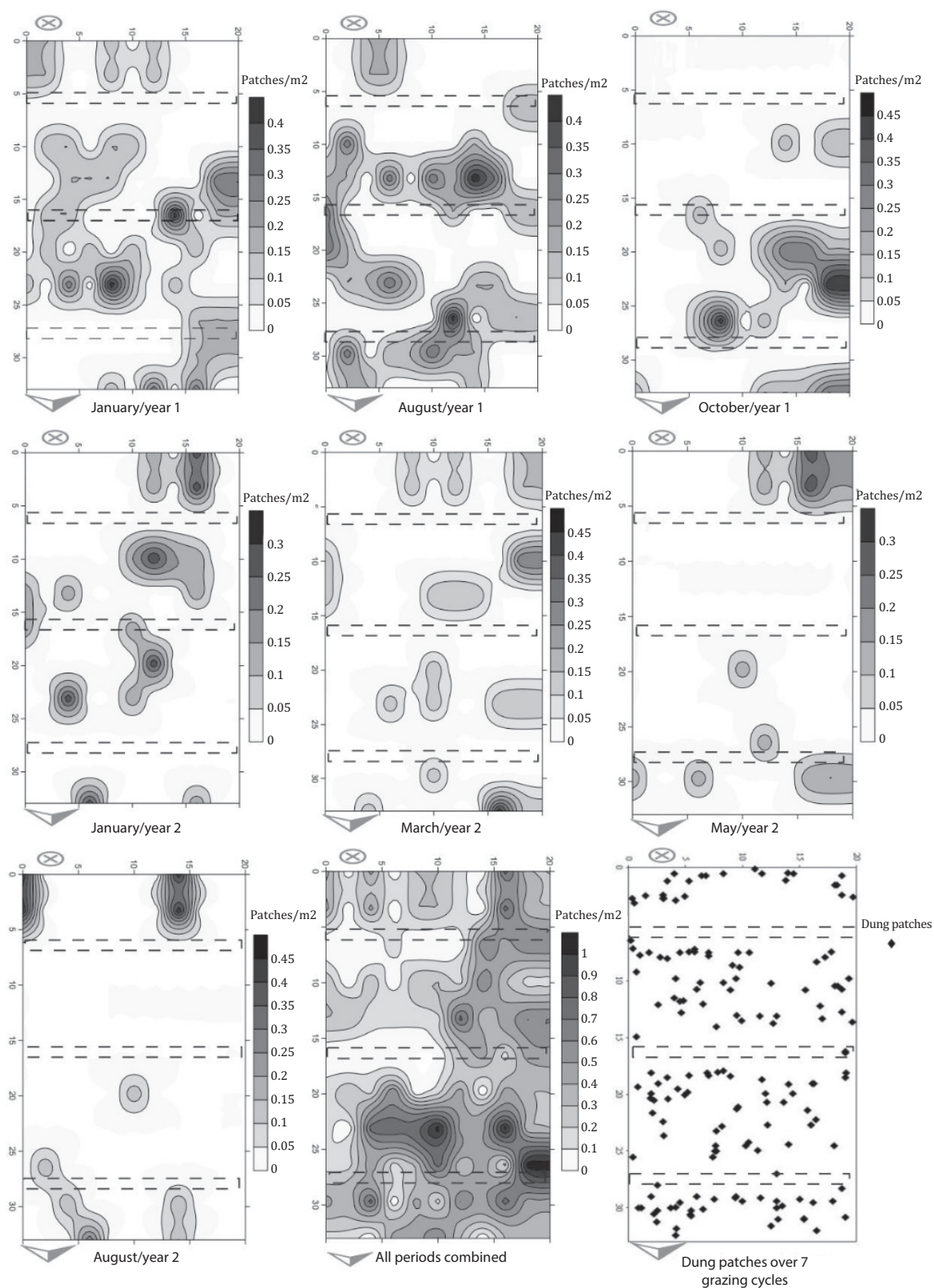
4.1. Forage nutrient concentrations

The average N concentration observed in gliricidia (41 g kg⁻¹) and sabia (31 g kg⁻¹) was similar to that observed by Herrera et al. (2021), who also observed greater N concentration in gliricidia (40 g kg⁻¹) than in sabia (30 g kg⁻¹). These findings corroborate Apolinário et al. (2016) and Lima et al. (2016), who also observed higher N concentrations in gliricidia than in sabia. This difference appears consistent and may be attributed to the intrinsic characteristics of both species, such as greater biological nitrogen fixation potential, higher leaf N concentration, and lower lignification in gliricidia, which promote faster nutrient cycling compared with sabia. The greater N concentration resulted in a lower C:N ratio in gliricidia than in sabia, indicating that gliricidia might have a greater contribution to nutrient cycling due to greater litter decomposition rates. Herrera et al. (2020) demonstrated greater decomposition rates of senescent leaves of gliricidia compared with sabia, indicating greater nutrient release to the soil. Besides greater N concentration and lower C:N ratio, gliricidia usually has lower lignin concentration than sabia, which might contribute to faster nutrient cycling (Apolinário et al., 2016). The higher N concentration in both species in the third evaluation (May) likely reflects increased soil



B+G: brachiaria grass (*Brachiaria decumbens*) + gliricidia (*Gliricidia sepium*) in a silvopastoral system; B+S: brachiaria grass + sabia (*Mimosa caesalpinifolia*) in a silvopastoral system; B+N: fertilized brachiaria grass in monoculture; B: unfertilized brachiaria grass in monoculture; B1 to B4: blocks 1, 2, 3 and 4; () Gate, () Watering trough, () Management alley, (- - -) Tree strips.

Figure 4 - Spatial distribution map of cattle dung in different grazing systems. Maps are summing up seven grazing periods of 48 hours across different seasons.



(X) Gate, (⊗) Watering trough, (- -) Tree strips. Each evaluation period corresponds to 48 hours of dung deposition.

Figure 5 - Spatial distribution map of cattle dung in a silvopastoral system established with brachiaria grass (*Brachiaria decumbens*) and sabia (*Mimosa caesalpinifolia*) over seven grazing cycles.

nitrogen availability and greater plant uptake, driven by higher cumulative precipitation and adequate temperatures (Figure 1). Increased soil moisture enhances microbial activity and organic matter mineralization, increasing inorganic N availability in the soil solution. In addition, favorable water conditions stimulate root activity, nutrient transport, and leaf growth, thereby increasing nitrogen assimilation and accumulation in plant tissues.

The higher N concentration in brachiaria grass in B+S during the first evaluation period (January) can be explained by the drought observed in the previous three months (October, November, and December; Figure 1). Although distributed throughout the year, most litterfall from *sabia* typically concentrates during the drought period (Apolinário et al., 2016). Thus, a significant amount of litter was likely decomposed in the soil through January, contributing to higher grass N concentrations in the grass-legume systems compared with the unfertilized grass system. This was confirmed by the greater N concentration of brachiaria grass in B+G and B+S in evaluation 1 (January) compared with the other evaluations. Despite the greater N concentration in *gliricidia* than in *sabia*, *sabia* usually has a greater tree volume and productive capacity than *gliricidia* (Herrera et al., 2021), resulting in greater amounts of litterfall (Apolinário et al., 2016). The lower amount of *gliricidia* litter might explain the similar N concentrations in brachiaria grass across B+G and the other treatments. Lower legume litterfall and higher brachiaria grass N concentrations during the favorable cumulative precipitation and temperature period likely contributed to similar brachiaria grass N concentrations across treatments. A previous study have also found similar N concentrations of brachiaria grass submitted to full sun or shading in silvopastoral systems with *gliricidia* and *sabia* (Lima et al., 2016). The lower N concentration of fertilized brachiaria grass in evaluation 2 (March) compared with the other periods might be explained by the significant reduction in the cumulative precipitation observed in that period (Figure 1). The brachiaria grass C:N ratio also followed the N variation patterns among treatments and across evaluations in response to the meteorological conditions. Beyond confirming previously reported species differences, these findings provide important insights into how tree species selection in silvopastoral systems can influence nutrient cycling dynamics through litter quality and seasonal nutrient availability. The consistently higher N concentration and lower C:N ratio observed in *gliricidia* suggest greater potential for rapid nutrient turnover and soil nutrient replenishment than in *sabia*, which may improve nutrient-use efficiency in grazing systems. Additionally, the observed seasonal variation highlights the strong interaction between climatic conditions and plant nutrient dynamics, indicating that nutrient return through litter and animal excreta is temporally variable rather than constant throughout the year. These results emphasize that both species selection and seasonal environmental conditions should be considered when designing silvopastoral systems to enhance ecosystem functioning and nutrient cycling efficiency.

In addition to incorporating N via biological N fixation in the system, the inclusion of both legume trees in B+G and B+S showed the potential to increase the supply of K in the system via litterfall, resulting in greater K concentration in the forage grass. The inclusion of *gliricidia* also resulted in higher Mg concentration in the forage grass, suggesting that legume trees enhance the availability of other plant macronutrients besides N, reducing the need for industrial fertilizer. Tree legumes typically have deeper and more extensive root systems compared with grasses. These deep roots can access nutrients that are located deeper in the soil profile, which might be unavailable to shallow-rooted grasses (Dubeux Jr. et al., 2017). This access allows tree legumes to take up more macronutrients that can be recycled through litter and animal excreta, becoming available to the grass after residue decomposition. This fact was also previously reported in the literature (Aguirre et al., 2016; Fioreli et al., 2018; Herrera et al., 2021). On the other hand, the reduction in the forage grass P concentration in B+G compared with B might be attributed to high competition due to the high stem density and the high arboreal basal area of *gliricidia* (Hernández and Castellanos, 2006), which is associated with the low mobility of this mineral in the soil.

4.2. Cattle dung nutrient concentrations

The high N concentration of the leguminous trees (30 to 40 g kg⁻¹) compared with the N concentration observed in the brachiaria grass (15 to 20 g kg⁻¹) did not result in a greater N return to the soil

through cattle dung in the silvopastoral systems compared with B+N and B. This can be explained by the low legume intake by the animals in both silvopastoral systems. Although we did not evaluate the proportions of grass and legumes in the diet, we visually observed low voluntary intake of the legume components by the animals. Apolinário et al. (2015) explained that the lignin concentration in the leaves of both species, as well as other compounds such as tannins and coumarin, might limit voluntary intake. Similarly, Costa et al. (2021) reported that grazing cattle preferred brachiaria grass over gliricidia or sabia in silvopastoral systems. Despite soil N inputs from biological nitrogen fixation and legume litter, legume trees were likely competing for the inorganic N available in the soil, resulting in similar N concentrations in the brachiaria grass across treatments over the evaluated periods. However, further evidences are required to confirm this hypothesis. Additionally, biological nitrogen fixation does not necessarily result in effective nitrogen transfer to companion grasses, which may also explain the similar N concentrations in the brachiaria grass. A previous study looking at the soil fertility (e.g., pH, P, Mg^{+2} , and K^{+}) over time in silvopastoral systems integrating brachiaria grass and gliricidia or sabia concluded that silvopastoral systems reduced soil fertility regardless of the legume tree species (Herrera et al., 2023). Thus, the N concentration in the animal diet was likely similar across treatments, leading to similar N recycling through dung deposition.

Despite the greater concentrations of K in the forage grass in B+G and B+S and the greater concentration of Mg in the forage grass in B+S, the concentrations of all evaluated macronutrients in the dung of animals grazing in silvopastoral systems with legume trees were similar to the concentrations of the dung of animals fed with brachiaria grass in monoculture with and without fertilization. This can be explained by the greater bioavailability of essential minerals provided by organic sources compared with inorganic minerals and the high absorption coefficients (>0.90) of these nutrients by ruminants (Castro et al., 2019; Costa e Silva et al., 2023), resulting in a low return through excretion.

The lower N excretion through cattle dung observed during the period of greater forage N concentration suggests improved nitrogen-use efficiency by the animals under higher forage quality conditions. Diets with greater crude protein concentration and higher leaf proportion generally enhance ruminal microbial activity and protein digestibility, increasing nitrogen incorporation into microbial biomass and animal tissues rather than excretion. Consequently, a larger fraction of ingested nitrogen may have been retained by the animals, reducing fecal N losses. The likely increase in the leaf:stem ratio during this period may have further contributed to improved forage digestibility by reducing structural fiber concentration and increasing nutrient availability. Although digestibility was not directly measured, these results highlight a potential seasonal shift in nitrogen partitioning between animal retention and nutrient return to the soil. This finding has important implications for nutrient cycling in grazing systems, as periods of higher forage quality may reduce immediate nutrient return via dung while potentially improving animal productivity and overall nitrogen-use efficiency. A similar excretion pattern was observed for K, Mg, and P, with an overall lower excretion in evaluation 1 compared with evaluation 3, enforcing our hypothesis.

4.3. Dung distribution

Cattle dung deposition in pastures varies depending on climatic conditions, the dry matter intake, and the inherent characteristics of the animal, altering both the excreted volume and frequency. The daily number of defecations per animal (11) and the average area of each dung pad (0.06 m^2) observed in this study were similar to those observed by Araújo et al. (2017), who reported 6 to 8 dung pads of 0.05 m^2 per day in pastures of *Brachiaria brizantha* cv. Marandu in monoculture or integrated with babassu (*Attalea speciosa*). The authors indicate that the number and distribution of dung pads vary with season and tree density. They noted that as the babassu density increases ($80\text{-}160 \text{ trees ha}^{-1}$), the area covered by dung pads also increases, leading to greater nutrient cycling through dung deposition in these systems. We also observed seasonal variation in cattle dung distribution, with greater DD and PC coinciding with the period of lower average temperature in year 1.

The lower temperature likely affected the animals' grazing behavior, increasing grazing time and spatial distribution in response to the favorable thermal conditions. Most of the changes in dung spatial distribution across evaluations observed in our study may be attributed to daily variations in the microclimate, which alter grazing behavior. For example, a moderate spatial dependence ($25\% < \text{SDI} < 75\%$) was observed during evaluations 1 and 6, coinciding with the periods of greater cumulative precipitation, whereas all the other evaluations showed strong spatial dependence on the dung pads ($\text{SDI} < 25\%$). Even when monthly meteorological data showed low variability, changes in dung distribution likely reflected changes in animal grazing behavior due to daily fluctuations in the microclimate, especially temperature. A reduced grazing time during dry periods and when the ambient temperature exceeded $26\text{ }^{\circ}\text{C}$ has been previously reported (Araújo et al., 2017). Moreover, the temporal variability has been also attributed to animal behavior and, consequently, dung spatial distribution to fluctuations in temperature and daylight (Hassan-Vásquez et al., 2022). Despite the environmental impact on temporal variation in dung distribution, we did not find shading effects on the spatial distribution of cattle dung, as hypothesized. This might have occurred due to the small plot size used for the current study (660 m^2) and high grazing pressure. The small area associated with the intense grazing likely led to homogeneous grazing over the plot, regardless of the system, resulting in a similar pattern of dung deposition among treatments. Additionally, the legume trees were still young during the evaluations (approximately 2 years) and did not have as much shade as a developed tree. Thus, the shading level may not have contributed to the increase in forage N concentration or to changes in animal spatial distribution in the silvopastoral systems. Further evaluations are required across larger areas and in developed areas with mature trees to confirm our findings.

In our study, an average deposition of 11 dung pads $\text{animal}^{-1}\text{ day}^{-1}$, occupying an average area of $0.06\text{ m}^2\text{ pad}^{-1}$, was observed. Considering 10 grazing cycles of 35 days (three days of stay and 32 days of rest) over the year, it is estimated that 3% ($11\text{ pads} \times 0.06\text{ m}^2 \times 3\text{ days of grazing} \times 10\text{ cycles}$) of the pasture area (660 m^2) would be covered by dung without overlapping, equivalent to approximately $0.2\text{ ha}^{-1}\text{ year}^{-1}$. This estimation would be even lower if we assume a likely dung pad overlapping in some spots over subsequent grazing cycles, since the dung distribution occurred in an aggregated way ($\text{DI} > 1$ and $\text{SMI} > 0.5$) in all systems. The average C_s and C_k coefficients for the dung distribution among treatments and evaluations ($C_s = 1.85$ and $C_k = 4.92$) showed a positive skewness and leptokurtic tendency of the variables. This suggests that there was a higher frequency of lower values, with some higher values causing the dung distribution to lean to the right. In addition, the C_k values indicate a more pronounced peak and longer tails compared with a normal distribution pattern. Figures 4 and 5 demonstrate the aggregate pattern of spatial dung distribution among treatments and across evaluations, with an evident overlapping of dung pads when considering all the evaluated periods. These findings indicate that the nutrient cycling via cattle dung deposition is unevenly distributed and might not be enough to ensure sustainable production.

However, previous studies reported greater cattle dung spatial distribution in silvopastoral systems compared with unshaded pastures, especially with increasing tree density (Karki and Goodman, 2010; Araújo et al., 2017; Carnevalli et al., 2019; Carpinelli et al., 2020). The presence of trees in silvopastoral systems might increase the spatial distribution of cattle dung compared with monocultures in larger areas under lower grazing intensity. This is because large areas with a high herbage allowance offer greater selection opportunities to the animals, leading to a heterogeneous grazing behavior and spatial distribution. In these conditions, animals will likely search for areas with a favorable microclimate, making the shading distribution crucial to avoid specific hotspots of animal grazing or resting (e.g., gates, watering troughs, and single fixed shades; Carnevalli et al., 2019; Hassan-Vásquez et al., 2022; Bretas et al., 2023).

Our study demonstrates that geostatistics, together with mathematical models, can be applied to monitor the spatial distribution of dung deposition in grazing lands. Kriging maps allow visualization of spatial distribution arrangements of dung pads in pastures and resting areas, which facilitates the identification of dung deposition zones based on the characteristics of each pasture location. This information allows the definition of management practices to reduce the potential environmental

impacts of dung deposition and nutrient overloading in fixed spots, while enhancing the efficiency of nutrient cycling in grasslands. From an environmental and management perspective, the aggregated spatial distribution of dung observed across all systems has important implications for nutrient cycling efficiency and pasture sustainability. Concentrated dung deposition promotes localized nutrient accumulation, potentially increasing nutrient losses through runoff, volatilization, or leaching while limiting nutrient availability in underutilized areas of the pasture (Dubeux Jr. and Sollenberger, 2020). This spatial heterogeneity may reduce nutrient-use efficiency and contribute to uneven forage growth, reinforcing the need for management strategies that improve nutrient redistribution. Practices such as adjusting stocking density, modifying grazing rotation schemes, strategically positioning water and mineral supplementation points, or optimizing shade distribution may help promote more uniform animal movement and excreta deposition.

5. Conclusions

The use of *sabia* or *gliricidia* as a leguminous component of silvopastoral systems with *brachiaria* grass increased K and Mg concentrations in the forage grass compared with unfertilized pastures and can enhance N cycling through litter deposition during dry periods, with greater litterfall. However, the inclusion of the leguminous trees did not affect the nutrient excretion via cattle dung nor the spatial distribution of cattle dung within the tree arrangement and conditions evaluated in the current study.

The results of this study reflect conditions in a silvopastoral system at an early stage of tree development, when shading intensity and associated environmental stresses were still limited. Therefore, the observed responses should be interpreted as representative of initial system interactions, and responses may differ as canopy development progresses and shading becomes more pronounced.

Data availability

The entire dataset supporting the results of this study is available upon request to the corresponding author.

Author contributions

Conceptualization: Dubeux Junior, J. C. B. and Santos, M. V. F. **Data curation:** Lira, C. C. and Camelo, D. **Formal analysis:** Dubeux Junior, J. C. B. and Santos, M. V. F. **Funding acquisition:** Dubeux Junior, J. C. B. **Investigation:** Lira, C. C. **Methodology:** Lira, C. C.; Camelo, D. and Santos, E. R. S. **Project administration:** Dubeux Junior, J. C. B. **Resources:** Dubeux Junior, J. C. B. **Supervision:** Dubeux Junior, J. C. B. **Visualization:** Freitas, E. V. and Bretas, I. L. **Writing – original draft:** Lira, C. C.; Camelo, D. and Bretas, I. L. **Writing – review & editing:** Dubeux Junior, J. C. B.; Santos, M. V. F.; Freitas, E. V.; Santos, E. R. S. and Bretas, I. L.

Conflict of interest

The authors declare no conflict of interest.

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