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Crop-livestock-forestry systems as a strategy for mitigating greenhouse gas emissions and enhancing the sustainability of forage-based livestock systems in the Amazon biome

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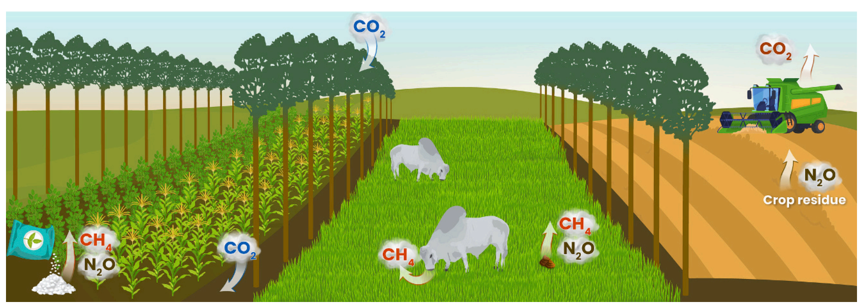
HIGHLIGHTS

- Integrated systems increased meat and grain production and offset GHG emissions.
- Systems with crops produced 3 times more human-edible protein.
- Systems with crops incorporated more 270 kg N/ha over four years.
- Forestry systems can sequester from 15.9 to 20.4 Mg CO_2eq/ha/year.

GRAPHICAL ABSTRACT



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ABSTRACT

Intensification of livestock systems becomes essential to meet the food demand of the growing world population, but it is important to consider the environmental impact of these systems. To assess the potential of forage-based livestock systems to offset greenhouse gas (GHG) emissions, the net carbon (C) balance of four systems in the Brazilian Amazon Biome was estimated: livestock (L) with a monoculture of Marandu palisade grass [*Brachiaria brizantha* (Hochst. ex A. Rich.) R. D. Webster]; livestock-forestry (LF) with palisade grass intercropped with three rows of eucalyptus at 128 trees/ha; crop-livestock (CL) with soybeans and then corn + palisade grass, rotated with livestock every two years; and crop-livestock-forestry (CLF) with CL + one row of eucalyptus at 72 trees/ha. Over the four years studied, the systems with crops (CL and CLF) produced more human-edible protein than those without them (L and LF) (3010 vs. 755 kg/ha). Methane contributed the most to total GHG emissions: a mean of 85 % for L and LF and 67 % for CL and CLF. Consequently, L and LF had greater total GHG emissions (mean of 30 Mg CO_2eq/ha/year). Over the four years, the system with the most negative net C balance (i.e., C storage) was LF when expressed per ha (−53.3 Mg CO_2eq/ha), CLF when expressed per kg of carcass (−26 kg CO_2eq/kg carcass), and LF when expressed per kg of human-edible protein (−72 kg CO_2eq/kg human-edible

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protein). Even the L system can store C if well managed, leading to benefits such as increased meat as well as improved soil quality. Moreover, including crops and forestry in these livestock systems enhances these benefits, emphasizing the potential of integrated systems to offset GHG emissions.

1. Introduction

Increasing global food production is essential since the world population is projected to reach 9.7 billion by 2050 (UNDESA, 2022). At the same time, environmental impacts of agriculture need to be mitigated, since it contributes 23 % of total anthropogenic greenhouse gas (GHG) emissions (IPCC, 2019a). Understanding how agriculture contributes to climate change helps identify ways to mitigate emissions while ensuring food security and promoting sustainable agricultural practices. Moreover, it can help support alternatives aligned with the goals of the Glasgow Climate Pact to limit the increase in global warming (UNFCCC, 2022a) and Brazil's Nationally Determined Contributions (UNFCCC, 2022b).

Livestock production is a significant source of GHG emissions, contributing 14.5 % of total global emissions, with 40 % of these emissions coming from enteric methane (CH_4) (Gerber et al., 2013). This sector plays a key role in the Brazilian economy by contributing to the world's demand for animal protein, since Brazil is the world's second-largest producer of beef (FAO, 2022). Therefore, it becomes necessary to establish sustainable strategies that increase productivity while simultaneously reducing greenhouse gas (GHG) emissions, especially enteric CH_4 (Berchielli et al., 2012).

Forage-based systems for producing beef cattle offer a promising solution as it have lower environmental impacts and decrease feed-food competition (Dumont et al., 2020; Mottet et al., 2018). Also, forage-based livestock systems have been combined with crop and forestry production as an alternative, leading to more sustainable livestock systems (Carvalho et al., 2019; Domiciano et al., 2020; Silva et al., 2020).

Since 2005, the adoption of integrated systems (i.e. livestock-forestry, crop-livestock, or crop-livestock-forestry) in Brazil has increased from 1.9 to >17 million ha in 2021 (ILPF Network, 2021). These systems have the potential to sequester carbon (C) through the combined effects of enhanced soil organic matter and the presence of trees that capture atmospheric C (Nascimento et al., 2018). In addition, the tree shade lowers soil temperatures, reducing the activity of microorganisms and thus the decomposition of organic compounds, which is responsible for C loss from the soil, mainly as carbon dioxide (CO2) (Hoosbeek et al., 2018). Strategically placing trees also improves animal welfare by providing shade, regulating heat production and energetic metabolism of the animal, and impacting enteric CH_4 production (Brower, 1965). Rotations with crops help increase forage productivity and quality due to the use of residual fertilizer applied to the crops (Carvalho et al., 2019), which increases nutrient cycling and soil organic matter.

Improving our understanding of the dynamics of integrated systems may support the development of solutions that benefit agriculture and contribute to the rational use of natural resources. To our knowledge, no study has examined the net C balance (i.e., total GHG emissions minus C sequestration) of integrated systems at the edge of the Brazilian agricultural frontier (Amazon biome). We hypothesized that integrated systems have lower GHG emissions, which results in a neutral or negative net C balance. To test this hypothesis, four forage-based integrated systems were examined as case studies in a field experiment, and their net C balances were estimated and compared. The objectives of the present study were (1) to estimate GHG emission of 4 production systems; and (2) to assess the potential of integrated forage-based livestock systems to offset GHG emissions.

2. Materials and methods

2.1. Description of the data, system boundaries, and experimental management

The boundary for this study was a 4-year cycle (Fig. 1) that contained four annual cycles of beef cattle [from yearling (~340 kg BW) to slaughter (~490 kg BW)]. The GHG emissions were calculated using IPCC methodology (IPCC, 2019a) and country-specific equations, combined with the dataset for the large-scale integrated-system experiments developed at the Embrapa Agrossilvipastoril, Sinop, Mato Grosso, Brazil.

The experiment was performed in a randomized complete block design with four systems (treatments) and four replicates (blocks), for a total of 16 experimental units. Each plot had an area of 2 ha (200 m east-west × 100 m north-south), for a total of 32 ha of experimental area.

The systems evaluated were livestock (L), livestock-forestry (LF), crop-livestock (CL), and crop-livestock-forestry (CLF) (Fig. 1) (Magalhães et al., 2019). Marandu palisade grass [*Brachiaria brizantha* (Hochst. ex A. Rich.) R. D. Webster] was used as the forage source for all systems. In L, palisade grass was planted as a monoculture (control). In LF, palisade grass was intercropped with three rows of eucalyptus trees (*Eucalyptus urograndis*; a hybrid of *Eucalyptus grandis* W. Hill ex Maiden and *Eucalyptus urophylla* S. T. Blake), spaced 3 × 3.5 × 30 m, yielding 135 trees/ha, but due to some failures the real density was 128 trees/ha. System CL consisted of rotating the crop (no-till) and livestock components every two years. The crop component consisted of soybean (October–February), followed by corn (February–July), intercropped with palisade grass (July–October). After two years, the livestock component, managed in the same way as that for L, was introduced. System CLF followed the same crop and livestock rotation as CL but with one row of eucalyptus spaced every 3 × 37 m, yielding 90 trees/ha, but due to some failures the real density was 72 trees/ha. In LF and CLF, the trees were approximately 4 years old at the beginning of the experiment.

In all systems, grazing was managed using continuous stocking, with a variable stocking rate (Allen et al., 2011) using Nellore steers (*Bos taurus indicus*). The cattle were divided into two groups: testers, which remained on pasture during the entire experiment, and grazers, which maintained the target canopy height of 30 cm ± 5 (Carvalho et al., 2019). These animals received a protein supplement (82 % ground corn, 5 % sodium chloride, 5 % calcium carbonate, 5 % urea, and 3 % calcium phosphate) calculated as 0.1 % of body weight (BW) in the first year and 0.2 % of BW, the second year on.

The animal production dataset was generated from June 2015 to May 2018, including both dry and rainy seasons. The animals were weighed every 28 days after 16-h fasting (feed and water). The stocking rate was determined by dividing the average weight of the animals (testers and grazers) by the number of days in the experimental units (Petersen and Lucas, 1968). For the average daily gain (ADG) calculation, only testers were used. Values for the fourth year of the livestock component were estimated as means of the three previous years.

Forage mass was quantified every 28 days. In the L and CL systems, four exclosure cages (0.64 m^2 and 1.2 m tall) were strategically positioned within each experimental unit. In the LF and CLF systems, eight exclosure cages were placed, four on the north and four on the south face of the central rows of trees. Two cages were positioned at 7.5 m and two at 15 m. Forage mass was measured by clipping all vegetation to soil level inside a circular quadrat (0.64 m^2) in a paired site with a similar canopy height (Carvalho et al., 2019; Silva et al., 2020).

To assess the nutritive value, forage samples were collected using the hand-plucked technique. Within each experimental unit, 50 representative sites with average canopy conditions were chosen. Subsequently, the collected samples were dried at 55 °C in a forced-circulation dryer, ground to pass through a 1-mm screen, and subjected to analysis to determine crude protein (CP; AOAC, 1990) and neutral detergent fiber (NDF; Van Soest et al., 1991).

To determine the amount of grain produced by soybean and corn, the crops were manually harvested in two adjacent rows, each 5 m long (4.5 m^2). In systems with trees, the same area was harvested at 4, 7.5, and 15 m from the rows on the northern and southern sides, and used to calculate the plot average. and then mechanically threshed assuming a baseline moisture content of 13 % (Magalhães et al., 2020). The details of each system, including the external inputs and production parameters associated with cattle, crops, and eucalyptus, varied (Tables 1 and S6).

The GHG estimated were CH_4 from enteric fermentation and manure, direct N_2O emissions (from manure, fertilizer, and crop residues), indirect N_2O emissions (from volatilization and leaching), and CO_2 emissions from the production of supplements, fertilizers, the electricity consumed, and combustion of diesel fuel for machinery (Fig. 2). Most of the calculations were conducted using data collected on field (primary data), such as animal performance, forage and crop production, tree height, soil carbon stock.

Emissions from seed and pesticide production were excluded since they were assumed to be negligible (Cardoso et al., 2016; Ruviaro et al., 2015). Emissions from tree planting, transport of animals and grain to or from the system, and fields and the slaughterhouse were also excluded. The system's output was limited to meat and grain, since the trees were not harvested during the experiment, and timber production was not considered. The analysis considered only emissions during the 4-year cycle.

The emissions were summed over the 4-year cycle and expressed as kg CO_2eq per ha and, to consider carbon footprints, per kg of carcass produced and per kg of human-edible protein (considering both the meat and grain produced). Carcass yield was 53 %, 56 %, 52 %, and 54 % for years 1, 2, 3, and 4, respectively. To consider human-edible protein, the mean protein content was assumed to be 232 g/kg of meat (Williams, 2007), 400 g/kg of soybean grain (Grieshop and Fahey Jr, 2001), and 100 g/kg of corn grain (Paes and Bicudo, 1997), which were then multiplied by their Digestible Indispensable Amino Acid Scores (DIAAS) (121 %, 98 %, and 48 %, respectively (Adhikari et al., 2022)) as a correction factor to estimate effects of protein quality (McAuliffe et al., 2023). Total GHG emissions were converted to CO_2eq using AR5 global warming potentials: 1 for CO_2, 28 for CH_4, and 265 for N_2O (IPCC, 2014).

2.2. CH_4 emissions

2.2.1. Enteric fermentation

Enteric CH_4 emissions were measured during the experiments using the GreenFeed® system for 6 months for L and 3 months for LF, upon equipment availability. Consequently, models were used to predict CH_4 emissions for all four years for all systems. To increase the accuracy and consistency of these predictions, the accuracy of seven models was assessed (Table S2) by comparing their predictions to the GreenFeed® measurements (n = 21) and calculating the root mean square prediction error (RMSPEp expressed in % of CH_4 mean; Table S3). Once predicted by the most suitable model van Lingen et al. (2019), (ID 4, Table S2), enteric CH_4 emissions were estimated according to the following equation: CH_4 (g/ d) = 23.8 + 13.5 x DMI + 0.844 x NDF and expressed according to three units: kg CH_4/animal/year, kg CH_4/ha/year, and kg CH_4/animal unit (AU)/ha/year. In addition, to determine which input parameters influenced model predictions the most and to develop a CH_4 emission model that was more suitable for forage-based systems, a global sensitivity analysis was performed (Tables S4 and S5) using the sensobol R package (Puy et al., 2022) with Monte Carlo simulations (n = 40,000)

2.2.2. Manure

The IPCC (2019b) Tier 1 approach was used to estimate CH_4 emissions from manure deposited on pasture. We assumed a maximum CH_4-producing capacity of 0.19 m^3 CH_4/kg of volatile solids and a CH_4 conversion factor of 0.47 % for manure management in grazing systems.

Methane absorbed in the soil was not considered in our study due to the complexity and variability of CH_4 absorption processes in the soil.

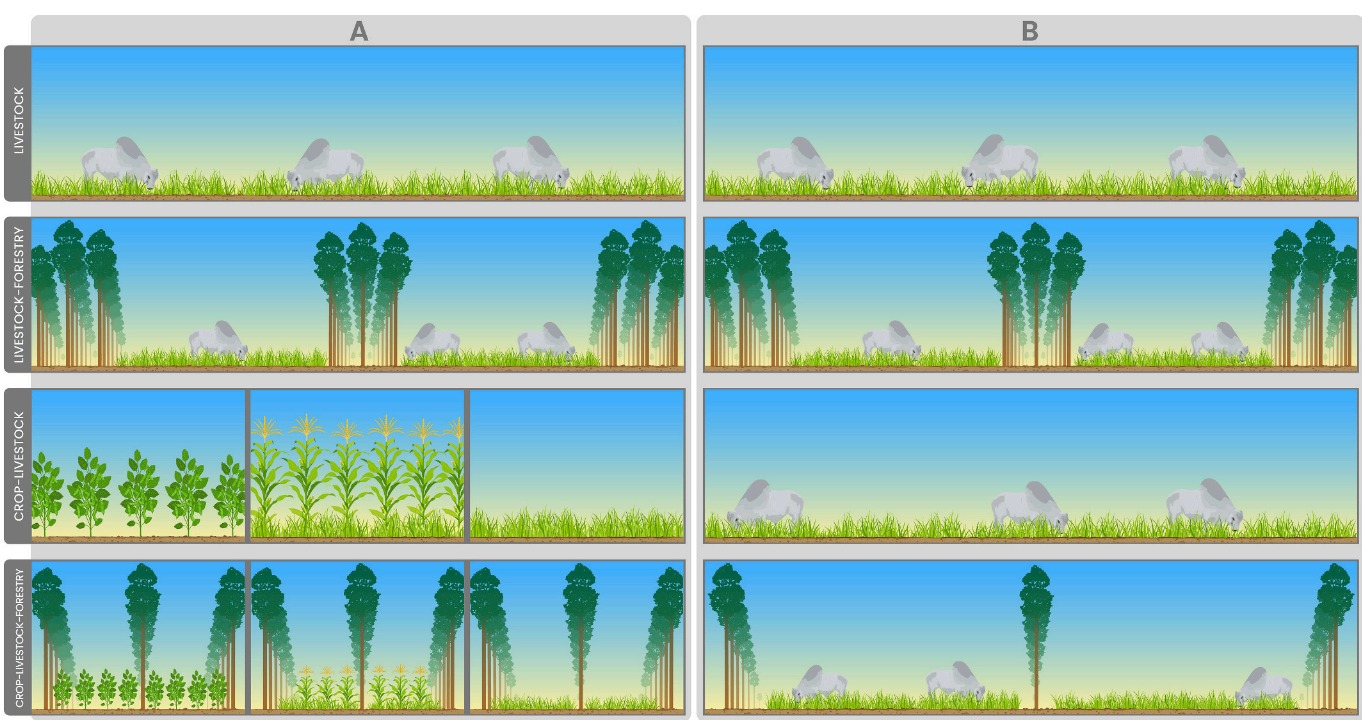

Fig. 1. Diagram of each system in years 1 and 2 (A) and 3 and 4 (B).

Table 1
Production parameters for livestock (L), livestock-forestry (LF), crop-livestock (CL), and crop-livestock-forestry (CLF) systems over a 4-year cycle.

Parameter	n	System				SEM	p-Value
		L	LF	CL	CLF		
Forage and crop area (ha)	–	2	1.5	2	1.5	–	–
Forestry area (ha)	–	0	0.5	0	0.5	–	–
Cumulative tree biomass (m^3/ha)	4	–	302[a]	–	165[b]	11	0.0011
Mean forage mass (kg DM/ha)	32	4200[b]	3400[c]	5100[a]	3500[c]	360	< 0.0001
Mean NDF (g/kg DM)	32	672[a]	667[a]	635[b]	623[b]	4.5	< 0.0001
Mean CP (g/kg DM)	32	95[c]	103[bc]	116[ab]	127[a]	2.0	0.0053
Number of livestock cycles completed	–	4	4	2	2	–	–
Mean stocking rate (AU/ha)	32	2.5[c]	2.4[c]	2.8[b]	3.2[a]	1.21	< 0.0001
Mean average daily gain (kg/day)	32	0.63[b]	0.67[ab]	0.71[a]	0.70[a]	0.12	0.0393
Cumulative meat production (kg/ha)	4	2760[a]	2620[a]	1460[c]	1730[b]	70	< 0.0001
Cumulative soybean production (kg/ha)	4	–	–	5930[a]	5540[a]	300	0.0535
Cumulative corn production (kg/ha)	4	–	–	7250[a]	5870[b]	400	0.0091
Cumulative human-edible protein production (kg/ha)[1]	4	770[b]	740[b]	3080[a]	2940[a]	100	< 0.0001

CP: crude protein; DM: dry matter; AU: animal unit (1 animal of 450 kg); NDF: neutral detergent fiber.
Means with different letters in the rows differ significantly ($p < 0.05$) according to Student's t-test.

[1] Human-edible protein of 28 % for meat, 39 % for soybean, and 4.8 % for corn.

2.3. N_2O emissions

2.3.1. Manure

The IPCC (2019b) Tier 2 approach was used to estimate N_2O emissions from manure deposited on pasture by applying a country-specific factor for N lost as N_2O. Total N intake was calculated from forage crude protein content and dry matter intake. The N deposited was estimated by subtracting the N in animal carcasses (2.5 % of average daily gain; Scholefield et al., 1991) from total N intake. As recommended by Lessa et al. (2014), direct N_2O emission factors (EFs) for urine and dung depended on the season: 1.93 % and 0.14 %, respectively, during the rainy season, and 0.01 % and 0.00 %, respectively, during the dry season. We assumed that 21 % of the N in the manure deposited on pasture was volatilized (of which 1 % was lost as N_2O) and that 24 % was leached or lost in runoff (of which 1.1 % was lost as N_2O) (IPCC, 2019d).

2.4. Synthetic N fertilizer and crop residues

The systems used different amounts of synthetic N fertilizer (Table S6). Urea (45%N) was used as N fertilizer. The Tier 2 approach (IPCC (2019b) was applied to estimate its N_2O emissions, with direct N_2O EFs of 0.93 % based on urea applied to an Oxisol (Mazzetto et al., 2020). As for manure, we assumed that 21 % of the N applied in fertilizer was volatilized (of which 1 % was lost as N_2O) and that 24 % was leached or lost in runoff (of which 1.1 % was lost as N_2O) (IPCC, 2019c). Total N_2O emissions associated with N released from crop residues (i.e. soybean, corn, and forage used as straw), which included both direct and indirect emissions from N mineralization during decomposition, were included. The amount of N from crop residues that returned to the soil was estimated from mean crop yields, default ratios for the above−/below-ground residue yield, and the N content of the residue returned to the soil (IPCC, 2019c). All crop residues were assumed to decompose within 1 year. Since this decomposition releases N into the soil (Chen et al., 2014), the CO_2 emissions avoided by using crop residues instead of urea as N fertilizer were estimated from the N content of crop residues. For this calculation, the total N accumulated by the crop residue over the 4-year cycle was multiplied by the EF of 2.1 kg CO_2 eq/kg of urea produced (Ledgard and Falconer, 2019).

2.5. CO_2 emissions

The EFs used to calculate GHG emissions from fertilizer production

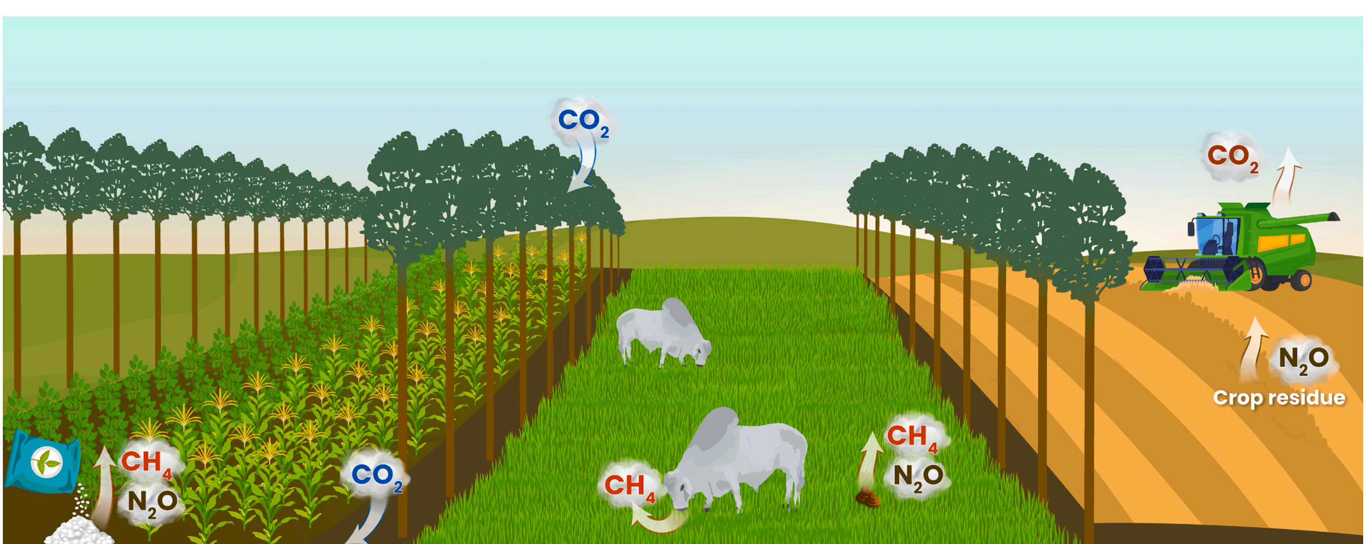

Fig. 2. Conceptual diagram of the carbon balance (CO_2eq) in the system.

(N, phosphorus (P), and potassium (K)) came from Ledgard and Falconer (2019). The EFs were 2.1 kg CO_2eq/kg N (i.e., 0.97 kg CO_2/kg urea assuming 45 % N), 0.426 kg CO_2eq/kg P (i.e. 0.156 kg CO_2/kg simple superphosphate assuming 16 % P_2O_5), and 0.62 kg CO_2eq/kg K (i.e. 0.45 kg CO_2/kg potassium chloride assuming 60 % K_2O). For lime, the EF was 0.47 CO_2/kg of lime (IPCC, 2006).

The CO_2 emissions associated with producing each ingredient in the supplement were considered to determine the total GHG emissions of the supplement. The EFs used were 0.28 kg CO_2/kg ground corn (Jayasundara et al., 2014), 0.40 kg CO_2/kg sodium chloride (Morais et al., 2018), 0.40 kg CO_2/kg calcium carbonate (Morais et al., 2018), 0.97 kg CO_2/kg urea (Ledgard and Falconer, 2019), and 0.50 kg CO_2/kg calcium phosphate (Morais et al., 2018), which yielded an EF of 0.34 kg CO_2eq/kg supplement.

The CO_2eq emissions associated with production of the electricity consumed were calculated assuming that L and LF consumed 31.9 kWh/ha/year and CL and CLF consumed 33.1 kWh/ha/year (Reis et al., 2021), and an EF of 88 kg CO_2eq/MWh of electricity (EPE, 2019). For diesel combustion, emissions were estimated assuming that L and LF used 3.19 L/ha/year and CL and CLF used 9.31 L/ha/year (Reis et al., 2021), and an EF of 3.53 kg CO_2eq/L diesel fuel (74.1 kg CO_2eq/G joule; IPCC, 2006). These values were assumed based on the estimation made by Reis et al. (2021) studying crop and livestock farms in the state of Mato Grosso (i.e. similar conditions).

2.6. Soil C sequestration

In our analysis, a soil C stock of 65.9 Mg/ha was considered for L and 58.4 Mg/ha for LF, CL, and CLF, based on the study of Baumgärtner (2022), who analyzed soil C stocks in the same experimental area. To estimate the soil C sequestration rate, soil C stocks from a poorly managed *Brachiaria decumbens* pasture planted in 1985 (Braz et al., 2013) and from a tropical humid area in the Amazon biome (Damian et al., 2023) were used as reference areas.

The formula used to estimate the soil C sequestration was: ((Soil C stocks from the systems – Soil C stocks from reference area) /age of the systems)) (Damian et al., 2023). The age of the systems was considered as the number of years passed since the onset of their implementation (2011) until the soil sampling date (2018).

2.7. Eucalyptus C sequestration

Eucalyptus has the potential to remove C from the atmosphere and store it in the soil and biomass (Pezzopane et al., 2021). Farmers in Brazil commonly use eucalyptus wood for several purposes, including fences, gates, roofs, tables, and doors (Figueiredo et al., 2017). To this end, we estimated its stem biomass excluding leaves and roots, since these parts are not usually used for wood, based on the equations of Pezzopane et al. (2021).

2.8. Net carbon balance

The net C balance was determined by subtracting total CO_2eq emissions (all in Mg CO_2eq/ha over the 4-year cycle) from the total C sequestration (i.e. in the soil and trees) and the CO_2 emissions avoided by using crop residues instead of urea. Total CO_2eq emissions summed all emissions: CH_4 (enteric and manure), N_2O (manure, fertilizer, crop residues, indirect), and CO_2 (supplements, fertilizer, energy, and diesel). Positive values indicated C released from the system (i.e. CO_2 emissions exceeded CO_2 sequestration), while negative values indicated C stored in the system.

2.9. Statistical analyses

After verifying homogeneity of variance for each linear predictor, quantil-quantile plots of the residuals, and the normality of residues using the Shapiro-Wilk test, data were analyzed using the mixed-model method (PROC MIXED) with parametric structure in the covariance matrix of SAS® software, version 9.4 (SAS, 2014).

For the enteric CH_4 variables, block and year were considered as random effects, and the system as a fixed effect in the following model:

$$Y_{ijk} = \mu + S_i + B_j + e_{ij} + Y_k + \varepsilon_{jk}$$

where Y_{ijk} = the ijk dependent variable, μ = overall mean, S_i = fixed effect of the i^{th} system, B_j = random effect of the j^{th} block, e_{ij} = error associated with system i in block j ~ normally and independently distributed, Y_k = random effect of year, and ε_{jk} = experimental error ~ normally distributed.

For the variables that were summed over the 4-year cycle (i.e. tree biomass, meat, soybean, and corn production), the block was considered as a random effect in the following model:

$$Y_{ij} = \mu + S_i + B_j + \varepsilon_{ij}$$

where Y_{ij} = the ij dependent variable, B_j = random effect of the j^{th} block ~ normally and independently distributed, and ε_{ij} = experimental error ~ normally distributed.

For forage production, NDF, crude protein, stocking rate, and average daily gain, the block, year, and season (dry and rainy) were considered as random effects in the following model:

$$Y_{ijkl} = \mu + S_i + B_j + e_{ij} + E_k + e_{ijk} + Y_l + \varepsilon_{jkl}$$

where Y_{ijkl} = the ijkl dependent variable, E_k = random effect of the k^{th} season, e_{ijk} = error associated with system i, season k, in block j ~ normally distributed, Y_l = random effect of the l^{th} year, and ε_{jkl} = experimental error ~ normally distributed.

The variance and covariance matrix was selected using the Akaike information criterion (Wolfinger and O'Connell, 1993). Treatment means were calculated using the LSMEANS statement and then compared using the probability of difference (PDIFF) procedure using Student's *t*-test ($p < 0.05$).

3. Results

3.1. Production parameters

There was a system effect on all parameters ($p < 0.05$), except for soybean production ($p = 0.0535$) (Table 1). Trees biomass was 83 % greater for livestock-forestry (LF) (128 trees/ha) than crop-livestock-forestry (CLF) (72 trees/ha). The systems with trees (LF and CLF) had lower forage mass (mean of 3450 kg DM/ha). The crop-livestock (CL) and crop-livestock-forestry (CLF) presented lower forage neutral detergent fiber (NDF) than L and LF (630 vs. 670 g/kg DM, respectively). The greater forage crude protein (CP) content was observed in CL and CLF (121.5 g/kg DM), while the lowest was observed in L. The stocking rate in the CLF was 30 % greater than in L and LF (3.20 vs. 2.45 AU/ha), but the lowest average daily gain was observed in L, 9.5 % lower than those in LF, CL, and CLF (mean of 0.69 kg/day).

The L and LF produced the greater amount of carcass meat over the 4-year cycle (2690 kg/ha), followed by CL and CLF (1595 kg/ha). Soybean production did not differ between CL and CLF, but corn production was 19 % lower in CLF than that in CL. The systems with crops (CL and CLF) produced the most human-edible protein over the 4-year cycle (3010 vs. 755 kg/ha).

3.2. Enteric CH_4 emissions estimates

The model performance of enteric CH_4 emission models varied (Table S3). The model of van Lingen et al. (2019) (ID 6, Table S2), which includes dietary forage percentage and body weight (BW), was ranked first because it had the smallest RMSPEp (22.36%), followed by the model of van van Lingen et al. (2019) (ID 4, Table S2), which includes dry matter intake and NDF, (RMSPEp = 23.34%). The model of the IPCC (2006) had the largest RMSPEp (55.27%). All models tended to overestimate total CH_4 emissions (Table S3).

For the models that used gross energy intake as the input variable (i.e. IPCC (2006) and Ribeiro et al. (2020)), most of the variance was explained by net energy for growth (Table S5). For the models of van Lingen et al. (2019) (ID 5, Table S2), and van Lingen et al. (2019) (ID 6, Table S2), 99% of the variance was explained by BW, while for the model of van van Lingen et al. (2019) (ID 4, Table S2), 94% of the variance was explained by BW and 5% by NDF. After evaluation, the model of van Lingen et al. (2019) (ID 4, Table S2) was selected because it contained NDF as an input variable, which helped distinguish the four systems.

There was no significant system effect on predicted enteric CH_4 emissions per animal ($p > 0.05$, Table 2) estimated using the model of van van Lingen et al. (2019) (ID 4, Table S2), which averaged 80.5 kg CH_4/animal/year. The greatest enteric CH_4 emission per area was observed in CLF, which was 13 % greater than in CL and 45 % greater than those in L and LF. However, when the emissions were expressed as kg CH_4/AU/ha/year, L had the greatest emissions, which were 1.4-fold higher than CL.

3.3. Total GHG emissions and net C balance

There was a system effect on the total GHG emissions ($p < 0.05$, Table 3), with greater emissions in L and LF (mean of 30 Mg CO_2eq/ha over the 4-year cycle). Total GHG emissions in CL and CLF were 26 % and 18 % lower, respectively, then that in L.

The CL had the lowest total CH_4 emissions, which were 12.5 % lower than those in CLF and 42.5 % lower than those in L and LF.

The N_2O emissions from manure deposited on pasture contributed ca. 2.8 % of total GHG emissions (Table 3). Systems with crops (CL and CLF) had the greatest direct N_2O emissions (3.35 Mg CO_2eq/ha over the 4-year cycle), with N fertilizer contributing the most (35 %). Consequently, CL and CLF also had the greatest indirect and total N_2O emissions, followed by LF and L, with a mean contribution of 9.2 % to total GHG emissions.

The least CO_2 emissions came from the L and LF (1.6 Mg CO_2eq/ha over the 4-year cycle), while CLF had the greatest, 108 % greater than those in L and LF, due to its greater mechanization.

Soil C sequestration in L was 40 % greater than those in LF, CL, and CLF (mean of 40.5 Mg CO_2eq/ha over the 4-year cycle). The LF (128 trees/ha) sequestered 78 % more than CLF did (72 trees/ha) over the 4-year cycle; thus, LF sequestered 28 % more total C than CLF did. By using crop residues instead of urea as fertilizer, CL and CLF avoided emitting a mean of 0.56 Mg CO_2eq/ha over the 4-year cycle. The net C balance differed among systems ($p < 0.05$, Table 3), but all systems had a negative balance. The LF had the most negative net C balance, which was 30 %, 92 %, and 173 % greater than those in CLF, L, and CL, respectively.

3.4. Carbon footprint

There was a system effect on total GHG ($p < 0.05$) for all gases (CH_4, N_2O, CO_2) and on the C balance when expressed using both carbon-footprint units (kg CO_2eq/kg carcass and kg CO_2eq/kg human-edible protein) (Table 4).

The CL had the greatest CH_4, N_2O, and CO_2 emissions, and thus the greatest total GHG emissions expressed as kg CO_2eq/kg carcass: 7 %, 32 %, and 42 % greater than those in CLF, LF, and L, respectively. The lowest N_2O and CO_2 emissions were observed in the L and LF. When the net C balance was expressed as kg CO_2eq/kg carcass, systems with trees had a more negative net C balance than those without trees, while CLF stored 77 % more than CL did and 135 % more than L did, while LF stored 52 % more than CL did and 102 % more than L did.

When emissions were expressed as kg CO_2eq/kg human-edible protein, LF had the greatest CH_4 and N_2O emissions, which were 1.0, 8.0, and 9.8-fold higher than L, CLF, and CL, respectively (Table 4). The LF had the greatest total GHG emissions when expressed as kg CO_2eq/kg human-edible protein: 1.1, 6.5, and 7.6-fold higher than L, CLF, and CL, respectively. When the net C balance was expressed as kg CO_2eq/kg human-edible protein, LF had the most negative net C balance: 2.0, 5.2, and 11.4-fold higher than L, CLF, and CL, respectively.

In all systems, CH_4 was the GHG that contributed most (Fig. 3), with a mean of 85 % in L and LF, and 67 % in CL and CLF. The mean N_2O contribution to total GHG emissions was 100 % greater in CL and CLF (9 %) than in L and LF (18 %). The mean CO_2 contribution to total GHG emissions was 14 % in CL and CLF and 5 % in L and LF.

4. Discussion

4.1. Enteric CH_4 emissions x NDF

Since enteric CH_4 is the main GHG emitted by the livestock sector (Arndt et al., 2022), it was crucial to select a model that accurately predicted it in the studied systems. Although the model of van Lingen et al. (2019) (ID 6, Table S2), with BW and dietary forage content as input variables, was the most accurate (Table S3), we selected the second-best model, van Lingen et al. (2019) (ID 4, Table S2), which considers BW, net energy for maintenance, and NDF as input variables, explaining 94 %, 1 % and 5 % of the variance, respectively (Table S5). This choice was based on the fact that it considers NDF, which is relatively simple to measure in the field and is especially important in forage-based systems. In addition, the sensitivity analysis identified BW as the input variable that influenced estimates of enteric CH_4 emissions the most (Table S5). Thus, these variables need to be carefully measured to predict CH_4 emissions with more accuracy and precision.

4.2. The crop-livestock-forestry system emitted more enteric CH_4 per ha

In our study, the animals presented the same enteric CH_4 emissions among the systems, (mean of 80.5 kg CH_4/animal/year) because BW that was the most influential input variable in the model was similar among systems. In addition, all four systems were designed to provide

Table 2
Enteric methane (CH_4) emissions from livestock (L), livestock-forestry (LF), crop-livestock (CL), and crop-livestock-forestry (CLF) systems estimated using the model of van Lingen et al. (2019) (ID 4, Table S2) and expressed according to three units.

Unit[1]	n	System				SEM	p-Value
		L	LF	CL	CLF		
kg/animal/year	16	79.90	79.95	81.45	80.97	1.15	0.1262
kg/ha/year	16	224.00[c]	211.75[c]	278.52[b]	315.78[a]	14.43	< 0.0001
kg/AU/ha/year	16	62.46[a]	47.16[c]	44.56[d]	59.88[b]	1.12	< 0.0001

AU: animal unit (1 animal of 450 kg); SEM: standard error of the mean.
Means with different letters in the rows differ significantly ($p < 0.05$) according to Student's t-test.
[1] Emissions considering 4 years for systems L and LF and 2 years for systems CL and CLF.

Table 3
- Estimated carbon (C) balance (Mg CO_2eq/ha) and greenhouse gas emissions (Mg CO_2eq/ha) of livestock (L), livestock-forestry (LF), crop-livestock (CL), and crop-livestock-forestry (CLF) systems over a 4-year cycle.

Emission sources (Mg CO_2eq/ha)	n	System				SEM	p-Value
		L	LF	CL	CLF		
Methane (CH_4)							
Enteric	4	25.2^a	25.6^a	14.6^c	16.7^b	0.60	< 0.0001
Manure	4	0.20^a	0.20^a	0.11^c	0.15^b	0.008	< 0.0001
Total CH_4 emissions	4	25.4^a	25.8^a	14.7^c	16.8^b	0.62	< 0.0001
Nitrous oxide (N_2O)							
Manure	4	0.70	0.87	0.65	0.83	0.06	0.0689
Fertilizers[1]	4	1.36	1.36	1.50	1.50	–	–
Crop residues	4	–	–	1.16^a	1.07^b	0.03	0.0009
Direct N_2O emissions	4	2.06^c	2.23^b	3.31^a	3.40^a	0.05	< 0.0001
Indirect N_2O emissions	4	0.57^c	0.61^b	0.97^a	0.95^a	0.006	< 0.0001
Total N_2O emissions	4	2.63^c	2.84^b	4.28^a	4.35^a	0.05	< 0.0001
Carbon dioxide (CO_2)							
Total CO_2 emissions	4	1.63^c	1.60^c	3.27^b	3.33^a	0.02	< 0.0001
Total GHG emissions	4	29.6^a	30.2^a	22.2^c	24.5^b	0.65	< 0.0001
C sequestration							
Soil C sequestration[1]	4	−56.4	−40.5	−40.5	−40.5	–	–
Eucalyptus C sequestration	4	–	−41.0^a	–	−23.0^b	1.90	0.0020
Total C sequestration	4	−56.4^c	−81.5^a	−40.5^d	63.5^b	1.35	< 0.0001
CO_2eq emissions avoided from using crop residues instead of urea	4	–	–	−0.58^a	−0.54^b	0.02	0.0006
Net C balance	4	−26.8^d	−51.3^a	−18.8^c	−39.5^b	1.12	< 0.0001

SEM: standard error of the mean.
Means with different letters in the rows differ significantly ($p < 0.05$) according to Student's t-test.
[1] Since replicates had equal values, no statistical analysis was performed.

Table 4
Estimated net C balance and greenhouse gas (GHG) emissions (in kg CO_2eq/kg carcass and kg CO_2eq/kg of human-edible protein) from livestock (L), livestock-forestry (LF), crop-livestock (CL), and crop-livestock-forestry (CLF) systems, produced over a 4-year cycle.

Variable	n	System				SEM	p-Value
		L	LF	CL	CLF		
kg CO_2eq/kg carcass							
Total CH_4	4	9.20^c	9.83^b	10.07^a	9.71^b	0.07	< 0.0001
Total N_2O	4	0.95^c	1.08^c	2.94^a	2.56^b	0.08	< 0.0001
Total CO_2	4	0.60^c	0.61^c	2.24^a	1.95^b	0.04	< 0.0001
Total GHG	4	10.75^d	11.52^c	15.25^a	14.22^b	0.16	< 0.0001
Net C balance	4	−9.70^d	−19.58^b	−12.90^c	−22.83^a	0.88	< 0.0001
kg CO_2eq/kg human-edible protein							
Total CH_4	4	39.68^b	42.40^a	4.30^d	5.28^c	0.28	< 0.0001
Total N_2O	4	4.11^b	4.68^a	1.25^c	1.36^c	0.11	< 0.0001
Total CO_2	4	2.55^a	2.64^a	0.96^b	1.04^b	0.04	< 0.0001
Total GHG	4	46.34^b	49.72^a	6.51^c	7.68^d	0.39	< 0.0001
Net C balance	4	−34.80^b	−69.32^a	−6.10^d	−13.43^c	1.61	< 0.0001

CH_4: methane; N_2O: nitrous oxide; CO_2: carbon dioxide.
SEM: standard error of the mean.
Means with different letters in the columns differ significantly ($p < 0.05$) according to Student's t-test.

the cattle with diets of similar quantity and quality. Nonetheless, the obtained emission factor was 46 % greater than the default emission factor used by the IPCC (2019b) for beef cattle in high-productivity systems in Latin America (55 kg CH_4/animal/year). The CLF emitted more enteric CH_4 (Table 2) due to its greater stocking rate (Table 1). Although CLF system had lower forage mass (kg DM/ha) as compared to L and CL systems, other studies conducted in the same experimental area showed that the CLF had greater forage accumulation) (Carvalho et al., 2019; Domiciano et al., 2020), which allows for greater stocking rates as the systems were managed based on canopy height (30 cm as a target).

In the context of sustainable production, it is important to emphasize that well-managed integrated systems can enhance land use efficiency. Stocking rates in the present study ranged from 2.4 to 3.2 AU/ha, which were 177–255 % greater than the mean stocking rate in Brazil (0.9 AU/ha; ABIEC, 2022). These greater stocking rates means producing more animals on the same land area or needing less land for the same number of animals. Increasing the efficiency of forage-based systems creates opportunities for other land uses, such as forestry, which can help decrease the pressure to clear old-growth forest areas and promotes biodiversity (Jose and Dollinger, 2019). It can also increase the area available to grow crops, thus increasing grain production.

4.3. Trees can decrease forage and crop production

Although trees can increase animal production, they can decrease forage and crop production by reducing photosynthetically active radiation (PAR) (Gomes et al., 2020), which drives plant growth. In the present study, trees decreased corn production by 19 % and tended to decrease soybean production by 6.5 % (Table 1), which highlights that soybean can produce more in a PAR-limited environment than corn can, as corn is a C4 plant that is more light-responsive (Pearcy and Ehleringer, 1984). Thus, potential trade-offs and synergies of agroforestry systems must be assessed carefully, considering the specific requirements of each component (crop, livestock, and forestry), to design

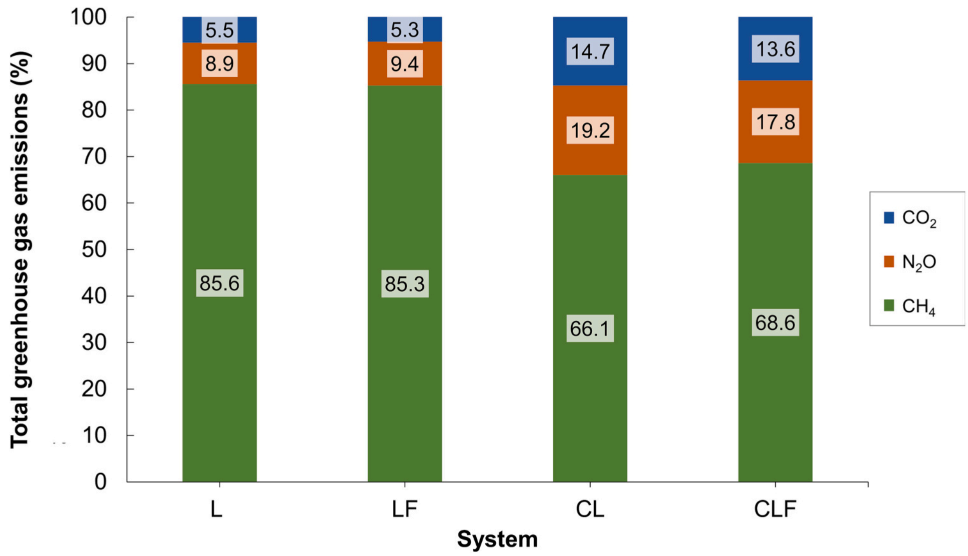

Fig. 3. Percentage contributions of greenhouse gases to total greenhouse gas emissions in livestock (L), livestock-forestry (LF), crop-livestock (CL), and crop-livestock-forestry (CLF) systems.

a system appropriately. However, trees can improve animal welfare (i.e. the thermal index) (Domiciano et al., 2018; Karvatte et al., 2016; Magalhães et al., 2020), increase soil organic matter (Hoosbeek et al., 2018), decrease soil temperature (Bosi et al., 2020) and, most importantly, increase C sequestration in aerial biomass (Pezzopane et al., 2021).

Regarding the influence of crops, the residual fertilization and decomposition of crop residues can improve soil quality (Fu et al., 2021) and increase forage mass in CL. Silva et al. (2020) and Carvalho et al. (2019) observed an increase of 40 % and 27 %, respectively, in forage mass in CL compared to that in L.

4.4. Integrated systems with crops emitted more N_2O but incorporated N into the soil

The CL and CLF had the lowest CH_4 emissions (Table 3) due to the shorter presence of cattle (i.e. only two of the four years). The CLF had the second-greatest total GHG emissions, likely due to greater stocking rates during the two years that cattle were present.

Systems with crops emitted 10 % more N_2O over the four years due to urea applications. The N_2O emissions from manure did not differ among systems, besides that, it is important to highlight that manure is a source of N for the soil (Lessa et al., 2014). Furthermore, decomposition of crop residues releases N in ammonium and nitrate, which are sources of N_2O emissions (Chen et al., 2013). Greater grain production in CL increased N_2O emissions from crop residues. Thus, the greatest N_2O emissions were due to the highest N fertilizer rate and the decomposition of crop residues, which increased indirect N loss through leaching and runoff.

Although crop residues increased N_2O emissions, they also incorporated N into the soil (Fu et al., 2021). In CL and CLF, crop residues provided 270 kg N/ha over the four years, which explained why the forage of these systems had the greatest crude protein concentrations in the forage (Table 1). Furthermore, combining crops and livestock in a system can increase the delivery of ecosystem services, such as the provision of crop residues and animal manure as inputs for crop and forage production (Assmann et al., 2014; Damian et al., 2023), which decreases the need for synthetic N fertilizers. Over the four years, CL and CLF avoided emitting 0.58 and 0.54 Mg CO_2eq/ha, respectively, by replacing some synthetic N fertilizers with N input from crop residues.

4.5. Including forestry in the forage-based system improves the net C balance

The greater CO_2 emissions in CL and CLF are due to greater fossil fuel consumption caused by the increased mechanization practices on crops. Besides that, tillage associated with the establishment of crops can contribute to the release of C that was stored in the soil (Haddaway et al., 2017). Furthermore, the CLF exhibit greater CO_2 emissions from feed supplements, as it had greater stocking rates.

When including C sequestration in the soil and trees, LF had the most negative net C balance, with 50 % sequestered in the soil and 50 % in the tree trunk. The allocation of C between the soil and trees in integrated systems is a dynamic process that involves various biological and environmental factors (i.e, photosynthesis, root exudation, litter decomposition, soil respiration) (Franklin et al., 2012), The inclusion of forestry components in the forage-based system positively influences the net C balance, with trees playing a significant role in enhancing C sequestration both above and below ground (Albrecht and Kandji, 2003). Understanding the intricate interactions between biological and root activities is crucial for optimizing the sustainable benefits of integrated systems in mitigating greenhouse gas emissions and promoting carbon accumulation in the soil and biomass.

The CLF had the second-largest sequestration due to its lowest tree density (72 trees/ha). If CLF had the same tree density as LF (128 trees/ha), it would have stored more C, but the increased shading may have decreased forage production and animal performance, changing the dynamics of emissions and C sequestration.

The fact that all systems had a negative net C balance emphasizes the importance of the contribution of design and management to more sustainable forage-livestock systems. The system with the most negative net C balance stored 53.3 Mg CO_2eq over the four years (i.e. 13.3 Mg CO_2eq/year). Figueiredo et al. (2017) reported a storage of 12.0 Mg CO_2eq/year for a CLF system with eucalyptus (476 trees/ha) and a release of 6.8 Mg CO_2eq/year. Even with a higher tree density, they estimated a lower net C balance than we did due to larger soil C stocks (0.44 vs. 3.0 Mg C/ha in our study).

According to Damian et al. (2023), adopting more intensive and diversified systems can increase soil C stocks. Given the estimated C sequestration in the systems we studied, soil clearly acts as a large C sink. In addition, we assumed a C stock of poorly managed pasture soils in Brazil as a baseline to calculate the soil C sequestration rate to highlight that well-managed livestock systems will not only help decrease GHG emissions but also help restore degraded areas. Good management is crucial because ca. 53 % of the pasture area in Brazil (∼163 million ha) has some degree of degradation (LAPIG, 2022). Therefore, the results of the present study could contribute to Brazilian public policies, such as the National Plan for Low Carbon Emission in Agriculture (ABC Plan), which aims to recover 30 million ha of degraded pasture by 2030 through adequate pasture management and the adoption of integrated systems (MAPA, 2022).

A greater soil C sequestration was observed in L when compared to CLF, due to the continuous presence of forage and animals all four years. *Brachiaria* significantly enhances soil quality through its large root system(Baptistella et al., 2020). Additionally, animal excreta incorporation into the soil increases soil C content (Damian et al., 2023). In addition, trees may favor litter decomposition and nutrient cycling, but they are not always associated with incorporation of C in soil organic matter, since their residues, especially branches and limbs, contain mainly cellulose, hemicellulose, and lignin. As a complex polymer, lignin strongly resists microbial decomposition, making tree residues more difficult to decompose (Krishna and Mohan, 2017), but it is important to highlight that the tree component plays an important role as a C store in the aerial and root biomass.

4.6. Integrated systems with crops emitted more CO_2eq per kg of carcass but less CO_2eq per kg of human-edible protein

There are several possible arrangements of integrated systems and different purposes for each component in Brazil and worldwide (Ryschawy et al., 2014). Each system arrangement has advantages and disadvantages, and the one chosen will depend upon soil and climate conditions and farmer skills. In the study region, other trees can be introduced besides eucalyptus, such as mahogany and teak (Behling et al., 2014). In northeastern Brazil, fruit trees or native regional trees are used more often, and the crops may be beans, sorghum, cassava, or peanuts (Rangel et al., 2015). It is important to assess differences in the emissions, sequestration, and benefits of each system.

Emission intensity allows us to evaluate which systems are more effective in reducing GHG emissions relative to final product. Per kg of carcass produced, L and CL had the lowest and highest total GHG emissions, respectively, because systems with crops (i.e., only two years with livestock) produced less carcass than systems without crops (L and LF). The CLF and L had the most and least negative net C balance, storing 23 and 10 kg CO_2eq per kg of carcass produced, respectively. In the literature, there is a wide variation in emissions across studies due to different systems boundaries, used GWP values, etc. Figueiredo et al. (2017) compared three different pasture management systems and reported emissions of 25 kg CO_2eq/kg carcass over a 10-year cycle for a CLF system. In the study of Silva et al. (2017), the net C balance ranged from 2.87 to 9.26 kg CO_2eq/kg carcass when considering the adoption of pasture restoration during the finishing phase in grazing systems.

Per kg of human-edible protein, total GHG emissions of systems without crops (L and LF) were a mean of 6.8-fold higher than those of systems with crops (CL and CLF), mainly because soybeans provide more human-edible protein (39 %) than meat (28 %), which diluted system emissions. However, although the systems without crops (L and LF) provided less human-edible protein, animal-based protein is important because it contains unique nutrients important for human diets that are usually absorbed and used more easily once consumed than those in plant-based protein (Beal et al., 2023). The higher DIAAS for meat (121 %) than for soybean (98 %) or corn (48 %) (Adhikari et al., 2022) highlights that animal-based protein has more bioavailable essential amino acids.

In the present study, the GHG emissions of L and LF averaged 48 kg CO_2eq/kg human-edible protein, while Herrero et al. (2013) reported a range of 58–1000 kg CO_2eq/kg human-edible protein in ruminant meat worldwide. Another important consideration is that grass-fed beef can be produced on non-arable land that is unsuitable for crop production, which decreases feed-food competition (Dumont et al., 2020; Mottet et al., 2018). It highlights the potential benefits of producing animal-based protein from ruminants that increase security of the global food system.

When estimating the net C balance per kg of human-edible protein, the response was the opposite: systems with crops (CLF and CL) had a less negative net C balance (−13 and −6 kg CO_2eq/kg of human-edible protein, respectively) than systems without crops (L and LF) did (−34 and −69 kg CO_2eq/kg of human-edible protein, respectively).

Since Brazil has a Nationally Determined Contribution to reduce CH_4 emissions by 30 % and total GHG emissions by 50 % by 2030, integrated systems can offset GHG emissions to help reach these goals. Furthermore, the results of the present study may help estimate GHG emissions more accurately in countries with hot and humid climates, such as Brazil and other countries with the same climate conditions. The climatic similarity of tropical regions is particularly useful for applying these results since these regions have high potential for similar plant and animal production. However, this approach can also be useful for estimating GHG emissions in temperate countries since it can be applied to any conditions.

5. Conclusions

Systems with crops emitted more enteric CH_4 per hectare due to greater stocking rates. However, they also incorporated more 270 kg N/ha over four years, which contributed to improved forage quality and reduced reliance on synthetic N fertilizers.

All integrated systems presented a negative net C balance, with the greatest sequestration observed in systems with the forestry component. Over four years, these systems sequestered from 15.9 to 20.4 Mg CO_2eq/ha/year. Per unit of output, systems with crops emitted 6.8-fold less GHG per kg of carcass than systems with no crops. However, the livestock-forestry system stored 10-fold more C per kg of human-edible protein than the crop-livestock system demonstrating that integrated forage-based livestock systems show promise in GHG mitigation and C sequestration.

The continuous presence of forage and animals in these systems contributed to greater soil C sequestration, while trees played a significant role as a C store in aerial biomass. Furthermore, the C sequestration in the soil underscores its importance as a substantial C sink, and adopting well-managed livestock systems can contribute to restoring degraded areas. as evidenced by the storage from 40.5 to 56.4 Mg CO_2eq/ha over four years.

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CRediT authorship contribution statement

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Barreto-Mendes: Validation, Formal analysis, Writing – review & editing, Visualization. **Audrey Fanchone:** Validation, Writing – review & editing, Visualization. **Diego P. Morgavi:** Validation, Writing – review & editing, Visualization. **Bruno C. Pedreira:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing – review & editing, Project administration, Funding acquisition. **Ciro A.S. Magalhães:** Investigation, Resources, Writing – review & editing. **Adibe L. Abdalla:** Conceptualization, Methodology, Validation, Writing – review & editing, Visualization. **Maguy Eugène:** Methodology, Validation, Formal analysis, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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