Silages of sorghum, Tamani guinea grass, and *Stylosanthes* in an integrated system: production and quality

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Crop−livestock integration systems are efficient technologies for diversifying production and promoting agricultural sustainability. However, less is known about the triple intercropping of crops for silage production. The objective of this study was to evaluate the dry mass production, fermentation profile, and nutritive value of sorghum silage intercropped with Tamani guinea grass and *Stylosanthes* cv. Bela in integrated systems. We used an experimental design with randomized blocks with four replicates. The treatments consisted of silage of (1) sorghum in monocropped; (2) Tamani guinea grass in monocropped (*Panicum maximum* cv. BRS Tamani); (3) *Stylosanthes* cv. Bela in monocropped (*Stylosanthes guianensis* cv. BRS Bela); (4) sorghum intercropped with Tamani guinea grass; (5) sorghum intercropped with *Stylosanthes* cv. Bela; (6) *Stylosanthes* cv. Bela intercropped with Tamani guinea grass; and (7) sorghum intercropped with Tamani guinea grass and *Stylosanthes* cv. Bela, totaling 28 experimental silos. Our results demonstrated that intercropping sorghum with tropical forages can be utilized in integrated silage production systems. This practice led to an increase in silage mass production per unit area while also providing pasture forage after the crop harvest for silage production, ultimately enhancing land-use efficiency in a sustainable manner. Silage produced from sorghum intercropped with Tamani guinea grass and *Stylosanthes* cv. Bela exhibited improved fermentative characteristics, as well as higher ether extract and total digestible nutrient contents compared with silage from monocropped forages. Tropical forages contributed to an increase in the crude protein content of monocropped sorghum silage, which could potentially reduce costs associated with acquiring protein salts for ruminant feed supplementation. Consequently, we recommend the triple intercropping of sorghum, Tamani guinea grass, and Bela for silage production, as it offers advantages for the cultivation of annual and tropical forage crops.

Introduction

The sustainability of agribusiness has become a prerequisite for maintaining a position in the global market, encompassing both socioenvironmental appeal and the necessity for integrated negotiation (Borsellino et al., 2016). In this regard, conservative production systems that integrate crops and livestock have emerged as an established strategy for intensifying food production while addressing environmental concerns (Simões et al., 2023). Integrated crop–livestock systems are widely recognized as one of the most sustainable and competitive technologies for advancing agribusiness (Dias et al., 2020).

The ecosystem services provided by integrated systems have been documented in numerous studies, including enhanced land use efficiency and increased grain production (Muniz et al., 2021); reduced soil compaction, improved water infiltration rates, and lower erosion risks (Linhares et al., 2020); maintenance of soil fertility through enhanced nutrient cycling (Dias et al., 2020); greater carbon sequestration, increased soil organic matter, and improved microclimatic conditions (Vincent-Caboud et al., 2019); pasture regeneration (Santos et al., 2020); and improved silage production during the dry season (Oliveira et al., 2020). However, due to the intricate interactions between annual and tropical forage crops, integrated systems have become dynamic and complex, requiring more sophisticated technologies to consolidate environmental and productive sustainability (Soussana and Lemaire, 2014).

Among the annual crops employed in integrated systems, *Sorghum bicolor* L. (sorghum) stands as a significant forage crop in numerous global regions owing to its adaptability across diverse environments (Perazzo et al., 2017), capability to flourish in low soil fertility conditions, potential for regrowth post-grain harvest, and high tolerance against water deficits (Buffara et al., 2018). These characteristics enable a wider range of sowing seasons and greater resilience to adverse environmental factors compared with maize (Mateus et al., 2016). Consequently, sorghum has been recognized as crucial in recent years for the production of preserved forage (Oliveira et al., 2020), offering high dry mass productivity, preservation of nutritional value, and favorable fermentation patterns (Cruz et al., 2020).

However, sorghum silage typically exhibits a lower crude protein content compared with tropical forages (Ribeiro et al., 2017), necessitating the adoption of cultivation strategies to enhance the crude protein content of sorghum silage. Notably, Oliveira et al. (2020) demonstrated the advantageous effects of intercropping annual and tropical forage crops in integrated systems for silage production. Intercropping not only enhances the quality of sorghum silage but also intensifies production systems, resulting in higher yields and improved silage nutritional value. Moreover, this approach mitigates the challenges associated with silage fermentation in exclusive grass and legume systems.

Among tropical forages, Tamani guinea grass possesses significant potential as a conserved forage resource due to its high mass yield, slender stems and leaves, and high tillering capacity (Dias et al., 2020). The exceptional quality and adaptability of this grass make it suitable for silage production (Paludo et al., 2020). Additionally, tropical legume silage has gained attention due to its superior nutritional value (Silva et al., 2023). Recently introduced *Stylosanthes* cv. Bela (*Stylosanthes guianensis* cv. BRS Bela) has shown positive results in ruminant production, owing to its elevated crude protein content (Braga et al., 2020) and ability to fix biological nitrogen. Given the escalating costs of mineral fertilizers and their significant contribution to greenhouse gas emissions, the integration of legumes into integrated systems emerges as a promising technology for increasing crop productivity and ensuring enhanced sustainability (Epifanio et al., 2019a,b). This approach allows for the partial or complete replacement of mineral (nitrogen) fertilizers, ensuring adequate plant nutrition, soil conservation, fertility maintenance, and carbon sequestration (Bourscheidt et al., 2023; Silva et al., 2023).

In this context, sorghum silage, tropical grasses, and legumes present a favorable combination for achieving a balanced nutritional value, improved qualitative characteristics in dry matter (DM), and higher nutrient production per unit area (Perazzo et al., 2017). Moreover, their versatility in use establishes them as significant alternative food sources during the off-season period (Oliveira et al., 2020).
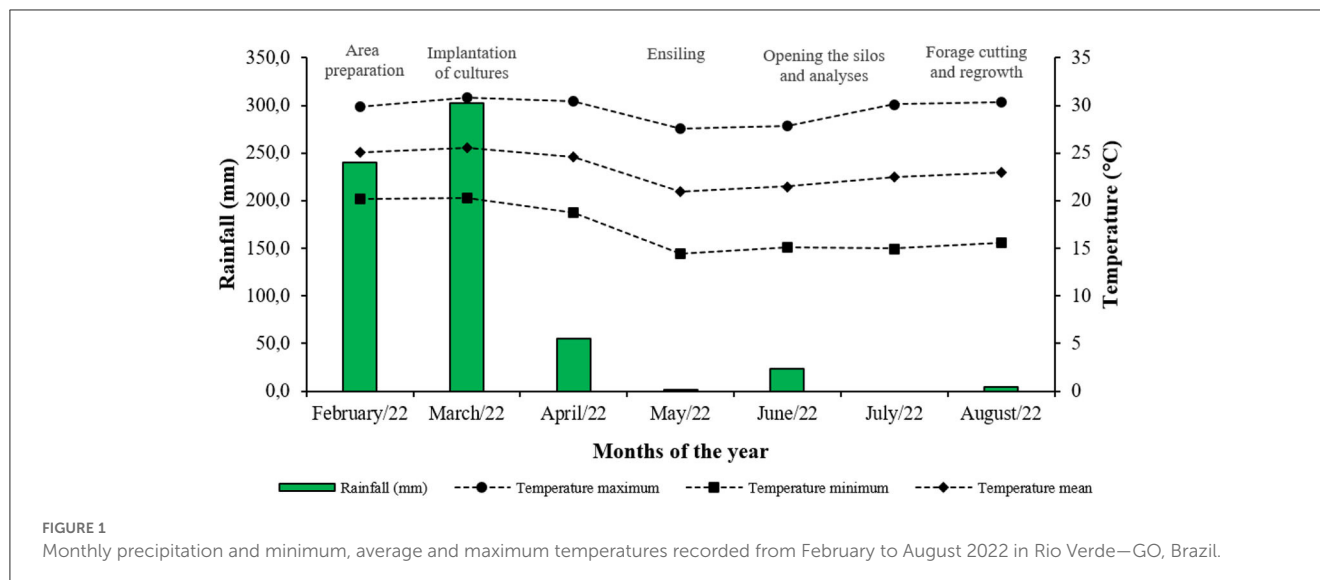
However, limited knowledge exists regarding triple intercropping (sorghum + tropical grass + legumes) for silage production. Understanding the optimal intercropping approach can enhance both silage production and quality within integrated systems while also facilitating crop diversification to meet the demand for high-quality feed during periods of limited forage availability. Therefore, we hypothesized that double and triple intercropping of sorghum with forage crops would have a positive impact on the bromatological characteristics and yield of the ensiled mass, without compromising the fermentation process required for silage preparation. The objective of this study was to assess the production of dry mass, fermentation profile, and nutritive value of silage derived from sorghum intercropped with Tamani guinea grass and *Stylosanthes* cv. Bela in integrated systems.

Materials and methods

Description of the area and crop establishments

This experiment was conducted under field conditions at Instituto Federal Goiano, Campus Rio Verde, located in the municipality of Rio Verde, State of Goiás, Brazil ($17°48′22″$S, $50°54′11″$W; 832 m altitude). According to the Köppen–Geiger classification, the climate of the region is defined as tropical (Aw), with a dry season in winter.

Before the implementation of the experiment, soil samples were collected from the 0 to 20 cm layer for physicochemical characterization. The soil in the experimental area was characterized as Dystroferric Red Latosol (Santos et al., 2018). The soil characteristics were as follows: it has 364, 83, and 553 g kg^{-1} of clay, silt, and sand, respectively; pH in $CaCl_2$: 5.4; Ca: 2.69 $cmol_c$ dm^{-3}; Mg: 1.00 $cmol_c$ dm^{-3}; Al: 0.01 $cmol_c$ dm^{-3}; Al + H: 3.79 $cmol_c$ dm^{-3}; K: 0.69 $cmol_c$ dm^{-3}; cation exchange capacity: 8.6 $cmol_c$ dm^{-3}; current base saturation of the soil (V1): 56%; P (Mehlich): 3.8 mg dm^{-3}; S: 8.5 mg dm^{-3}; Cu: 3.7 mg dm^{-3}; Zn: 1.0 mg dm^{-3}; Fe: 17.3 mg dm^{-3}; organic matter (OM): 39.8 g

FIGURE 1
Monthly precipitation and minimum, average and maximum temperatures recorded from February to August 2022 in Rio Verde—GO, Brazil.

dm^{-3}. No irrigation system was installed during the experiment. Precipitation, maximum, average, and minimum temperatures were monitored throughout the duration of the study, as shown in Figure 1.

Experimental design and treatments

The experimental design consisted of randomized blocks with four replicates. The treatments consisted of the silage of (1) sorghum in monocropped; (2) Tamani guinea grass in monocropped (*Panicum maximum* cv. BRS Tamani); (3) *Stylosanthes* cv. Bela in monocropped (*Stylosanthes guianensis* cv. BRS Bela); (4) sorghum intercropped with Tamani guinea grass; (5) sorghum intercropped with *Stylosanthes* cv. Bela; (6) *Stylosanthes* cv. Bela intercropped with Tamani guinea grass; and (7) sorghum intercropped with Tamani guinea grass and Bela *Stylosanthes*, totaling 28 experimental silos. The sorghum used was grain sorghum (AG 1077) with an early cycle and high production potential.

For monocropped cultures, a spacing of 0.5 m between rows was used (Figures 2A–C). In the double intercropping of annual and forage crops, sorghum was sown at 0.5 m spacing and Tamani guinea grass and/or legume between rows was sown at a 0.25 m distance from the sorghum row (Figures 2D, E). In the double intercropping of forage plants, Tamani guinea grass was sown at 0.25 m of the *Stylosanthes* cv. Bela (Figure 2F). In the triple intercropping, sorghum was sown at 0.9 m spacing, with Tamani guinea grass and *Stylosanthes* cv. Bela sown between the rows at a 0.3 m distance from the sorghum row, as shown in Figure 2G. No herbicides were applied to suppress grass or legume growth during the intercropping.

The seeding of forage systems was carried out manually on 7 March 2022, and 120 kg ha^{-1} of P$_2$O$_5$ and 20 kg ha^{-1} of FTE BR 12 (9% Zn, 1.8% B, 0.8% Cu, 2% Mn, 3.5% Fe, and 0.1% Mo) were applied in the planting furrow using simple superphosphate and Frit sources, respectively. A total of 12 sorghum seeds were used

per meter, and for grass and legumes, 3 kg of pure viable seeds were used per hectare.

When sorghum plants were at the stage of three and six fully developed leaves, two fertilizer applications of 80 and 60 kg ha^{-1} of N and K$_2$O from urea and potassium chloride, respectively, were applied to the following systems: sorghum in monocropped, Tamani guinea grass in monocropped, and sorghum intercropped with Tamani guinea grass. For the systems intercropped with legumes (sorghum intercropped with *Stylosanthes* cv. Bela, Tamani guinea grass intercropped with *Stylosanthes* cv. Bela, and sorghum intercropped with Tamani guinea grass and Bela), 60 kg ha^{-1} of K$_2$O and only half of the dose of nitrogen (i.e., 40 kg ha^{-1}) were applied, aiming to utilize the nitrogen obtained through the biological fixation by legumes. For the *Stylosanthes* cv. Bela monocropped system, only 60 kg ha^{-1} K$_2$O was applied.

To *Spodoptera frugiperda* and *Dalbulus maidis*, we applied the insecticides Klorpan (active ingredient Chlorpyrifos) and Connect (active ingredient Beta-cyfluthrin and Imidacloprid) at the rate of 0.4 and 0.1 L ha^{-1} of commercial product, respectively. Both applications were performed using knapsack sprayers.

Crop silage

Harvesting of cultures for silage was carried out 93 days after sowing the cultures. During this period, sorghum, Tamani guinea grass, and *Stylosanthes* cv. Bela were harvested at 340.66, 276.16, and 285.04 g kg^{-1} dry matter (DM), respectively. To evaluate the dry mass production and proportion of the ensiled material (Table 1), the material was collected, separated, and weighed to determine the proportions of sorghum, Tamani guinea grass, and *Stylosanthes*. Subsequently, the material was placed in an oven at 55°C until it reached a constant mass, and its dry weight was determined and expressed in kg ha^{-1}.

To make the silage, the material was ground together in the treatments of the intercropping systems, in particles of ∼10 mm size. The material was stored in experimental PVC silos measuring
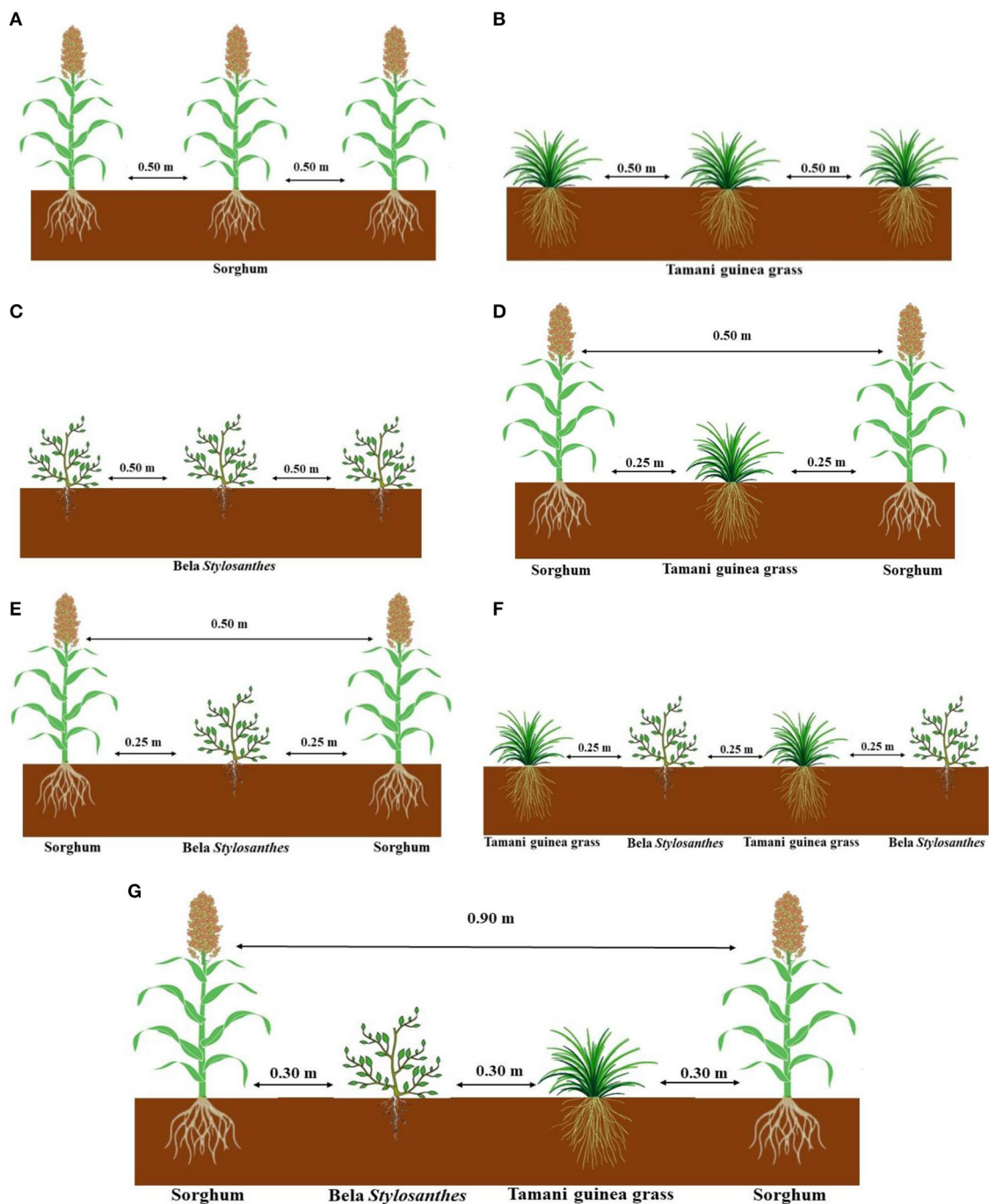
FIGURE 2
Arrangement of crops: Sorghum in monocropped **(A)**, Tamani guinea grass in monocropped **(B)**, *Stylosanthes* cv. Bela in monocropped **(C)**, Sorghum intercropped with Tamani guinea grass **(D)**, Sorghum intercropped with *Stylosanthes* cv. Bela **(E)**, *Stylosanthes* cv. Bela intercropped with Tamani guinea grass **(F)** and Sorghum intercropped with Tamani guinea grass and *Stylosanthes* cv. Bela **(G)**.

10 cm in diameter and 40 cm in length. The material was compacted with an iron pendulum, closed with a PVC lid, and sealed with adhesive tape to prevent the entry of air. They were then stored at room temperature and protected from rain and sunlight.

In the material *in natura* (before ensiling), bromatological analyses were performed (Table 2) to determine the dry matter (DM), method 934.01; crude protein (CP), method 920.87; lignin, method 973.18; ether extract (EE) contents, method 920.85,

and mineral matter (MM), method 924.05, according to the methodologies described by the AOAC (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured as described by Mertens (2002). Total digestible nutrients (TDN) were calculated using the equation proposed by Chandler (1990). For the determination of *in vitro* dry matter digestibility (IVDM), the technique described by Tilley and Terry (1963) was used, adapted to the artificial rumen, developed by ANKON®, using the

TABLE 1 Proportion of material ensiled from sorghum intercropped with Tamani guinea grass and *Stylosanthes* cv. Bela.

Cultivation system	Proportion of ensiled material (%)		
	Sorghum	Tamani guinea grass	*Stylo santhes*
Sorghum in monocropped	100	0	0
Tamani guinea grass in monocropped	0	100	0
Stylosanthes cv. Bela in monocropped	0	0	100
Sorghum + Tamani guinea grass	68.3	31.7	0
Sorghum + *Stylosanthes* cv. Bela	70.8	0	29.2
Tamani guinea grass + Bela *Stylosanthes*	0	60.3	39.69
Sorghum + Tamani guinea grass + Bela	56.7	24.4	18.9

TABLE 2 Chemical–bromatological composition (g kg^{-1}) of sorghum, Tamani guinea grass, and *Stylosanthes* cv. Bela in monocropped and intercropped before ensiling.

Cultivation system	DM	CP	MM	EE	IVDMD
Sorghum in monocropped	340.66	76.78	45.92	41.65	613.88
Tamani guinea grass in monocropped	276.16	130.80	65.90	21.89	600.72
Stylosanthes cv. Bela in monocropped	285.04	155.35	62.66	22.41	619.72
Sorghum + Tamani guinea grass	315.74	110.91	54.24	28.72	606.35
Sorghum + *Stylosanthes* cv. Bela	318.58	125.68	53.81	29.11	616.65
Tamani guinea grass + Bela *Stylosanthes*	283.55	141.38	66.56	22.82	615.53
Sorghum + Tamani guinea grass + Bela	323.26	127.57	53.56	29.26	613.69
SEM	2.656	2.283	1.450	1.013	5.879
	TDN	NDF	ADF	Lignin	
Sorghum in monocropped	61.90	612.17	348.70	43.03	
Tamani guinea grass in monocropped	53.15	660.26	375.05	27.69	
Stylosanthes cv. Bela in monocropped	56.93	596.16	352.44	26.59	
Sorghum + Tamani guinea grass	58.38	621.06	359.95	35.52	
Sorghum + *Stylosanthes* cv. Bela	56.83	605.38	360.91	33.13	
Tamani guinea grass + Bela *Stylosanthes*	53.46	636.55	361.59	28.50	
Sorghum + Tamani guinea grass + Bela	57.44	621.07	366.30	38.49	
SEM	1.286	3.988	3.617	0.833	

DM, dry matter; CP, crude protein; MM, mineral matter; EE, ether extract; IVDMD, *in vitro* dry matter digestibility; NDF, neutral detergent fiber; ADF, acid detergent fiber; TDN, total digestible nutrients; SEM, standard error of mean.

instrument "Daisy incubator" by Ankom Technology (*in vitro* true digestibility—IVTD). The ruminal collection was carried out in dairy cattle fed on pasture and receiving corn silage twice a day. The liquid was collected through a ruminal cannula, in the morning, 3 h after feeding the animal. The initial project was approved by the Research Ethics Committee (REC) of the Federal Goiano Institute, protocol 53752405-16.

Analysis of the fermentation and bromatological characteristics of the silages

After 50 days of fermentation, the silos were opened, and the upper and lower portions of each silo were discarded. The central portion of the silo was homogenized and placed in a plastic tray. Part of the *in natura* silage was separated for the analysis of fermentation parameters: buffering capacity, pH, and ammoniacal nitrogen in total nitrogen (N-NH$_3$/NT), following the method described by Bolsen et al. (1992).

The pH and buffering capacity analyses were performed when the silos were opened to avoid changes in the expected values due to heat and humidity. To determine ammoniacal nitrogen, the silage was frozen to inactivate the activity of anaerobic bacteria, thus avoiding the volatilization of nitrogen, and the samples were later thawed for juice extraction (Bolsen et al., 1992). Total dry matter loss and effluent production were determined according to the methodology proposed by Jobim et al. (2007). Organic acids were determined using high-performance liquid chromatography (HPLC), according to the method described by Kung and Shaver (2001) for the determination of lactic, acetic, propionic, and butyric acids.

The other portion of the material (~0.5 kg) was weighed and dried in a forced ventilation oven at 55°C until it reached a constant mass. The samples were, then, ground in a knife mill with a 1 mm

sieve and stored in plastic containers. Subsequently, the chemical–bromatological characteristics of the silage were analyzed following the methodology described above for the *in natura* material.

Statistical analysis

The variables were subjected to an analysis of variance using the R program version R-3.1.1 (R Core Team, 2014) and the ExpDes package (Ferreira et al., 2014). Means were compared using Tukey's test at 5% probability.

To understand the cause-and-effect relationship between the variables, Pearson's correlation analyses (low: $r \leq 0.30$; moderate: $0.30 < r \leq 0.70$: and high $r > 0.70$) and trail analysis were performed, considering pH and IVDMD as dependent variables

FIGURE 3
Dry mass production of silage material from sorghum, Tamani guinea grass, and *Stylosanthes* cv. Bela in monocropped and intercropping. Means followed by different letters, differ by Tukey's test ($p < 0.05$). The vertical bars represent the standard error of the mean.
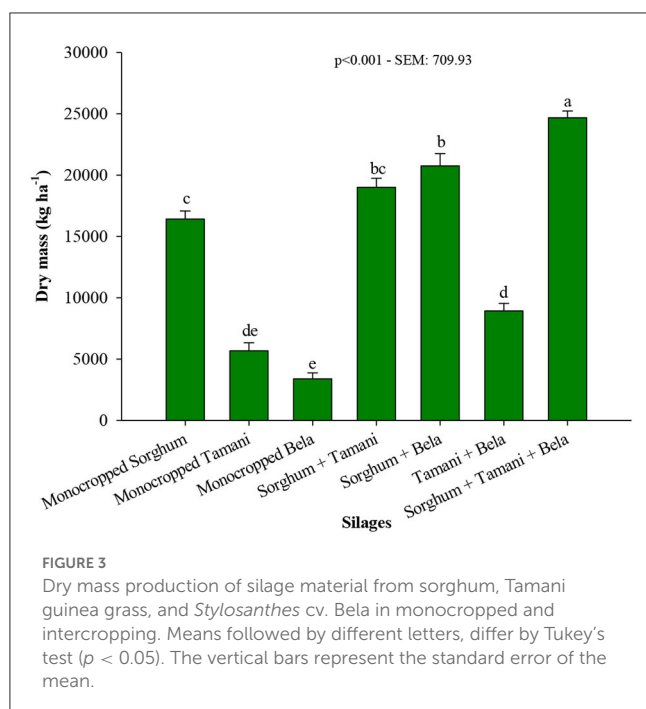
due to the importance of these variables for the fermentative profile and nutritional value of silages. To define the causal diagram, multiple linear regression analysis was performed using the "stepwise" procedure with the "backward" option (Coimbra et al., 2005; Charnet et al., 2008). Subsequently, multicollinearity was diagnosed based on the condition factor (ratio between the highest and lowest Eigenvalues), and the number of conditions (NC) < 100 was verified, indicating that multicollinearity is weak and does not constitute a problem for the analysis (Cruz et al., 2014).

The contributions of the direct and indirect effects of the variables were quantified as percentages. Contributions above 50% were considered high direct effect (Botelho et al., 2019; Ribeiro et al., 2019). For the statistical analysis, the "corrplot," "lavaan," and "semPlot" packages of the R development computational program were used.

Results

Dry mass production

The cropping systems had a significant impact ($p < 0.05$) on dry mass production for silage (Figure 3). The highest production was observed in the triple intercropping of sorghum + Tamani guinea grass + Bela, followed by the double intercropping of sorghum + Tamani guinea grass and sorghum + Bela. Monocropped forages exhibited lower silage mass production. The intercropping systems resulted in a 36.20% increase in silage mass in the triple intercrop and a 22.09% increase in the double intercrop compared with monocropped sorghum.

Fermentation characteristics

Fermentation characteristics, including pH, buffering capacity, dry matter (DM), and N-NH$_3$ content, were influenced by the different silages (Figure 4). Silages derived from Tamani guinea grass and *Stylosanthes* cv. Bela in both the monocropped and intercropping systems exhibited the highest pH values (4.42, 4.30, and 4.28, respectively). In the intercropping systems with sorghum, there was an average reduction of 10.5% in the pH values of the silage for sorghum + Tamani guinea grass + Bela, sorghum + Bela, and sorghum + Tamani guinea grass consortia compared with the Tamani guinea grass and *Stylosanthes* cv. Bela silages in monocropped systems. The sorghum silage displayed the lowest pH value (3.47). Similar results were observed for buffering capacity (Figure 4B), with a reduction of 13.9% for the triple and double intercrop silages with sorghum compared with the monocropped silages of the respective forages.
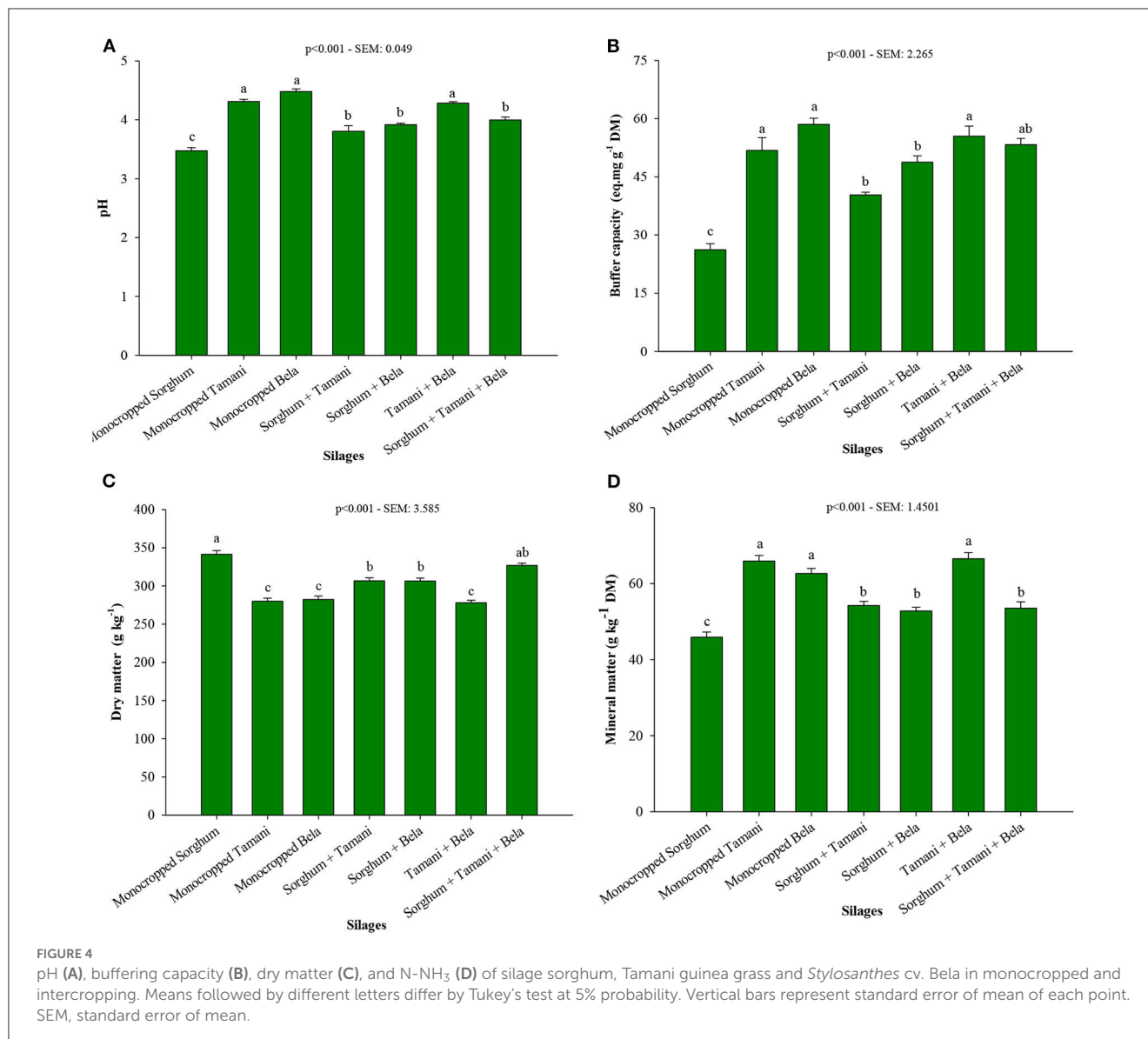
Sorghum silage exhibited the highest dry matter (DM) content (341.50 g kg^{-1}), followed by sorghum + Tamani guinea grass + Bela silage (326.91 g kg^{-1}; Figure 4C). Silages of sorghum + Tamani guinea grass and sorghum + Bela showed similar results, averaging at 306.64 g kg^{-1} DM. Monocropped silages of the forages displayed the lowest DM contents (279.99 g kg^{-1} for Tamani guinea grass and 282.35 g kg^{-1} for *Stylosanthes* cv. Bela, respectively).

The monocropped silage of *Stylosanthes* cv. Bela exhibited the highest N-NH$_3$ value (72.74 g kg^{-1} DM), followed by Tamani guinea grass silage in monocropped and Tamani guinea grass + Bela silage, with an average of 63.43 g kg^{-1} DM, which did not differ significantly from the silage of sorghum + Bela and sorghum + Tamani guinea grass + Bela consortia. The silages of sorghum in monocropped and sorghum + Tamani guinea grass had the lowest N-NH$_3$ values (39.85 and 47.80 g kg^{-1} DM, respectively; Figure 4D).

The silages of *Stylosanthes* cv. Bela in monocropped and Tamani guinea grass + Bela exhibited the highest dry matter losses (26.47 and 27.15 g kg^{-1} DM, respectively), followed by the silages of Tamani guinea grass in monocropped, sorghum + Bela, and sorghum + Tamani guinea grass + Bela, which did not differ significantly from the silage of sorghum + Tamani guinea grass. Sorghum silage showed the lowest DM losses at 13.30 g kg^{-1} DM (Figure 5A).

The highest effluent productions were observed in the silages of Tamani guinea grass (17.89 kg t^{-1} FM), *Stylosanthes* cv. Bela (17.44 kg t^{-1} FM), and *Stylosanthes* cv. Bela + Tamani guinea grass (18.39 kg t^{-1} FM; Figure 5B). Intercropping sorghum with forage crops contributed to an average reduction of 17.16% in effluent production compared with the silages of Tamani guinea grass and *Stylosanthes* cv. Bela in monocropped. Sorghum silage exhibited the lowest effluent production (11.42 kg t^{-1} FM).

Monocropped sorghum silage exhibited the highest lactic acid content (44.43 g kg^{-1} DM) (Figure 5C). In contrast, silages of Tamani guinea grass, monocropped *Stylosanthes*, and *Stylosanthes* cv. Bela + Tamani guinea grass had the lowest values, with an average of 19.31 g kg^{-1} DM. The intercropped systems with sorghum silage showed a 39.36% increase in lactic acid content compared with the silages of grass and legumes in monocropped and the Bela + Tamani guinea grass combination.

FIGURE 4

pH **(A)**, buffering capacity **(B)**, dry matter **(C)**, and N-NH$_3$ **(D)** of silage sorghum, Tamani guinea grass and *Stylosanthes* cv. Bela in monocropped and intercropping. Means followed by different letters differ by Tukey's test at 5% probability. Vertical bars represent standard error of mean of each point. SEM, standard error of mean.
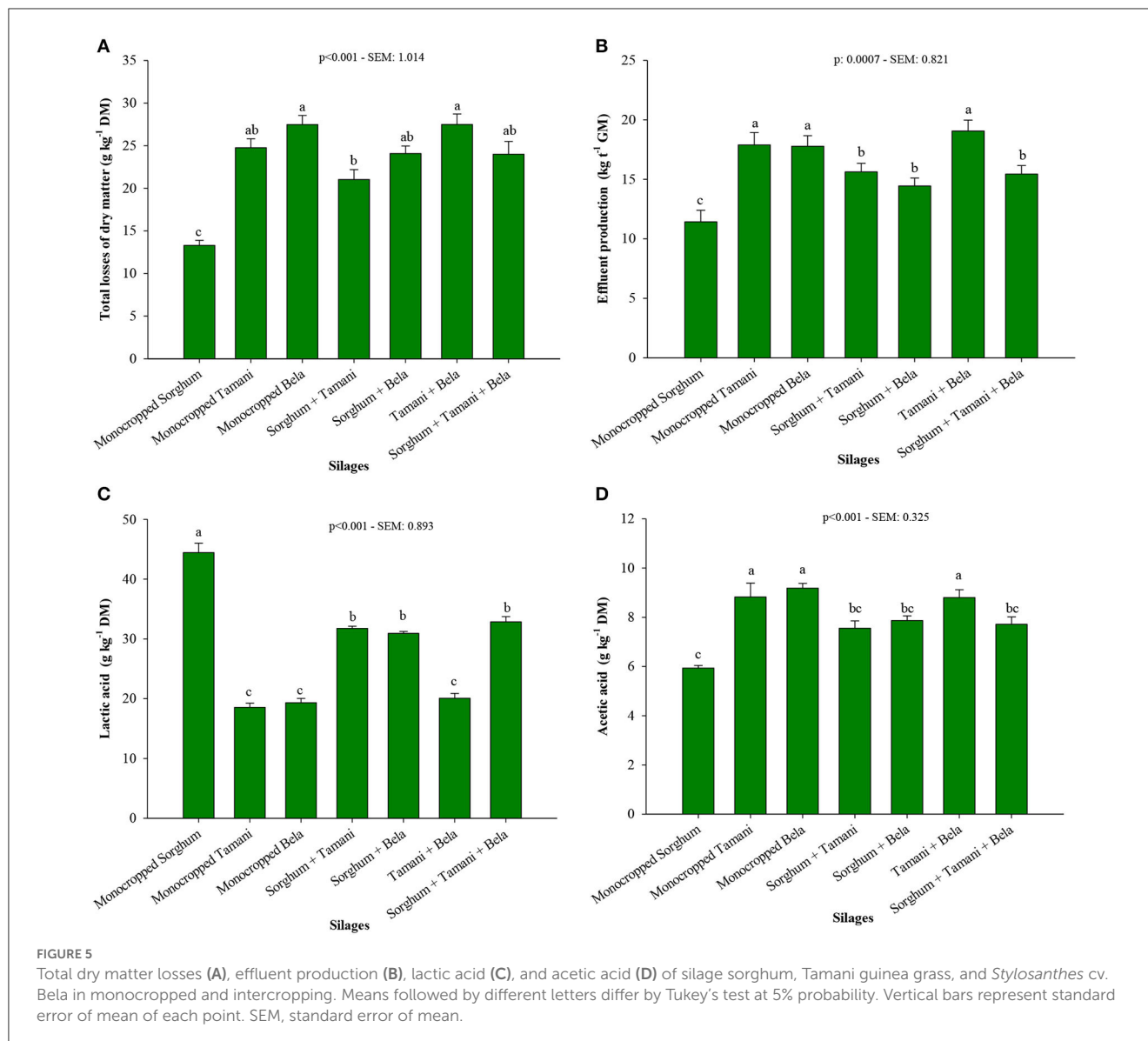
Silage of *Stylosanthes* cv. Bela and Tamani guinea grass in monocropped and the Bela + Tamani guinea grass combination exhibited the highest values of acetic acid (Figure 5D), with an average of 8.93 g kg^{-1} DM. On the other hand, silages from intercropped systems containing sorghum in their composition showed a 13.78% reduction in acetic acid. Sorghum silage in monocropped exhibited the lowest values (5.94 g kg^{-1} DM) of acetic acid.

Bromatological characteristics

Bromatological characteristics such as NDF, ADF, lignin, mineral matter, crude protein, ether extract, IVDMD, and TDN were significantly influenced by the different silages (Figures 6, 7). Monocropped sorghum silage exhibited the lowest NDF (542.08 g kg^{-1} DM) and ADF (297.90 g kg^{-1} DM) contents (Figures 6A, B). Conversely, the highest NDF (609.12 g kg^{-1} DM) and ADF

(336.20 g kg^{-1} DM) contents were observed in the monocropped Tamani guinea grass silage. Silages from intercropped systems (double and triple-cropped) showed intermediate values.

The monocropped sorghum silage exhibited the highest lignin content (Figure 6C) at 43.02 g kg^{-1} DM. On the other hand, the lowest levels were observed in the silages of *Stylosanthes* cv. Bela (26.59 g kg^{-1} DM) and Tamani guinea grass (27.69 g kg^{-1} DM) in monocropped and double-cropping systems (28.50 g kg^{-1} DM). The intercropping systems effectively reduced lignin concentration, with reductions of 10.52, 17.43, and 23.01% observed in the sorghum + Tamani guinea grass + Bela, sorghum + Tamani guinea grass, and sorghum + Bela silages, respectively, compared with monocropped sorghum silage. Regarding mineral matter (Figure 6D), the highest values were observed in the Tamani guinea grass + Bela (66.55 g kg^{-1}), Tamani guinea grass (65.91 g kg^{-1}), and *Stylosanthes* cv. Bela (62.66 g kg^{-1}) silages in monocropped. The double sorghum + Tamani guinea grass and sorghum + Bela, as well as the triple sorghum + Tamani guinea grass + Bela silages,

FIGURE 5
Total dry matter losses **(A)**, effluent production **(B)**, lactic acid **(C)**, and acetic acid **(D)** of silage sorghum, Tamani guinea grass, and *Stylosanthes* cv. Bela in monocropped and intercropping. Means followed by different letters differ by Tukey's test at 5% probability. Vertical bars represent standard error of mean of each point. SEM, standard error of mean.

showed a 16.2% reduction in mineral matter compared with the forage silages in monocropped.
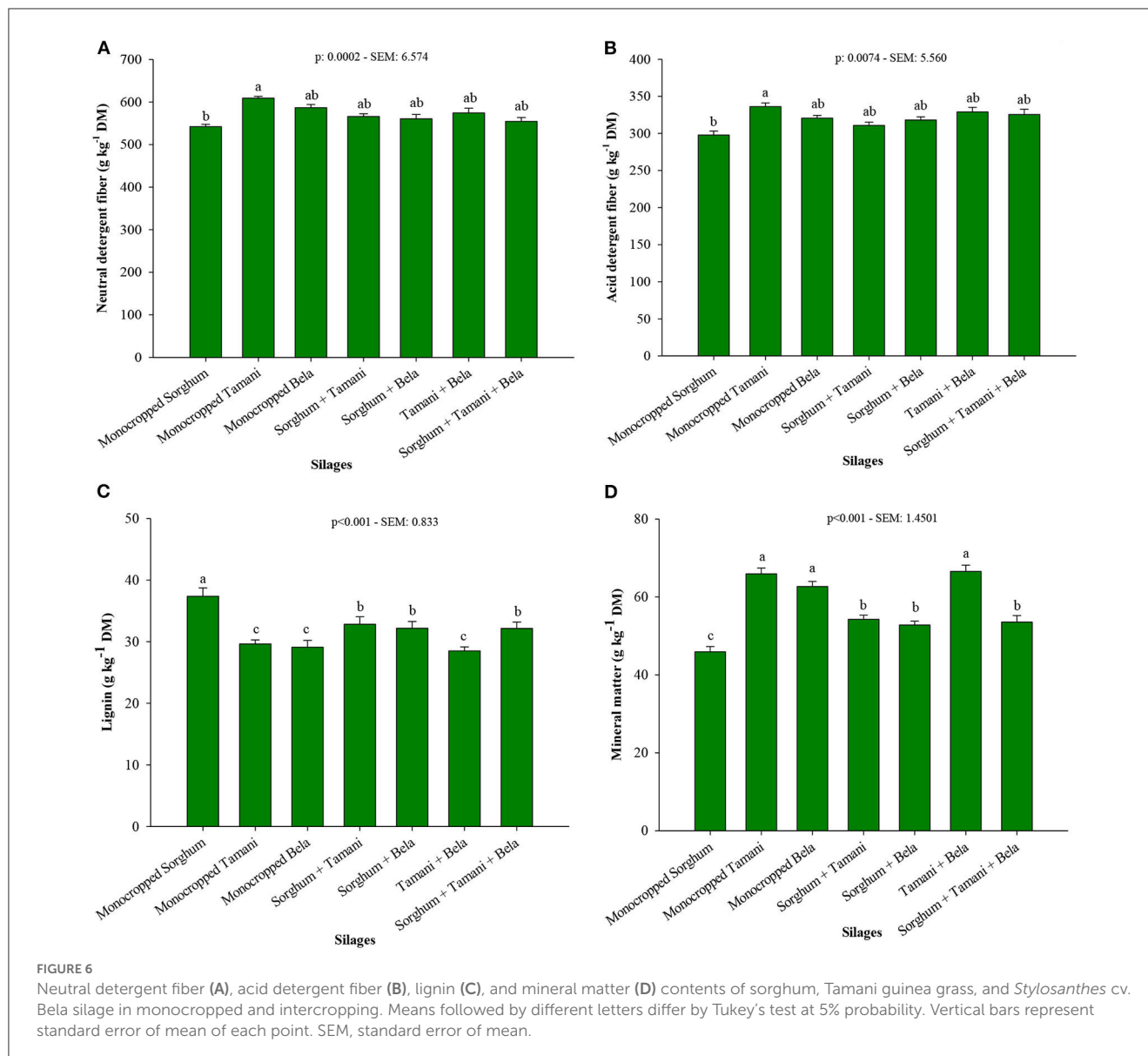
Silage of *Stylosanthes* cv. Bela in the monocropped had the highest crude protein content (152.33 g kg^{-1}), followed by *Stylosanthes* cv. Bela + Tamani guinea grass at 140.88 g kg^{-1} DM (Figure 7A). The silages of Tamani guinea grass in the monocropped, sorghum + Tamani guinea + Bela, and sorghum + Bela showed similar results, with an average of 126.43 g kg^{-1} DM. There was 35.32 and 41.88% increase in the crude protein content of the sorghum + Tamani guinea grass and sorghum + Bela double intercrop silages, respectively, and 43.54% for the triple intercrop silage when compared with the silage of sorghum in monocropped, which showed a crude protein content of 71.46 g kg^{-1} DM.

The sorghum silage had the highest ether extract content (40 g kg^{-1} DM; Figure 7B). There was 20.05, 32.4, and 32.4% reduction in the ether extract content for silages of sorghum + Tamani guinea grass + Bela silage, sorghum + Tamani guinea grass, and sorghum + Bela, respectively, compared with silage of sorghum

in the monocropped. Silages of *Stylosanthes* cv. Bela + Tamani guinea grass and Tamani guinea grass and *Stylosanthes* cv. Bela in monocropped showed the lowest ether extract values (20.32, 19.89, and 19.81 g kg^{-1} DM, respectively). The content of IVDMD (Figure 7C) was similar among the silages, with an average of 612.36 g kg^{-1} DM.

Silage of sorghum in monocropped showed the highest value of TDN (61.90 g kg^{-1} DM), followed by silage of sorghum + Tamani guinea grass, sorghum + Bela, and sorghum + Tamani guinea grass + Bela, showing increases of 5.8, 3.41, and 4.27%, respectively, for the intercrop silages compared with the silages of the forages in monocropped (Figure 7D).

In the correlation analysis (Figure 8), IVDMD was the only variable that did not show a correlation with any analyzed variable. Moreover, the formation of two groups of variables was observed: Group 1 was composed of NDF, N-NH$_3$, acetic acid, pH, CP, effluent production, buffering capacity, DM loss, and ADF, whereas Group 2 was composed of DM, lactic acid, EE, lignin, and TDN.

FIGURE 6
Neutral detergent fiber **(A)**, acid detergent fiber **(B)**, lignin **(C)**, and mineral matter **(D)** contents of sorghum, Tamani guinea grass, and *Stylosanthes* cv. Bela silage in monocropped and intercropping. Means followed by different letters differ by Tukey's test at 5% probability. Vertical bars represent standard error of mean of each point. SEM, standard error of mean.

The variables within the same group showed positive correlations and those between distinct groups showed negative correlations.

Through the causal diagram obtained from the multiple regression analysis using the "stepwise" procedure with the "backward" option, it was found that the variables IVDMD, ADF, TDN, CP, buffering capacity, EE, and lactic acid were maintained in the model to explain the pH, with significance for the first four variables and a coefficient of determination of 0.96 (Table 3). The model to explain IVDMD showed a coefficient of determination of 0.72 and was composed of the variables, such as pH, buffering capacity, ADF, NDF, TDN, and CP, all of which were significant in the model.

In the causal diagram of pH (Figure 9), it was verified by the decomposition of correlation that CP, ADF, and IVDMD had direct effects with contributions above 50% of the correlation coefficient and positive values for the first two variables and negative values for IVDMD (Table 3). The other variables showed low direct effects and a high correlation with pH. For the causal diagram of IVDMD
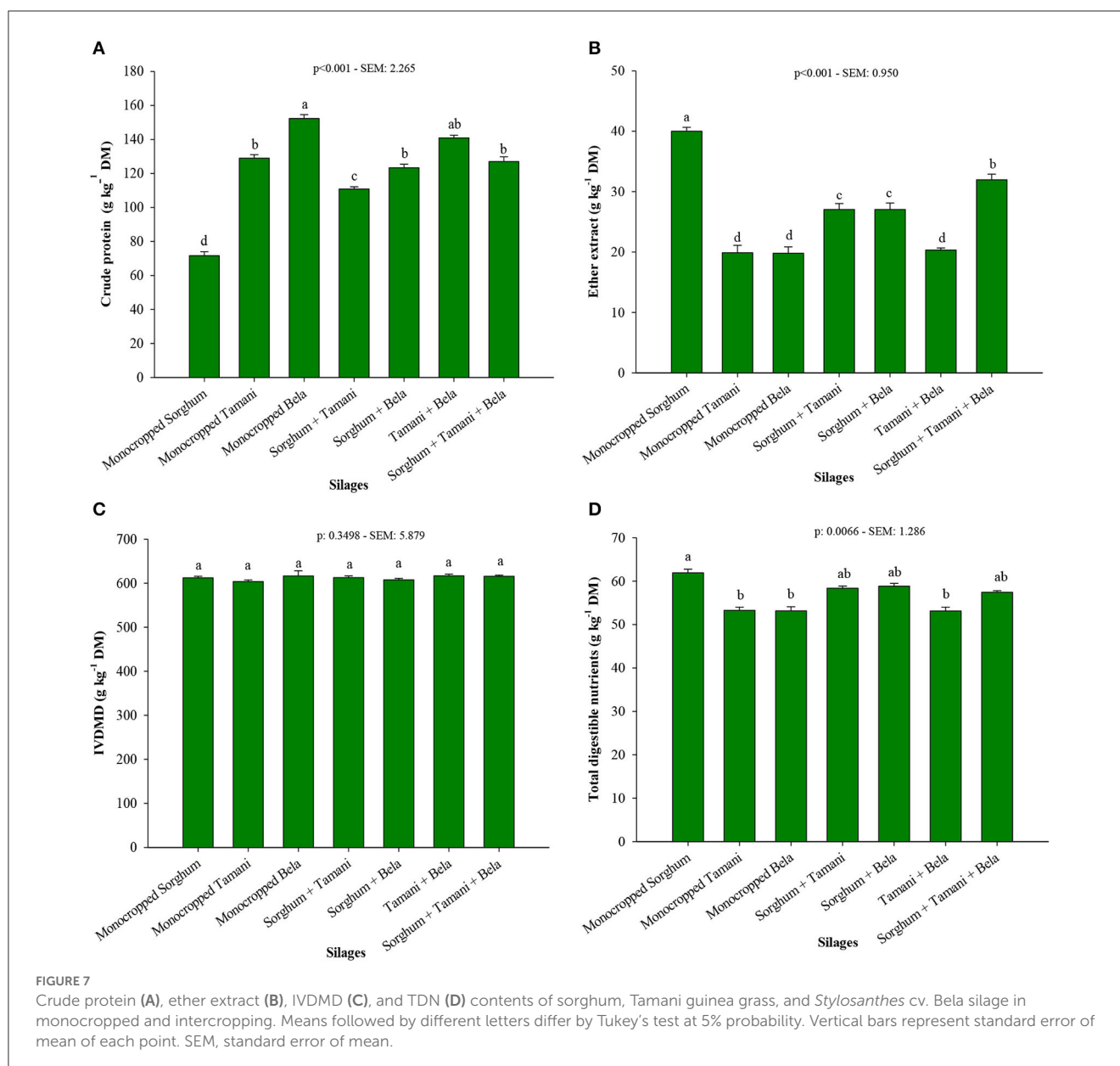
(Figure 10), there was a high contribution of direct effects to the correlation for pH, CP, NDF, and ADF, and the correlation was negative for all variables except ADF.

Discussion

Dry mass production

Integrated systems promote diversification of production and food production without the need for clearing new areas, making them a sustainable approach (Costa et al., 2016) that contributes to global food security (Sekaran et al., 2021).

In this study, the triple intercropping system demonstrated the highest dry mass production due to the simultaneous cultivation of three crops, enabling better utilization of the available land and resulting in increased silage yield. These findings highlight the benefits of intercropping annual and tropical forage crops

FIGURE 7
Crude protein **(A)**, ether extract **(B)**, IVDMD **(C)**, and TDN **(D)** contents of sorghum, Tamani guinea grass, and *Stylosanthes* cv. Bela silage in monocropped and intercropping. Means followed by different letters differ by Tukey's test at 5% probability. Vertical bars represent standard error of mean of each point. SEM, standard error of mean.

(grasses and legumes) for crop-livestock integration, as it enhances the yield of dry mass for silage compared with monocropped systems. Furthermore, after harvesting crops for silage production, the formed pasture can be utilized for low-cost animal grazing during the off-season when weather conditions are unfavorable for most crops (Santos et al., 2020). Therefore, this cultivation strategy is an effective means to improve land-use efficiency (Costa et al., 2016), particularly in tropical regions (Oliveira et al., 2020).

In addition to these advantages, legumes contribute to integrated systems by reducing the need for nitrogen application in grasses. Through biological nitrogen fixation, legumes increase the availability of this macronutrient in the soil, thereby enhancing the sustainability of cropping systems and reducing the reliance on mineral nitrogen fertilizers in the system (Bolson et al., 2022).

Fermentation characteristics

The findings of this study are highly significant in evaluating the fermentation characteristics and nutritional value of silage produced in integrated systems. Legumes and grasses possess a high buffering capacity, low soluble carbohydrate concentration, and low dry matter content, which makes it challenging to lower the pH during ensiling (Hawu et al., 2022). This explains the observed results for Tamani guinea grass and *Stylosanthes* cv. Bela silages in monocropped and combined (Bela + Tamani guinea grass) systems, where the pH stabilized above 4.2 (Figure 4A). Such pH values promote the growth of undesirable microorganisms during silage fermentation, thereby compromising the final quality (Kung et al., 2018).

On the other hand, the silages from intercropped systems with sorghum were effective in achieving pH reduction, displaying

values within the recommended range of 3.7–4.2 for good-quality silage classification, as suggested by Mcdonald et al. (1991). Grasses such as sorghum and maize exhibit good stability due to their adequate levels of soluble carbohydrates and lactic acid, which

contribute to pH reduction when ensiled with appropriate levels of dry matter (Rodrigues et al., 2020).

The higher buffering capacity of *Stylosanthes* cv. Bela and Tamani guinea grass silages in monocropped and combined systems (*Stylosanthes* cv. Bela + Tamani guinea grass; Figure 4B) can be attributed to the inherent buffering capacity of forage grasses and legumes compared with sorghum silage in

FIGURE 8
Pearson's correlation between the variables of the fermentation profile and nutritional value of silages of sorghum, Tamani guinea grass, and *Stylosanthes* cv. Bela in monocropped and intercropping systems.

FIGURE 9
Causal diagram showing the correlations between the independent variables and the cause−effect relationships with pH.
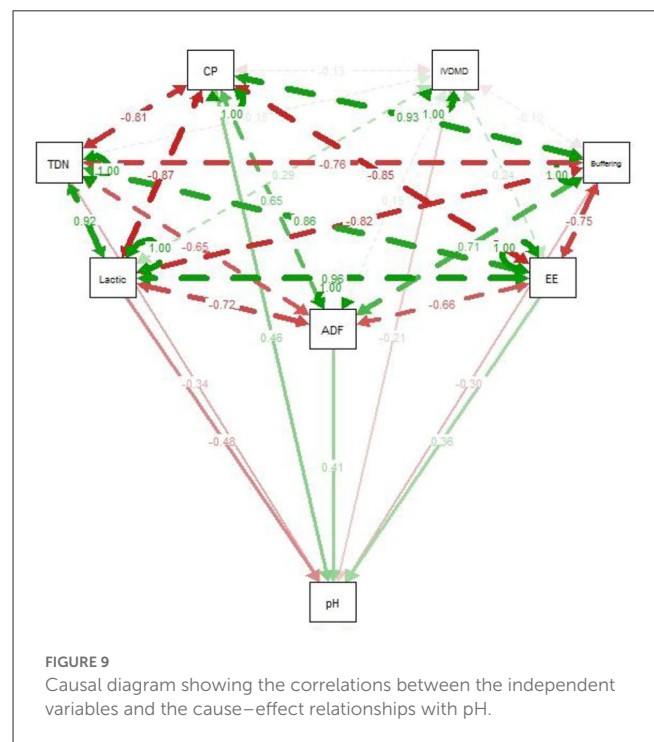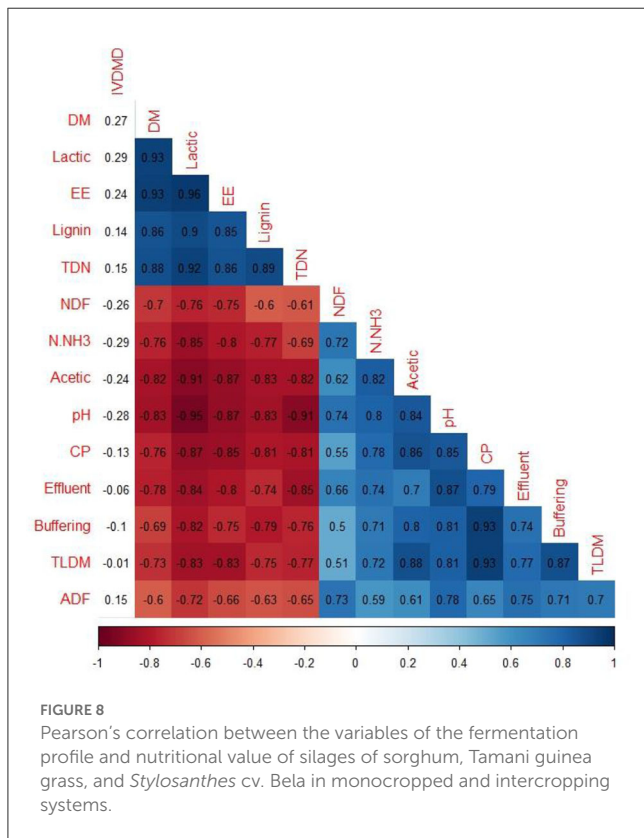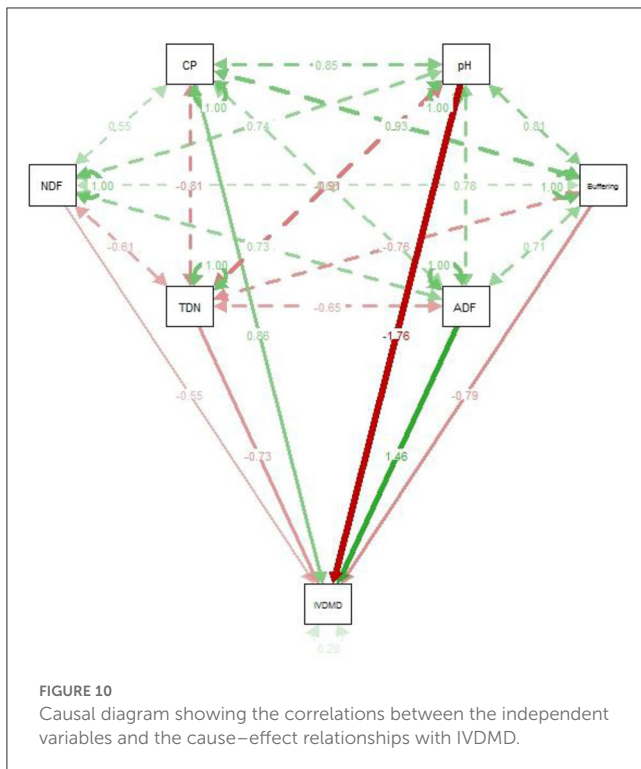
TABLE 3 Direct and indirect effects, correlation, and coefficient of determination of the causal models.

Variables dependent	Variables independent	Effect direct		Effect indirect		Correlation	R^2
		Coefficient	%	Coefficient	%		
pH	IVDMD	−0.212*	75.71	−0.0680	24.29	−0.28	0.97
	Buffering	−0.303	21.40	1.1130	78.60	0.81	
	EE	0.365*	22.81	−1.2350	77.19	−0.87	
	ADF	0.408*	52.31	0.3720	47.69	0.78	
	Lactic	−0.475	50.00	−0.4750	50.00	−0.95	
	TDN	−0.340*	37.36	−0.5700	62.64	−0.91	
	CP	0.461*	54.24	0.3890	45.76	0.85	
IVDMD	pH	−1.759*	54.32	1.4790	45.68	−0.28	0.72
	Buffering	−0.788*	53.39	0.6880	46.61	−0.10	
	CP	0.858*	46.48	−0.9880	53.52	−0.13	
	NDF	−0.553*	65.37	0.2930	34.63	−0.26	
	ADF	1.459*	52.71	−1.3090	47.29	0.15	
	TDN	−0.732*	45.35	0.8820	54.65	0.15	

%, percentage of contribution; *significance at 5%.
IVDMD, *in vitro* dry matter digestibility; buffering, buffering capacity; EE, ether extract; ADF, acid detergent fiber; lactic, lactic acid; TDN, total digestible nutrients; CP, crude protein; NDF, neutral detergent fiber.

FIGURE 10
Causal diagram showing the correlations between the independent variables and the cause−effect relationships with IVDMD.

monocropped systems (Oliveira et al., 2020). This increased buffering capacity makes them more susceptible to proteolysis during the fermentation process, impeding pH reduction and resulting in inadequate forage preservation (Gomes et al., 2021). The intercropping of sorghum with forage crops contributed to a reduction in buffering capacity due to the higher proportion of sorghum in the ensiled mass (Table 1). Similar findings were reported by Oliveira et al. (2020), who evaluated silage production of sorghum intercropped with Paiaguas palisadegrass and observed that a higher proportion of sorghum effectively reduced the buffering capacity of monocropped grass silage and contributed to pH reduction. Studies by Carvalho et al. (2016) and Aloba et al. (2022) also observed improvements in the fermentation characteristics of monocropped legume silage with the inclusion of sorghum.

The dry matter (DM) content of the ensiled material significantly impacts the final silage quality as it affects material compaction and the fermentation process (Teixeira et al., 2021). Recommended DM values for good-quality silage classification range from 300 to 350 g kg^{-1} DM (Mcdonald et al., 1991), indicating that the fermentation process of silages from intercropping systems with sorghum occurred adequately. However, the silages of monocropped forage crops exhibited DM contents below 300 g kg^{-1} (Figure 4C), leading to increased effluent production and higher activity of Clostridium bacteria, compromising silage quality (Muck and Shinners, 2001). Additionally, as previously mentioned, tropical forage silages in monocropped systems without additives can display inadequate fermentation characteristics (Oliveira et al., 2020; Hawu et al., 2022). These results underscore the importance of silage production in integrated systems, as intercropping can

reduce inadequate fermentation characteristics and increase the DM concentration of grass and legume silages, ensuring proper preservation of the ensiled material.

N-NH$_3$ is a parameter used to assess the quality of the silage fermentation process because an increase in its production can neutralize the acids necessary for the proper fermentation of the ensiled material (Veriato et al., 2018). Silages with adequate fermentation, as defined by Kung et al. (2018), should have values below 100 g kg^{-1} of N-NH$_3$, similar to the results observed in this study. Silages from intercropping systems with sorghum were more effective in reducing N-NH$_3$ compared with silages of grass and legumes in monocropped and combination systems. Sorghum contains a sufficient amount of soluble carbohydrates and represents the largest proportion in intercropped silages (Table 1), enabling proper fermentation and lower nutrient loss, which aligns with the findings of Ni et al. (2018) and Li et al. (2022).

Higher DM losses were observed in Stylosanthes cv. Bela in monocropped and Bela + Tamani guinea grass systems (Figure 4A) due to the higher moisture content and lower DM content of legumes and grasses at the time of cutting for ensiling compared with monocropped sorghum (Table 2). Tropical forages typically have higher moisture content than annual crops at harvest (Bernardes et al., 2018). Additionally, high water activity and low concentrations of soluble carbohydrates can contribute to DM losses during secondary fermentation (Borreani et al., 2018).

Silage production should be optimized to achieve favorable fermentation patterns and preserve the nutritive value of the ensiled material, ultimately providing high-quality feed. One key management strategy to attain this objective is to minimize forage losses (Köhler et al., 2019). In the current study, silages from intercropping systems with sorghum demonstrated the potential in reducing total DM losses in tropical forage silages due to the higher proportion of sorghum (Table 1) incorporated in these silages, along with an appropriate DM content (340.66 g kg^{-1}) at harvest.

Regarding effluent production, monocropped Tamani guinea grass and Stylosanthes cv. Bela silages, as well as the intercropping of Bela + Tamani guinea grass, exhibited the highest effluent production (Figure 5B) due to the high moisture content typically present in grasses and legumes at harvest, as previously mentioned. High effluent production leads to nutrient losses through leaching, which can result in nutritional degradation of the feed and potential environmental contamination (Araújo et al., 2020).

The intercropping systems with sorghum exhibited a significant reduction in effluent production, which was directly influenced by the higher proportion of sorghum present in these silages (Table 1). The high DM content of sorghum efficiently absorbed excess moisture from Tamani guinea grass and Stylosanthes cv. Bela, highlighting the close relationship between effluent production and the DM content of the ensiled material (Paludo et al., 2020). These results underscore the importance of silage production in intercropping systems for enhancing the fermentation process of tropical forage silages in monocropped systems and ensuring proper conservation of the ensiled material.

Silage production is a microbial fermentation process in which the lactic acid bacterial community plays a crucial role. These bacteria quickly establish dominance by consuming nutrients in the silage, creating a low-pH environment that inhibits the growth of undesirable protease and other bacterial communities, thus

facilitating an adequate fermentation process (Wang et al., 2021). In this context, silages from intercropping systems with sorghum were more effective in increasing lactic acid values compared with silages of tropical forages in monocropped and combination systems, as shown in Figure 5C. Similar findings were reported by Meng et al. (2022), who evaluated the effects of different proportions of soybean and maize in an intercropping system and observed that intercropping enhanced the microbial community, particularly *Lactobacillus* and *Weissella* (co-producers of lactic acid), thereby improving fermentation and silage quality.

The silages of monocropped forages and their combination exhibited higher acetic acid values (Figure 5D). The intercropping systems resulted in a 13.78% reduction in acetic acid compared with the silages from monocropped and combination forages. However, the observed acetic acid values in all produced silages did not exceed $20 \, g \, kg^{-1}$ DM, which is considered suitable for classifying silage quality and ensuring proper preservation of the ensiled material, as reported by Kung et al. (2018). Therefore, the production of acetic acid did not negatively affect the stability of the silages, and the predominant production of lactic acid ensured the adequate preservation of the ensiled material (Oliveira et al., 2020).

Bromatological characteristics

Regarding the fibrous fractions (Figure 6), the NDF content represents the hemicellulose, cellulose, and lignin fractions, while the ADF content represents the cellulose and lignin fractions. These two fractions are important indicators of forage quality, with lower levels indicating better quality forage (Umesh et al., 2022), as they are negatively correlated with ruminant intake and digestibility (Tang et al., 2018). NDF values above $600 \, g \, kg^{-1}$ DM can reduce dry matter consumption due to rumen filling (Paludo et al., 2020). For ADF, values above $400 \, g \, kg^{-1}$ DM reduce fiber digestibility due to the unavailability of degradable structural carbohydrates, and the lignin content in the material hinders the adherence of rumen microbiota and the subsequent enzymatic hydrolysis of components such as cellulose and hemicellulose (Van Soest, 1994). In the present study, the ADF and NDF contents of all silages were lower than those previously reported.

The silages from the intercropping systems demonstrated the potential to reduce the fibrous fractions of the silages due to the lower NDF and ADF levels in sorghum (Table 2), resulting in the production of high-quality feed. Supporting these results, Oliveira et al. (2020) also observed a dilution of NDF and ADF contents when sorghum was intercropped with guava grass and when a higher proportion of sorghum was included in the silage. However, monocropped sorghum silage had the highest lignin content (Figure 6C). This can be attributed to the higher proportion of stalks in the crop, which is responsible for higher lignin accumulation (Oliveira et al., 2021).

The silage of Tamani guinea grass and *Stylosanthes* cv. Bela in monocropped and combination showed the lowest lignin contents due to the higher leaf blade-to-stalk ratio of Tamani guinea grass (Muniz et al., 2022), which contributed to the low accumulation of lignin in the forage and allowed for better digestibility (Paludo et al., 2020). Additionally, legumes such as *Stylosanthes* cv. Bela,

which have herbaceous growth habit characterized by soft stems and a high number of leaves, have reduced lignin content (Castro-Montoya and Dickhoefer, 2020).

Thus, in the present study, it was observed that the double consortia of sorghum + Bela and sorghum + Tamani guinea grass, as well as the triple intercropping of sorghum + Tamani guinea grass + Bela, was efficient in diluting the lignin content of monocropped sorghum silage when ensiled. This emphasizes the importance of intercropping in the production of high-quality silage.

Silage production using integrated production systems is of fundamental importance for improving the nutritional characteristics of traditional maize and sorghum silage. The intercropping of cereals, grasses, and/or legumes primarily aims to increase the crude protein content (Ligoski et al., 2020) of the ensiled mass (Oliveira et al., 2020), as well as to enhance nitrogen use efficiency through biological fixation and minimize the use of mineral fertilizers (N), thereby reducing potential environmental impacts (Zhang et al., 2022) and promoting greater sustainability in food production.

The higher mineral matter content observed in the silages of Tamani guinea grass and Bela in monocropped and mixed systems is attributed to their lower DM content, higher pH, buffering capacity, and N-NH$_3$ levels (Table 2). These factors contribute to an inadequate fermentation process with DM losses during fermentation, resulting in increased mineral matter content (Oliveira et al., 2020). In contrast, the higher proportion of sorghum in the intercropped silages (Table 1) leads to a reduction in mineral matter, as cereals generally have a low mineral matter content (Paula et al., 2016).

In the present study, we observed that the double consortia of sorghum + Tamani guinea grass, sorghum + Bela, and Bela + Tamani guinea grass, as well as the triple intercropping (sorghum + Tamani guinea grass + Bela), were more effective in increasing the crude protein content of the silage compared with sorghum silage in monocropped systems. Intercrops that included legumes had higher crude protein content than those with Tamani guinea grass (Figure 7A). The inclusion of Tamani guinea grass and *Stylosanthes* cv. Bela in the silage resulted in an increase in crude protein levels, with respective values of 130.80 and $155.35 \, g \, kg^{-1}$ DM. Thus, silages from integrated systems serve as not only an efficient alternative to increase silage mass production per unit area (Souza et al., 2019) but also a cost-effective approach as they maximize nutrient production per unit area in a sustainable manner (Umesh et al., 2022).

In addition to the nutritional benefits for animals, intercropping systems of grasses and legumes offer agronomic advantages such as reducing insects and pests, producing biomass for no-till farming systems, and lowering fertilizer costs through nutrient cycling, particularly with the presence of legumes that can supply adequate amount of nutrients to the soil-plant system (Ligoski et al., 2020; Bourscheidt et al., 2023). These systems also contribute to pasture recovery (Santos et al., 2020), providing sufficient high-quality feed for animals during the dry season. After forage regrowth, the established pasture can be utilized by animals (Oliveira et al., 2020), facilitating the sustainable intensification of the production system (Herrera et al., 2023).

The higher ether extract content observed in monocropped sorghum silage is attributed to the higher fat content in sorghum grains (Oliveira et al., 2021). These findings further support the observations of Bueno et al. (2020), who reported that well-preserved silages exhibit similar ether extract levels to those found in the original ensiled material. The IVDMD contents were similar among the studied silages, with an average value of 612.36 g kg^{-1}. IVDMD can be used to assess the nutritional value and animal feed intake (Xie et al., 2022) and is considered one of the main determinants of forage quality (Daniel et al., 2019).

Silages from the intercropping systems demonstrated efficiency in increasing ether extract and TDN contents compared with silages from monocropped and combined forages, primarily due to the higher proportion of sorghum present in the material (Table 1). The increase in TDN content associated with sorghum in the intercrop was also observed by Ribeiro et al. (2017). It is worth noting that TDN content plays a crucial role in ruminant production as it, along with protein, can be a limiting factor (Oliveira et al., 2020).

Positive correlations between variables within the same group indicate a direct relationship, meaning that an increase in one variable leads to an increase in another, either directly or indirectly through other variables. On the other hand, negative correlations between variables from different groups indicate an inverse relationship, where an increase in one variable causes a reduction in the other and vice versa. Therefore, these results contribute to a better understanding of the relationship between the fermentation profile and the nutritional value of silage (Htet et al., 2021).

The regression models obtained for pH and IVDMD showed high coefficients of determination ($R^2 > 0.70$), indicating that the independent variables included in the models are significant in determining pH and IVDMD. It is recommended to use regression models for bromatological variables when R^2 is higher than 0.70 (Paludo et al., 2020; Rodrigues et al., 2020). Previous studies in the literature have also reported trail analyses with R^2 values below 0.7, providing support for conducting this analysis (Crevelari et al., 2020; Ligoski et al., 2020).

The causal diagram of pH revealed high direct effects on CP, ADF, and IVDMD contents, suggesting that these variables are less influenced indirectly by other variables in their correlations with pH. However, despite having the highest percentage of direct effects, IVDMD showed a non-significant correlation with pH. Therefore, the variables with the greatest impact on pH were CP and ADF contents. On the other hand, CP, EE, lactic acid, and TDN exhibited low direct effects but had high correlations with pH, indicating that their effects occur indirectly through other variables in the model. Consequently, their inclusion is of limited importance in determining the effects of independent variables on pH.

The high direct effects of pH, CP, NDF, and ADF, along with their low correlation with IVDMD, indicate that these independent variables can provide significant benefits for estimation purposes when used in analyses together with other independent variables. However, they should not be relied upon solely. On the other hand, CP and TDN exhibited weak direct effects and correlations with IVDMD, suggesting that these variables have limited utility in the causal model involving IVDMD. These findings imply that the bromatological variables studied have minimal interference with IVDMD. Future research should explore other bromatological variables or different crops to determine how IVDMD can be improved, as it is a crucial variable in animal production.

Thus, our study highlights the importance of integrated systems, particularly the triple intercropping of annual and tropical forage crops (grasses and legumes), as a promising technique for silage production and the restoration of degraded areas or establishment of new pastures (Santos et al., 2020). Furthermore, this system ensures sustainable food production (Simões et al., 2023), mitigates greenhouse gas emissions (Eugène et al., 2021), promotes greater soil carbon sequestration, diversifies production (Bourscheidt et al., 2023), and reduces costs, particularly those associated with mineral nitrogen fertilizer inputs in the system (Bolson et al., 2022).

Conclusion

In conclusion, our study supports the use of sorghum intercropped with tropical forages in integrated silage production systems. This approach enhances land-use efficiency by increasing the production of ensiled mass per area and providing pasture after crop harvest. The intercropping improves fermentation characteristics and increases EE and TDN contents in monocropped forage silages. Additionally, the inclusion of tropical forages in sorghum silage reduces the need for protein salts in ruminant feed, leading to cost savings. Overall, the triple intercropping of sorghum + Tamani guinea grass + Bela is recommended for the production of high-quality silage and annual and tropical forage crops.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

LP, KC, and LS wrote the manuscript. LP, LS, JC, and JS collected data in the field and processed the data. KC, AC, EH, and ES conceived and designed the experiments. All authors contributed to the revision of the manuscript and approved the submitted version.

Acknowledgments

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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