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Harnessing cactus biomass for biogas and biofertilizer production under temporal fermentation strategies

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ABSTRACT

Two experiments were conducted to optimize biogas production from cactus feedstock through temporal feeding strategies and evaluate the biofertilizer potential of the effluent. In experiment 1, treatments included cactus inclusion to primed dung at different time points and replacement of cactus with cattle dung once a week: (1) Cattle dung on day 0 + cactus, (2) Cattle dung day 0 + cactus + replacement, (3) Cattle dung day 21 + cactus, (4) Cattle dung day 21 + cactus + replacement, (5) Cattle dung day 42 + cactus, (6) Cattle dung day 42 + cactus + replacement, and two controls (7) cactus only and (8) cattle dung only. A randomized complete block design (RCBD) with three replicates for 20 weeks was used. In experiment 2, the effluent from treatments 5 through 8 was applied at 0, 9.5, 18.9, and 28.4 g DM pot^{-1} in a RCBD with three replicates to evaluate ryegrass (*Lolium multiflorum*) growth in a greenhouse. Pre-fermentation of cattle dung and weekly replacement of cactus kept pH within the optimal range (6.5–7.7), and produced the highest cumulative CH_4 (64 mL CH_4 g^{-1} VS). Over 50% of CH4-C came from cactus, which peaked when cactus was pre-fermented and replaced weekly. Cactus treatments (D42, D42+R) showed intermediate (0.4-0.6 g pot^{-1}) aboveground DM content compared to the control and cattle dung alone. In addition, above-ground biomass increased with increasing effluent application rate (P=0.0138). These results confirm that temporal feeding strategies in cactus co-digestion maximize methane output while producing effluent that improves plant growth.

1. Introduction

Many households across the dryland regions of the Global South depend on wood fuel for cooking [1]. This reliance places a heavy burden on women and children, who spend several hours daily collecting wood from increasingly degraded landscapes, and exposes families to harmful smoke and pollutants from traditional low-efficiency stoves [2,3]. Coincidentally, these rangelands are also experiencing degradation due to the invasion of invasive cactus species, which can negatively affect grazing lands and reduce forage availability [4]. Ironically, this invasive cactus, often viewed as a nuisance, represents an underutilized biomass resource that could simultaneously address the challenges of energy scarcity and land degradation [5].

Cacti (*Opuntia* spp.) are highly drought-tolerant and capable of

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producing substantial biomass on marginal soils where conventional crops often fail [6], making them a promising energy crop for small, off-grid households in arid and semi-arid areas [5]. For example, in semi-arid northeast Brazil, managed rainfed systems have achieved cactus yields of 20-30 Mg dry matter (DM) ha^{-1} $year^{-1}$ [7]. Typically, a cactus plant has high water-use efficiency, reaching up to 35 kg DM ha^{-1} mm^{-1}, outperforming many traditional biomass crops under similar conditions [8]. In addition to its agronomic advantages, cactus biomass is highly fermentable due to its high levels of non-structural carbohydrates [9], making it suitable for anaerobic digestion. The methane potential of *Opuntia* ranges between 289 and 364 m^3 methane (CH_4) Mg^{-1} of volatile solids, comparable to that of conventional energy crops [10].

However, cactus alone is not a complete biogas feedstock. Its high C:N ratio limits microbial growth and methanogenesis, while its acidic pH (3.5-5.5) can suppress methane-forming microorganisms [5,6]. Typically, a pH below 5.5 results in biogas with reduced energy yield and poor combustibility [10]. Consequently, optimizing its digestion requires strategies that balance nutrients and stabilize pH. Studies have shown that co-digesting cactus with cattle dung can overcome these constraints by improving buffering capacity [10], enhancing microbial activity [11], and increasing methane yield [12]. While this approach is well established, most studies focus on chemical pretreatments of cactus [13,14], which are largely impractical in resource-limited rural settings. Optimizing biogas production through temporal feeding strategies offers a practical, low-cost approach to enhancing biogas production. While co-digestion and inoculum conditioning strategies are well established, limited information exists on how temporal feeding strategies, specifically cattle dung pre-fermentation duration prior to cactus inclusion and intermittent cactus replacement with cattle dung, influence biogas production and process stability in cactus-based systems. Similarly, no studies have quantified the contribution of cactus-derived carbon to methane production using isotope-based techniques.

In this study, two main questions were answered: (1) whether different durations of anaerobic pre-fermentation (priming) of cattle dung prior to cactus inclusion enhance pH stability, total gas, and methane yield, and (2) whether periodic (weekly) replacement of cactus with dung further improves these parameters. Although co-digestion of cactus and cattle dung has been previously investigated, the influence of temporal feeding strategies on long-term digestion performance remains poorly understood. Additionally, most cactus-based biogas studies are short-term, typically ranging from 14 days [15,16] to 30-31 days [17,18] and often rely on single-feeding (batch) systems that provide only a snapshot of performance. In contrast, this study employed a continuous feeding approach lasting for 20 weeks that better mimics real biodigester operation, enabling a more robust evaluation of digestion dynamics, process stability, and sustained biogas production.

In a complementary experiment, we evaluated the value of the resulting digestate as a biofertilizer by assessing its effects on ryegrass (*Lolium multiflorum*) growth under greenhouse conditions. While digestate use as a biofertilizer is well established for various feedstocks, its properties are highly dependent on feedstock characteristics. Similarly, studies evaluating biogas optimization from *Opuntia* feedstock are scarce and most have focused on spineless cultivated types, especially in Mexico [19] and Brazil [20]. This study contributes to existing knowledge by evaluating practical feeding strategies to improve biogas production from invasive cactus biomass and assessing the value of digestate as a biofertilizer. The findings provide useful insights for improving resource utilization and energy generation in dryland production systems. Valorization of invasive cactus biomass through anaerobic digestion could provide a sustainable pathway to improve renewable energy generation, reduce reliance on traditional biomass fuels, and promote circular nutrient flows in dryland regions affected by cactus invasion worldwide.

2. Materials and methods

2.1. Experiment 1: Anaerobic digestion study

2.1.1. Experimental design and treatments

The experiment was carried out at the North Florida Research and Education Center, University of Florida, in Marianna, FL (30°52′N, 85°11′W, 35 m asl), in a greenhouse, from May to September 2023 (20 weeks). The greenhouse was maintained at 39 °C during the experiment. The experiment was conducted using a randomized complete block design with three replicates and a 3 × 2 + 2 factorial arrangement to evaluate eight treatment combinations of cattle dung and cactus (*Opuntia* sp.; Table 1). The factors included three time points, levels of cactus inclusion following cow dung priming, and two levels of cactus replacement with dung. The dung was sourced from steers grazing on a mixed-species cool-season pasture, consisting of 'RAM' oat (*Avena sativa* L.) and 'Prine' ryegrass (*Lolium multiflorum* Lam.), while cactus was harvested from the greenhouse where they had been planted and grown for more than two years.

Eight treatments were established to evaluate the effects of cattle dung pre-fermentation duration and periodic dung replacement on biogas production from cactus biomass. Pre-fermentation durations of 0, 21, and 42 days were selected to represent no pre-fermentation, moderate pre-fermentation, and extended pre-fermentation, respectively. The aim was to evaluate whether increasing stabilization of cattle dung before cactus introduction improves the performance of anaerobic digestion.

Treatment 1 received an initial mixture of cattle dung and cactus biomass on day 0, followed by daily feeding with cactus biomass throughout the experiment. Treatment 2 was similar, starting with cattle dung and cactus biomass on day 0 and fed with cactus biomass daily; however, one day per week, the cactus biomass was completely replaced with an equivalent amount (on a dry matter basis) of cattle dung. Treatments 3 and 4 initially contained only cattle dung, which was allowed to pre-ferment for 21 days before feeding with cactus biomass began. Treatment 3 subsequently received daily cactus biomass from day 21 onward, while Treatment 4 also received daily cactus biomass but had weekly replacement with cattle dung. Similarly, treatments 5 and 6 began with a 42-day pre-fermentation of cattle dung before introducing daily cactus biomass. Treatment 5 received cactus biomass daily from day 42 onwards, whereas treatment 6 received cactus daily, with once-weekly replacement of cactus biomass with cattle dung. Additionally, two control treatments were included. Treatment 7 consisted only of cactus biomass added daily from day 0 throughout the study, and Treatment 8 consisted solely of cattle dung added daily from day 0 throughout the study. Except for the controls, the initial treatment proportions comprised 80% cactus and 20% cattle dung, as reported by Ref. [8].

2.1.2. Biodigester prototype

A prototype biodigester was improvised using a 3.78-L plastic bottle.

Table 1
Treatment combinations for the biogas experiment.

Item	Treatment	Feeding strategy
1	Cattle dung on day 0 + cactus	Cactus daily throughout
2	Cattle dung day 0 + cactus + replacement	Cactus daily, but replaced with CD once a week
3	Cattle dung day 21 + cactus	Cactus daily throughout
4	Cattle dung day 21 + cactus + replacement	Cactus daily, but replaced with CD once a week
5	Cattle dung day 42 + cactus	Cactus daily throughout
6	Cattle dung day 42 + cactus + replacement	Cactus daily, but replaced with CD once a week
7	Cattle dung only (control)	Cattle dung throughout
8	Cactus only (control)	Cactus daily throughout

The design included an inlet for substrate feeding, an outlet for effluent collection, and a clear flexible polyvinyl chloride gas delivery tube (inner diameter 0.64 cm and outer diameter 0.95 cm) connected to an inverted measuring cylinder to facilitate the measurement of biogas production (Fig. 1). The inlet was located at the top of the bottle and was made of a feedstock delivery tube (26 cm). The tube was continuously immersed in the feedstock near the base, ensuring fresh material was delivered at the bottom of the bottle and displacing the digested material. A rubber stopper was used to seal the tube externally. An exit was made by drilling a hole (2.5 cm in diameter) slightly above the volume occupied by the digesta. An effluent delivery tube (8 cm) was inserted and closed on the outside using a rubber stopper. The feedstock in the biodigester was kept continuously above the level of this delivery tube to prevent gas leakage during effluent retrieval.

A gas delivery point was drilled into the bottle above the area occupied by the digesta, closer to the lid. A valve was inserted and connected to a gas delivery tube (33 mm diameter and 30 cm in length). The tube was connected to a gas delivery system via a U-shaped copper wire, secured with double strands of zip ties and attached to an inverted 1-liter measuring cylinder. The measuring cylinder was placed inside a bucket filled with water and was supported by a rack and a stone to provide weight. Each bucket had two biodigester prototypes on either side, each connected to a separate measuring cylinder. The measuring cylinders had a hole drilled at the base and a rubber stopper with a valve placed. Using a hand pump, the valve was used for gas sampling and to bring the water level back in the measuring cylinder. The measuring cylinder was immersed in water throughout the experiment to avoid gas leakage. Before the experiment, all bottles were tested for proper sealing and potential leakage to ensure anaerobic conditions. The leak test was conducted by blowing air into each bottle and observing for bubbles while submerged in water.

2.1.3. Feedstock preparation

We used two feedstocks, fresh cattle dung and fresh cactus cladodes. The fresh dung was obtained from steers grazing on the mixed-species pasture already described and located at the North Florida Research and Education Center in Marianna, Florida. The dung was collected immediately after defecation, directly from the pasture, ensuring it was fresh and residue-free. The differences in isotopic composition between cactus, a plant with a crassulacean acid metabolism (CAM) photosynthetic pathway, and cattle dung (from steers grazing C_3 cool-season pastures) were used to determine the methane-carbon (CH_4-C) contribution of each treatment through stable isotope analysis.

The material was homogenized, and subsamples were used for DM and organic matter (OM) analyses. DM was determined by oven-drying sub-samples at 105 °C for 24 h, and OM by ashing at 550 °C for 6 h in a muffle furnace. To adjust for total solids (TS), cattle dung was diluted with tap water to achieve the recommended 10% TS concentration [21, 22]. Sufficient cattle dung was prepared in advance to cover the entire experimental period. It was portioned into weekly quantities, sealed in individual zip-lock bags, and stored frozen. Material for each week was removed and thawed at room temperature before feeding.

Cladodes of cactus were harvested from a greenhouse at the North Florida Research and Education Center in Marianna, Florida. The cactus was established in May 2021 using locally sourced planting material from Florida. The plants had been grown in pots under greenhouse conditions and were over 2 years old at harvest. A vegetable chopper was used to dice the cactus pads into uniform sizes, ensuring consistency in particle size for feeding. Cladode samples were also analyzed for DM and OM to adjust for TS. Unlike cattle dung, TS adjustment for cactus was done during each feeding period. Additional sub-samples of cattle dung and cactus were analyzed for total carbon (C), total nitrogen (N), and $\delta^{13}C$ isotopic composition using the Dumas dry combustion method (Vario Micro Cube, Elementar, Hanau, Germany) linked to an isotope ratio mass spectrometer (IsoPrime 100, IsoPrime, Manchester, UK).

2.1.4. Feedstock composition

Physical and chemical characteristics of cactus and cattle dung

Table 2
Physical and chemical composition (DM basis) of feedstocks used in the anaerobic digestion experiment.

Item[a]	Cactus	Cattle dung
pH	3.7	7.6
DM (g kg^{-1} fresh matter)	170	121
VS (g kg^{-1} DM)	925	783
N (g kg^{-1} DM)	5	29
C (g kg^{-1} DM)	397	422
C:N	83:1	15:1
$\delta^{13}C$ (‰)	−16.3	−28.3

[a] DM = dry matter; VS = volatile solids; N = nitrogen; C = carbon; C:N = carbon-to-nitrogen ratio; $\delta^{13}C$ = stable C isotope ratio (per mil, ‰). Values represent the mean chemical composition of cactus and cattle dung used as substrates in the experiment.

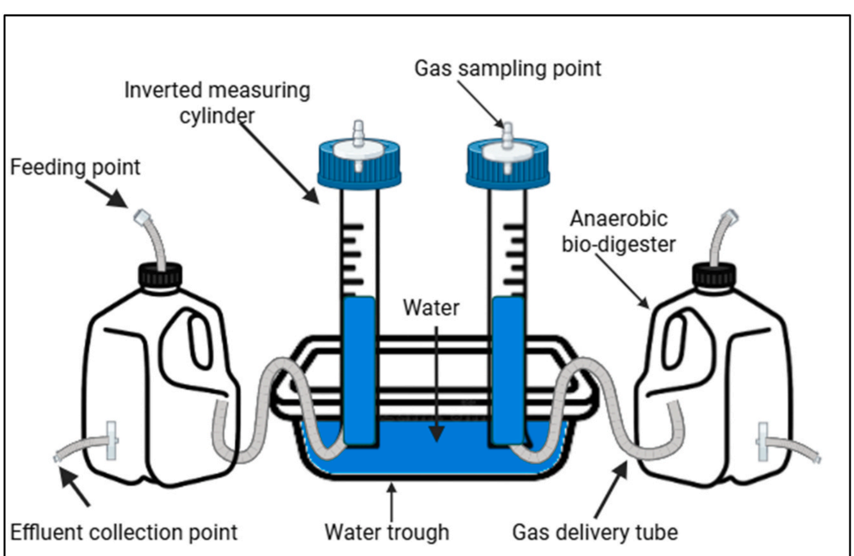

Fig. 1. An improvised biodigester prototype made using a 3.78 L plastic bottle.

feedstocks revealed clear contrasts that are important for anaerobic digestion (Table 2). Cactus had a markedly lower pH (3.7) compared with cattle dung (7.6), reflecting its acidic nature. It also had a greater DM concentration (170 g kg^{-1} fresh matter) and OM concentration (925 g kg^{-1} DM) relative to cattle dung (121 and 783 g kg^{-1} DM, respectively). By contrast, N concentration was much lower in cactus (5 g kg^{-1} DM) than in cattle dung (2.9 g kg^{-1} DM), resulting in a greater C:N ratio of 83:1 compared with 15:1 for dung. The isotopic composition also distinguished between the two feedstocks, whereby cactus displayed a less negative $\delta^{13}C$ value (−16.3‰) compared with cattle dung (−28.3‰).

2.1.5. Biodigester management

Feedstocks were introduced into the biodigester prototype on a DM basis. Each biodigester unit received 2.5 kg of DM, 80% cactus and 20% cattle dung according to Ref. [18]. The initial feedstock occupied approximately 66% of the total volume of each biodigester, allowing adequate headspace for gas collection. The inlets were sealed with rubber corks to exclude oxygen. Following this, each biodigester was fed with 2.5 g DM daily at 0700 h throughout the experiment. Similarly, there was no pressure buildup in the biodigesters; therefore, no pressure management strategies were employed in this experiment. Notably, all samples were collected without compromising the anaerobic conditions of the biodigester.

2.1.6. Response variables

Gas volume was measured daily before feeding using the water-displacement method [21] and adjusted by refilling the measuring cylinder to its original mark. Gas and effluent samples were collected every Wednesday throughout the study period. The gas samples were drawn from the biodigesters using a gas sampling septum and transferred into pre-evacuated bottles. After standardization and calibration, the samples were analyzed for CH_4, CH_4-$\delta^{13}C$, and CO_2 concentrations as previously described for C and N contents.

Cumulative methane production data were fitted using the modified Gompertz model to describe methane production kinetics during anaerobic digestion. The model assumes that methane production is associated with microbial growth and can be represented by a sigmoidal function. The modified Gompertz [23] equation was expressed as:

$$y = A \times \exp\{-\exp[R_{max} \times e \times (\lambda - t) / A + 1]\}$$

where y is the cumulative methane production (mL CH_4 g^{-1} VS) at digestion time (t) (days), A is the maximum cumulative methane yield (mL CH_4 g^{-1} VS), R_{max} is the maximum methane production rate (mL CH_4 g^{-1} VS d^{-1}), λ is the lag phase (days), and (e) is Euler's constant (2.71828).

Approximately 2.5 g DM of effluent was collected from the outlet once a week, and pH was measured immediately after sampling. Effluent samples were dried to constant weight at 55 °C and finely ground with a Wiley Mill (Model 4, Thomas-Wiley Laboratory Mill, Thomas Scientific, Swedesboro, NJ) to pass a 2-mm screen. At the end of the experiment, a composite effluent was prepared on a DM basis from the weekly samples and used in a separate trial to evaluate its potential as a biofertilizer. The sub-samples of the effluent from selected treatments were submitted to Waters Agricultural Laboratories, Inc. for the analysis of total N (using the macro-Kjeldahl method), and total phosphorus, potassium, sulfur, calcium, and magnesium using the Mehlich III extraction method.

2.2. Experiment 2: Biofertilizer evaluation

2.2.1. Experimental design and treatments

The experiment was conducted at the University of Florida, North Florida Research and Education Center (NFREC) greenhouse from January through March 2024, for 50 days. A randomized complete block design (RCBD) with a 4 × 3 + 1 factorial design was used to evaluate the effects of 4 effluent types at three application rates, including a control on the growth of Prine ryegrass.

Treatments 5 and 6 from experiment 1 were selected for effluent evaluation because they demonstrated the highest biogas production and methane concentration among all treatments. These treatments involved 42 days of pre-fermentation of cattle dung before cactus inclusion, resulting in improved system performance compared with shorter pre-fermentation durations and the control treatments. Therefore, effluents were obtained from four anaerobic digestion treatments: (5) cattle dung on day 42 + cactus, (6) cattle dung on day 42 + cactus with weekly cactus replacement by dung, and two controls, (7) cactus only, and (8) cattle dung only. Each effluent type was applied at three rates (9.5, 18.9, and 28.4 g DM pot^{-1} corresponding to 10, 20, and 30 t ha^{-1}, respectively, when scaled to a hectare basis assuming a soil bulk density of approximately 1.2 g cm^{-3}), along with a no-effluent control.

A total of 39 experimental units were established across three blocks, each comprising 13 pots. Experimental units consisted of 3.8-L plastic planting pots containing 1.5 kg of inert sand. Effluents were applied once at planting time by surface application. About 10 seeds were planted per pot. Two weeks after germination, seedlings were thinned to five plants per pot. Plants were irrigated daily at 0700 h by applying 150 mL of water per pot. A plate was placed beneath each pot to collect any excess water, which was later returned to the pot to minimize water and nutrient loss.

2.2.2. Response variables

The collected data included plant height, aboveground biomass, and root biomass. Plant height was measured once at the end of the experiment by placing a ruler at the base of the plant to the tallest point. Three plants were randomly selected and measured per pot, and the values were averaged to obtain a mean plant height for each experimental unit. All plants within each pot were clipped at the soil surface following height measurements. Aboveground biomass was collected, oven-dried at 55 °C for 72 h in a forced-air drying oven and weighed to determine dry mass. After the aboveground harvest, the remaining sand and root material from each pot was collected and dried intact at 55 °C in a forced-air oven until a constant weight was reached, after which the total mass was recorded. The dried sand-root mixture was then washed through an 850-μm sieve using repeated decantation with water to remove adhered sand and foreign material. After washing, roots were separated, oven-dried at 55 °C for 72 h and weighed. Root biomass was expressed on a soil mass basis (g kg^{-1} soil), whereas shoot biomass was expressed on a per pot basis (g pot^{-1}).

2.3. Statistical analysis

All data were analyzed using the GLIMMIX procedure in SAS (SAS Institute, 2013) following model diagnostics. The weekly methane output, pH dynamics, and CH_4-C concentrations were evaluated as repeated measures to address the potential autocorrelation in the time-series data. In addition, cumulative methane (obtained by summing weekly outputs) was analyzed separately to assess both time-course trends and endpoint statistical comparisons. Treatment was specified as a fixed effect, while block was treated as a random effect. To assess treatment effects on pH, planned contrasts were performed by categorizing treatments based on three factors: (1) whether cattle dung was pre-fermented (primed) or not before use, (2) whether cactus was replaced weekly with fresh cattle dung or not, and (3) whether cattle dung was included as a co-substrate or not. Least squares means (LSMEANS) were compared using the PDIFF option with the Tukey adjustment for multiple comparisons, and statistical significance was declared at $P < 0.05$. Nonlinear regression analysis was performed using PROC NLIN with the Marquardt iterative method to estimate model parameters. Model performance was evaluated using the coefficient of determination (R^2), comparing observed and predicted cumulative methane production values. Changes in biogas composition were evaluated by analyzing methane (CH_4) and carbon dioxide (CO_2) concentrations across three digestion phases: early (weeks 1–6), mid (weeks 7–13), and late (weeks 14–20). Weekly gas composition measurements were grouped into these phases to assess temporal shifts in biogas quality during anaerobic digestion. Mean CH_4 and CO_2 concentrations were calculated for each treatment within each phase. Treatment, phase, and their interaction were included as fixed effects, and block was a random effect. Repeated measurements were modeled using a first-order autoregressive covariance structure [AR(1)] to account for temporal correlation. Descriptive statistics were used to summarize the physical and chemical properties of cactus and cattle dung feedstocks.

3. Results

3.1. Methane production

Cumulative methane production increased over the 140-day incubation period, with all treatments showing a lag phase during the first 6 weeks (Fig. 2). Significant treatment effects were observed on day 140 ($P < 0.0001$). The highest methane yields were obtained when cattle dung (CD) was pre-fermented for 42 days before cactus inclusion, with CD day 42 + C + replacement (64.1 mL CH_4 g^{-1} VS) and CD day 42 + C (61.3 mL CH_4 g^{-1} VS) producing the greatest yields. Intermediate yields were observed for CD only (56.6 mL CH_4 g^{-1} VS) and CD day 21 + C + replacement (51.5 mL CH_4 g^{-1} VS). In contrast, CD day 21 + C without replacement produced substantially less methane (19.2 mL CH_4 g^{-1} VS). Methane production was negligible in treatments containing fresh cattle dung with cactus and in the cactus-only treatment (<1.5 mL CH_4 g^{-1} VS).

The modified Gompertz model adequately described cumulative methane production with high coefficients of determination (R^2) ranging from 0.9916 to 0.9968 (Table 3). Estimated methane production potential (A) ranged from 21.42 to 78.67 mL g^{-1} VS, with the highest value observed in CD day 42 + C + replacement, followed by CD day 42 + C, CD day 21 + C + replacement, and CD only. Estimated maximum methane production rate (R_{max}) ranged from 0.2671 to 0.9178 mL g^{-1} VS day^{-1}, with the highest value recorded for CD day 42 + C, closely followed by CD day 42 + C + replacement. Experimental methane production potential ranged from 19.22 to 64.09 mL g^{-1} VS, while experimental R_{max} ranged from 0.4237 to 1.3744 mL g^{-1} VS day^{-1}. Lag phase (λ) varied from 35.71 to 56.23 days, with the shortest lag observed for CD day 21 + C and the longest for CD day 21 + C + replacement.

A significant treatment × week interaction was observed for methane yield ($P < 0.0001$). Methane production varied markedly among treatments over the 20-week incubation period. The greatest methane yields were recorded when cactus was introduced after 42 days of cattle dung pre-fermentation, with or without replacement (Fig. 3e and f). Methane yield increased rapidly after week 6, peaking at approximately 0.40 mL CH_4 g^{-1} VS in the day-42 treatment and 0.28–0.30 mL CH_4 g^{-1} VS in the day-42 + replacement treatment between weeks 10 and 14, before gradually declining. The day-21 + replacement treatment (Fig. 3d) also produced substantial methane,

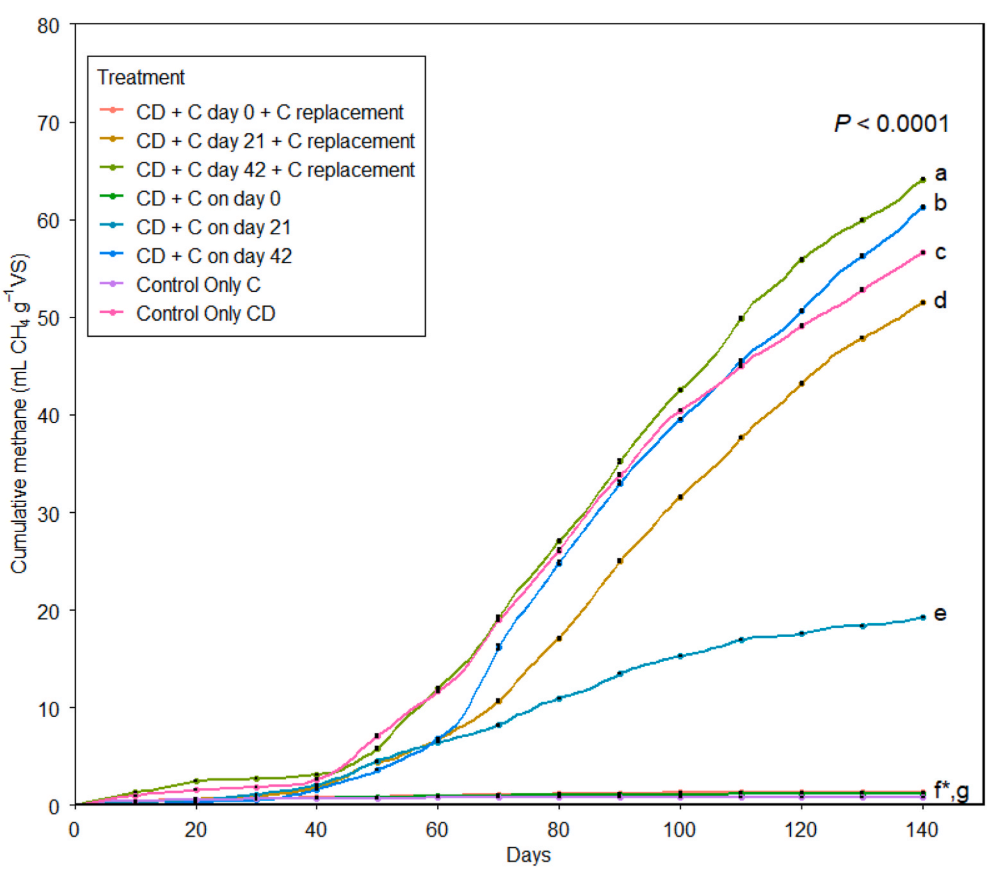

Fig. 2. Cumulative methane production during 140 days of anaerobic digestion. Treatments were: CD + C day 0, cattle dung plus cactus from day 0; CD + C day 0 + replacement, cattle dung plus cactus from day 0 with weekly cactus replacement; CD + C day 21, cattle dung pre-fermented for 21 days before cactus addition; CD + C day 21 + replacement, cattle dung pre-fermented for 21 days before cactus addition with weekly cactus replacement; CD + C day 42, cattle dung pre-fermented for 42 days before cactus addition; CD + C day 42 + replacement, cattle dung pre-fermented for 42 days before cactus addition with weekly cactus replacement; CD only, cattle dung only; C only, cactus only. The *P*-value refers to treatment differences at day 140. Different letters indicate significant differences among treatments at $P < 0.05$. The letters f* indicate no significant difference between CD + C day 0 + replacement and CD + C day 0, while g represents Control Only C VS=Volatile solids.

Table 3
Modified Gompertz kinetic parameters describing cumulative methane production during anaerobic digestion of cattle dung and cactus under different treatment combinations.

Treatment	[a]A (mL g^{-1} VS)		[b]R_{max} (mL g^{-1} VS day^{-1})		[c]λ (days)	[d]R^2
	Fit results	Exp results	Fit results	Exp results		
CD day 21 + C	21.4	19.2	0.3	0.4	35.7	0.9952
CD day 21 + C + replacement	67.6	51.5	0.8	0.9	56.2	0.9916
CD day 42 + C	67.9	61.3	0.9	1.4	53.5	0.9968
CD day 42 + C + replacement	78.7	64.1	0.9	1.1	49.0	0.9927
CD only	63.9	56.6	0.8	1.1	46.1	0.9961

Kinetic parameters were not interpreted for treatments CD day 0 + C, CD day 0 + C + replacement, and cactus only, because cumulative methane production was negligible and did not exhibit a distinct sigmoidal pattern, resulting in unstable lag-phase estimates.
[a] A = maximum cumulative methane yield.
[b] Rmax = maximum methane production rate.
[c] λ = lag phase time.
[d] R^2 = coefficient of determination, CD = cattle dung; C = cactus; Exp = Experimental.

reaching a maximum of approximately 0.26 mL CH_4 g^{-1} VS around weeks 12–13. In contrast, the day-21 treatment without replacement (Fig. 3c) exhibited lower methane yields, generally remaining below 0.11 mL CH_4 g^{-1} VS throughout the study.

Cattle dung alone (Fig. 3g) generated intermediate methane yields that increased steadily to a peak of approximately 0.28–0.30 mL CH_4 g^{-1} VS between weeks 10 and 14, then declined. Negligible methane production was observed when cactus was added on day 0, either with or without replacement (Fig. 3a and b), and in the cactus-only treatment (Fig. 3h), where methane yields remained near zero throughout the experiment. These results indicate that pre-fermentation of cattle dung, particularly for 42 days, created the most favorable conditions for methanogenesis, while weekly replacement enhanced methane production when cactus was introduced after 21 days of pre-fermentation.

The treatment × phase interaction significantly affected both CH_4 and CO_2 composition ($P < 0.0001$; Table 4). Treatments where cactus was added at day 0 and the cactus-only treatment maintained consistently low CH_4 concentrations throughout digestion (<8%). In contrast, CH_4 increased markedly in pre-fermented dung treatments, particularly CD day 21 + C + replacement, where CH_4 increased from 12.8% in the early phase to 51.4% in the late phase. Similarly, CD day 42 + C and CD day 42 + C + replacement reached the highest late-phase CH_4 concentrations (52.3% and 52.1%, respectively). Although CO_2 concentrations increased during mid to late digestion for CD day 21 + C + replacement, CD day 42 + C, and CD day 42 + C + replacement, their CH_4:CO_2 ratios remained substantially higher (1.7–2.7) than those of the other treatments.

3.2. Effects on pH

All contrasts showed significant effects on pH ($P < 0.0001$; Fig. 4). Priming, where cattle dung was pre-fermented before cactus inclusion, resulted in a significantly greater pH (6.4) compared with treatments without priming (4.8), maintaining values within the methanogenic range (Fig. 4a). Weekly replacement of cactus with fresh dung also produced a significantly higher pH (6.2) than treatments without replacement (5.6; Fig. 4b). Likewise, co-digestion of cactus with cattle dung maintained a significantly greater pH (6.1) compared with cactus incubated alone (3.7), which dropped to levels too acidic for efficient methanogenesis (Fig. 4c).

A significant treatment × week interaction was observed for pH during the 20-week incubation period ($P < 0.0001$; Fig. 5). Treatments in which cattle dung was pre-fermented for 42 days before cactus inclusion maintained the most stable and favorable conditions for anaerobic digestion. In particular, the combination with weekly cactus replacement (CD day 42 + C + replacement; Fig. 5f) sustained pH values above 7 throughout the experiment (range 6.0–7.4; week 20: 7.16). Similarly, treatment with 42 days pre-fermentation without

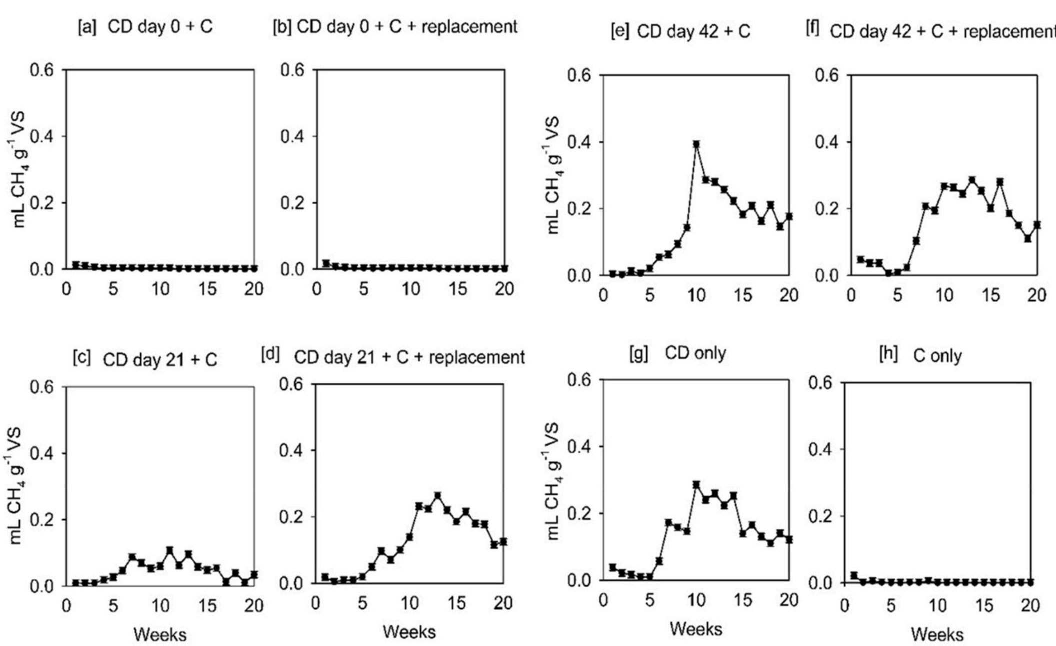

Fig. 3. Weekly methane production (g) over a 20-week anaerobic digestion period across treatments; (a) CD day 0 + C = cattle dung + cactus on day 0, (b) CD day 0 + C + replacement = cattle dung + cactus on day 0 + cactus replacement with cattle dung once a week, (c) CD day 21 + C = pre-fermented cattle dung + cactus start on day 21, (d) CD day 21 + C + replacement = pre-fermented cattle dung + cactus start on day 21 + cactus replacement with cattle dung once a week, (e) CD day 42 + C = pre-fermented cattle dung + cactus start on day 42, (f) CD day 42 + C + replacement = pre-fermented cattle dung + cactus start on day 42 + cactus replacement with cattle dung once a week, (g) CD only = Cattle dung used as the sole feedstock, (h) C only = Cactus used as the sole feedstock.

Table 4
Methane (CH_4) and carbon dioxide (CO_2) composition (%) during early, mid, and late phases of anaerobic digestion under different treatments.

Treatment x Phase	CH_4 (%)			SEM	*P*-value	CO_2(%)			SEM[a]	*P*-value
	Early[b]	Mid[c]	Late[d]			Early	Mid	Late		
CD day 0 + C	8.0 C^{a}	3.9 D^{a}	0.7 C^{a}	1.3	0.2765	18.5 A^{a}	20.0 AB^{a}	13.7 AB^{a}	11.6	0.3512
CD day 0 + C + replacement	6.2 C^{a}	4.7 D^{a}	1.6 C^{a}	1.3	0.4627	15.8 A^{a}	19.0 AB^{a}	21.3 AB^{a}	8.6	0.4608
CD day 21 + C	13.3 BC^{ab}	18.8 C^{a}	8.8 BC^{b}	5.7	0.0188	14.1 A^{a}	17.8 AB^{a}	10.7 B^{a}	5.1	0.2264
CD day 21 + C + replacement	12.8 BC^{c}	43.3 A^{b}	51.4 A^{a}	8.7	<0.0001	6.0 A^{b}	25.3 A^{a}	27.7 A^{a}	6.1	<0.0001
CD day 42 + C	7.8 C^{c}	33.8 B^{b}	52.3 A^{a}	6.9	<0.0001	9.8 A^{a}	24.0 A^{a}	21.7 AB^{a}	8.0	0.0027
CD day 42 + C + replacement	23.8 A^{c}	38.2 AB^{b}	52.1 A^{a}	9.0	<0.0001	10.1 A^{a}	16.5 AB^{a}	19.4 AB^{a}	5.6	0.1753
CD only	20.6 AB^{b}	34.6 B^{a}	13.3 BC^{b}	8.3	<0.0001	10.9 A^{a}	14.2 AB^{a}	9.0 B^{a}	4.3	0.4565
C only	7.4 C^{a}	2.9 D^{a}	0.0 C^{a}	4.5	0.4156	9.1 A^{a}	4.8 B^{a}	4.6 AB^{a}	5.3	0.6491
SEM	5.7	8.7	5.1			6.0	6.9	8.6		
P-value	0.0008	<0.0001	<0.0001			0.3123	0.0008	<0.0001		

Treatment × phase interaction was significant for both CH_4 and CO_2 ($P < 0.0001$). Means within a column followed by different uppercase letters differ significantly among treatments, whereas means within a row followed by different lowercase letters differ significantly among phases ($P < 0.05$).

[a] Pooled standard error of the mean (SEM).
[b] Early phase = Weeks 1–6.
[c] Mid phase = Weeks 7–13.
[d] Late phase = Weeks 14–20.

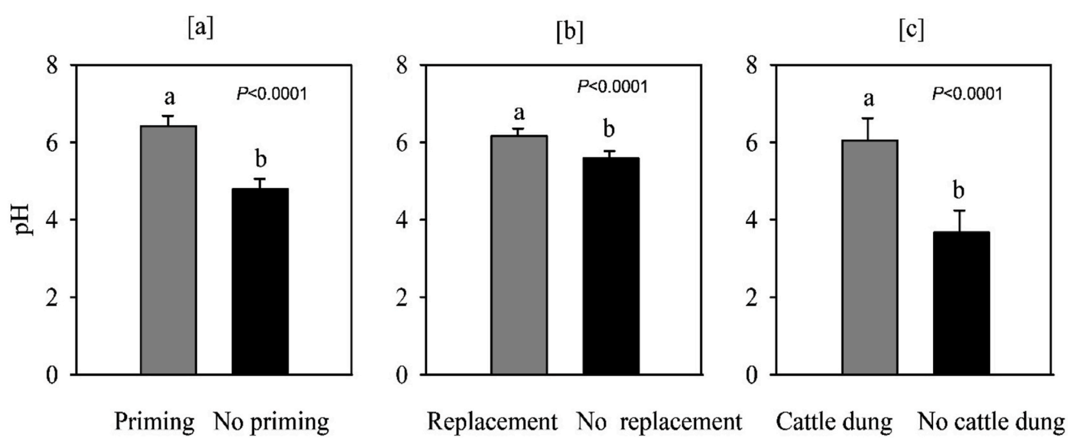

Fig. 4. Effect of priming, replacement, and cow dung inclusion on the pH of the anaerobic digestion mixture. Bars represent mean ± SE. Columns with different letters differ significantly ($P < 0.0001$), (a) Priming = treatments where cattle dung was pre-fermented before the inclusion of cactus, No priming = treatments where cattle dung and cactus were incubated simultaneously without pre-fermentation. Replacement = treatments where cactus was replaced with cattle dung once a week during the daily feeding. No replacement = treatments where cactus was fed daily throughout the experiment period. Cattle dung = treatments where cactus was co-digested with cattle dung. No cattle dung = treatments where cactus was the sole feedstock incubated.

replacement (CD day 42 + C; Fig. 5e) and cattle dung alone (CD only; Fig. 5g) stabilized the pH near neutral (6.5–7.4), with final values of 6.8 and 6.9, respectively. Weekly replacement after a shorter stabilization period (CD day 21 + C + replacement; Fig. 5d) also effectively buffered the system, with pH increasing to above 7 by mid-incubation (peak 7.5 at week 13) and remaining above 6.2 by week 20.

In contrast, treatments with no or limited stabilization showed continuous acidification. For example, fresh dung co-digested with cactus from day 0 (CD day 0 + C; Fig. 5a) declined steadily from an initial pH of 5.6 to 3.94 in week 20, while its replacement variant (CD day 0 + C + replacement; Fig. 5b) dropped from 5.6 to 4.41. Pre-fermentation for only 21 days without replacement (CD day 21 + C; Fig. 5c) provided only temporary buffering, as pH decreased from 6.5 to 4.54 by week 20. The most severe acidification occurred in the cactus-only treatment (C only; Fig. 5h), which started at pH 4.5 and fell to 3.13 by week 20.

3.3. CH_4-C from cactus

A significant interaction ($P < 0.0001$) was observed between treatment and week for the contribution of cactus-derived C to methane (CH_4-C). The effect of dung priming was evident in promoting more efficient conversion of cactus C into methane over the 20-week period. Across all treatments where cactus was co-digested with cattle dung, CH_4-C contributions generally exceeded 50% (Fig. 6). Treatments involving pre-fermented dung, either stabilized for 21 days (Fig. 6c and d) or 42 days (Fig. 6e and f), showed an increase in carbon contribution in the methane. Notably, CD day 21 + C (Fig. 6c) had the highest contributions of 81% in week 17 and maintained values above 70% from weeks 17 to 19, while CD day 42 + C + replacement (Fig. 6f) achieved the greatest late-phase contribution (78% in week 18).

In contrast, treatments where cactus was added to fresh dung without priming (Fig. 6a and b) displayed lower, less variable CH4-C values that generally ranged between 45 and 60% during the initial weeks. For example, CD day 0 + C (Fig. 6a) increased to 61% during week 4 and then leveled off, with final values (57% in week 20) still well below those of the primed treatments. CD day 0 + C + replacement showed a similar trend (Fig. 6b), with value levelling off around 50% despite some week-to-week variation.

3.4. Effluent nutrient composition

Effluent from cattle dung only (CD) had significantly greater concentration of N (34 g kg^{-1}), phosphorus (42 g kg^{-1}), potassium (27 g kg^{-1}), and sulfur (4 g kg^{-1}) than all the other treatments (Table 5). Macronutrient concentrations were intermediate in treatments where

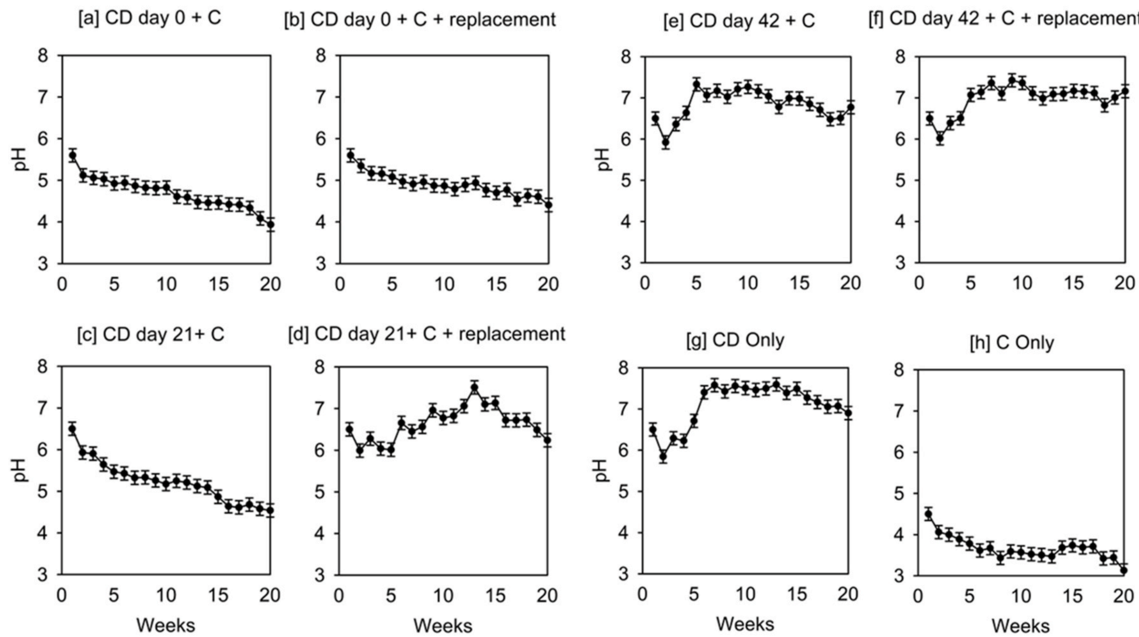

Fig. 5. Weekly pH dynamics during 20 weeks of anaerobic digestion across treatments; (a) CD day 0 + C = cattle dung + cactus on day 0, (b) CD day 0 + C + replacement = cattle dung + cactus on day 0 + cactus replacement with cattle dung once a week, (c) CD day 21 + C = pre-fermented cattle dung + cactus start on day 21, (d) CD day 21 + C + replacement = pre-fermented cattle dung + cactus start on day 21 + cactus replacement with cattle dung once a week, (e) CD day 42 + C = pre-fermented cattle dung + cactus start on day 42, (f) CD day 42 + C + replacement = pre-fermented cattle dung + cactus start on day 42 + cactus replacement with cattle dung once a week, (g) CD only = cattle dung used as the sole feedstock, (h) C only = cactus used as the sole feedstock.

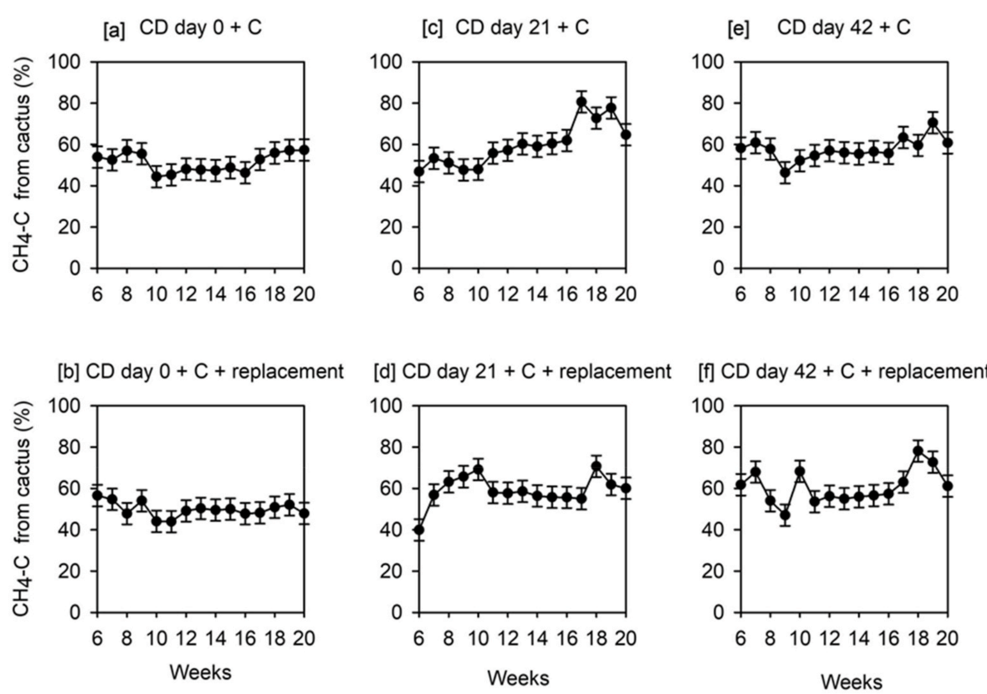

Fig. 6. Proportion of methane carbon derived from cactus (CH_4-C) over time for treatments where cactus was co-digested with cattle dung; (a) CD day 0 + C = cattle dung + cactus on day 0, (b) CD day 0 + C + replacement = cattle dung + cactus on day 0 + cactus replacement with cattle dung once a week, (c) CD day 21 + C = pre-fermented cattle dung + cactus start on day 21, (d) CD day 21 + C + replacement = pre-fermented cattle dung + cactus start on day 21 + cactus replacement with cattle dung once a week, (e) CD day 42 + C = pre-fermented cattle dung + cactus start on day 42, (f) CD day 42 + C + replacement = pre-fermented cattle dung + cactus start on day 42 + cactus replacement with cattle dung once a week.

cattle dung was pre-fermented for 42 days before cactus inclusion (D42) or when cactus was replaced weekly with dung after 42 days (D42+R), which did not differ from each other. In contrast, treatment with cactus only had the lowest concentrations of these elements (N = 6 g kg^{-1}, P_2O_5 = 6 g kg^{-1}, K_2O = 14 g kg^{-1}, S = 2 g kg^{-1}), indicating limited nutrient recovery in the absence of cattle dung.

Calcium concentrations were greatest in effluents from pre-fermented dung without replacement (26.6 g kg^{-1}) and with weekly replacement (26.3 g kg^{-1}), both significantly greater than in CD (24.5 g kg^{-1}) and cactus-only (24.2 g kg^{-1}). Magnesium concentrations

Table 5
Macronutrient concentrations (g kg^{-1} DM) in pooled effluents from different treatments over a 20-week incubation period.

Item[1]	N	P_2O_5	K_2O	S	Ca	Mg
	g kg^{-1} DM					
CD	33.8 ± 0.4^a	42.1 ± 0.7^a	27.2 ± 1.0^a	4.4 ± 0.1^a	24.5 ± 0.5^b	13.6 ± 0.4^a
D42+R	28.4 ± 0.8^b	30.2 ± 1.2^b	24.5 ± 0.5^b	3.6 ± 0.5^b	26.3 ± 0.2^a	13.6 ± 0.4^a
D42	28.0 ± 1.0^b	29.5 ± 2.5^b	24.0 ± 0.2^b	3.5 ± 0.2^b	26.6 ± 0.4^a	13.2 ± 0.3^a
Cactus	6.3 ± 0.1^c	6.4 ± 0.1^c	13.5 ± 0.2^c	2.4 ± 0.04^c	24.2 ± 0.2^b	8.1 ± 0.04^b
SE	0.68	1.44	0.57	0.13	0.36	0.31

Different superscript letters within a column indicate statistically significant differences among treatments ($P < 0.05$) based on Tukey's test; treatments sharing the same letter are not significantly different. [1]CD = Effluent from cattle dung only, D42+R = effluent from pre-fermented cattle dung + cactus start on day 42 + cactus replacement with cattle dung once a week, D42 = effluent from pre-fermented cattle dung + cactus start on day 42, (8) C only = Cactus used as the sole feedstock.

were similar in CD (13.6 g kg^{-1}), D42+R (13.6 g kg^{-1}), and D42 (13.2 g kg^{-1}), but were significantly lower in the cactus-only treatment (8.1 g kg^{-1}).

3.5. *Plant height and root biomass*

Plant height differed significantly among effluent types ($P < 0.0001$; Fig. 7a), while there were no effects of dose or interaction. The tallest plants were recorded in treatment with effluent from cattle dung only (CD; 41 cm), which was significantly greater than all other treatments. Heights were intermediate for D42+R (28 cm) and D42 (25 cm), which did not differ from each other. The control treatment produced the shortest plants (18.7 cm), while the cactus-only effluent resulted in the lowest values (9.7 cm).

Root biomass also varied significantly across effluent types ($P < 0.0001$; Fig. 7b) with no effects of dose or interaction. Effluent from CD yielded the greatest root mass (0.3 g kg^{-1} soil), significantly exceeding all other treatments. D42+R (0.2 g kg^{-1}) and D42 (0.1 g kg^{-1}) were intermediate and not statistically different from one another. Root biomass from the control (0.024 g kg^{-1}) and cactus-only (0.059 g kg^{-1}) treatments had the lowest DM and did not differ from each other.

3.6. *Aboveground biomass*

Significant treatment effect ($P < 0.0001$) was observed for the aboveground DM yield in ryegrass (Fig. 8a). The greatest DM was recorded in treatment with cattle dung only (CD; 1 g pot^{-1}), which was significantly greater than all other treatments. Intermediate yields were obtained with D42+R (0.6 g pot^{-1}) and D42 (0.4 g pot^{-1}), which did not differ statistically. The control (0.1 g pot^{-1}) and cactus-only effluent (0.2 g pot^{-1}) produced the lowest biomass, both significantly less than CD and D42+R. Even though the cactus effluent from D42+R and D42 produced lower biomass than that from cattle dung only, they still yielded significantly greater biomass than the control, indicating their potential value as biofertilizer.

A similar pattern was observed across effluent levels (Fig. 8b). Biomass production significantly ($P = 0.0138$) increased with increasing effluent application, indicating a clear level-dependent effect. The highest effluent application resulted in the greatest yield (0.7 g pot^{-1}), significantly greater than the lowest level (0.5 g pot^{-1}), but did not differ significantly from the intermediate level.

4. Discussion

In this study, cactus exhibited a markedly lower pH compared to cattle dung, confirming its acidic nature [11]. It also showed a much higher C:N ratio, indicating a nutrient imbalance [18], which inhibits methanogenesis and limits the buffering capacity of cactus feedstock when used alone [24] According to Liu et al. and Quiroz et al. [10,12], co-digestion of cactus with other feedstocks can enhance and stabilize methane yield, providing an effective strategy to overcome this limitation. For example, cattle dung serves as an important source of N, contributing to the maintenance of an optimal C:N ratio [25], promoting rapid microbial growth [26], and stabilizing microbial activity during

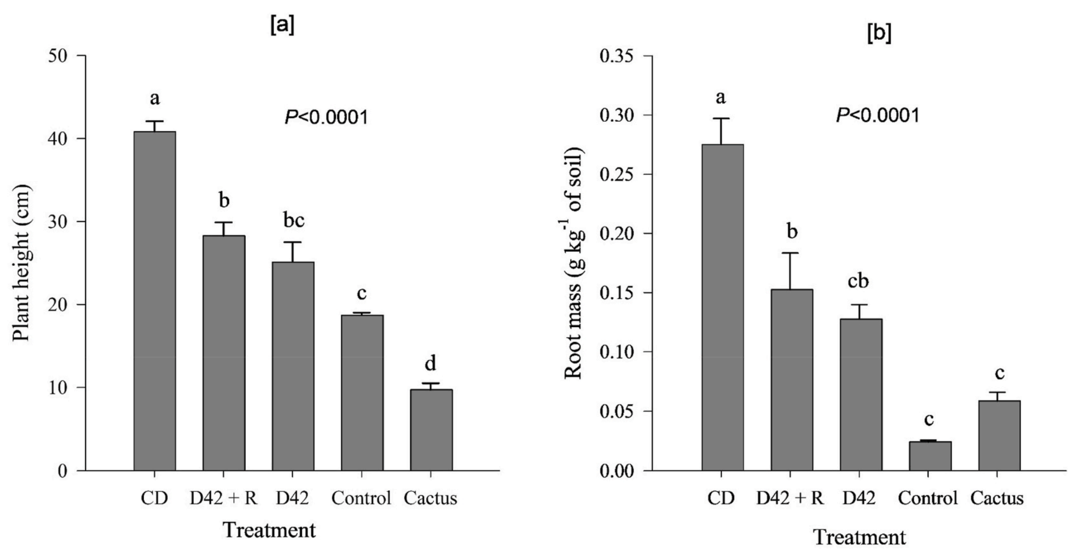

Fig. 7. Effect of different treatments on (a) plant height and (b) root biomass. Bars represent least squares means. Letters indicate significant differences according to Tukey's test at $P < 0.05$. Treatments: CD = effluent from cattle dung only; D42 + R = effluent from treatment where cactus was introduced on day 42 in pre-fermented dung, thereafter, fed daily with cactus except for once a week when cactus was replaced with cattle dung; D42 = cactus introduced on Day 42 without replacement; control = no fertilizer and cactus = effluent from treatment with cactus only.

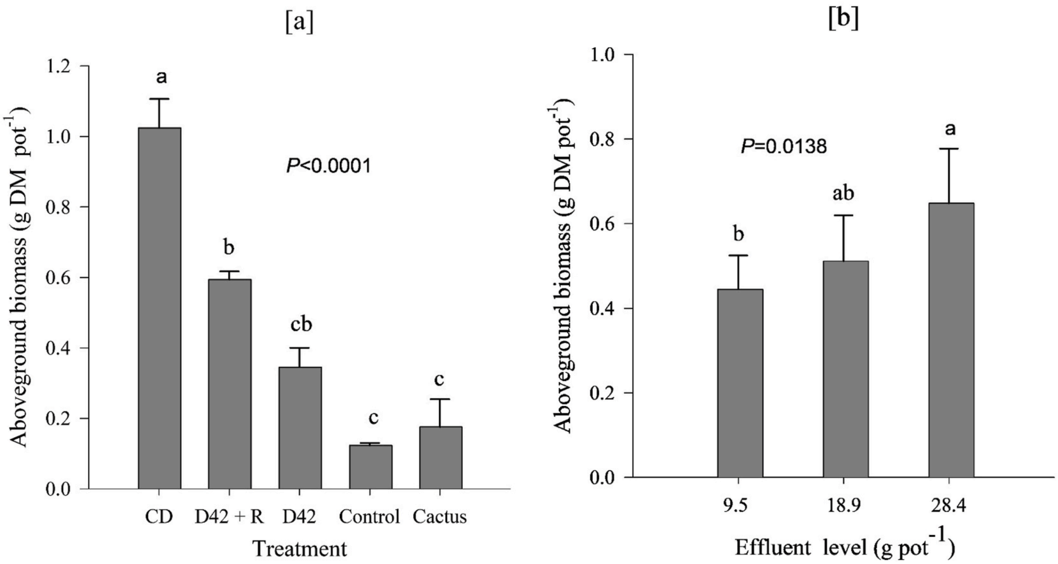

Fig. 8. Effect of (a) treatment and (b) effluent level on dry matter yield of ryegrass. Bars represent least squares means. Letters indicate significant differences according to Tukey's test at $P < 0.05$. Treatments: CD = cattle dung only; D42 + R = cactus introduced on Day 42 with once weekly replacement; D42 = cactus introduced on Day 42 without replacement; Control = no fertilizer; Cactus = cactus only without cattle dung, effluent levels: 9.5 g pot^{-1} equivalent to 10 t ha^{-1}; 18.9 g pot^{-1} equivalent to 20 t ha^{-1}; 28.4 g pot^{-1} equivalent to 30 t ha^{-1}.

anaerobic digestion [20]. However, the greater organic matter concentration in cactus relative to cattle dung suggests a higher biogas production potential per unit of dry mass. The distinct isotopic fingerprints between cactus and cattle dung (from cattle that exclusively grazed on C3 pasture) provide a useful basis for tracing the relative carbon contributions of each substrate to methane production in co-digestion systems.

The greatest methane yield was observed in the treatment in which cattle dung was pre-incubated for 42 days before the introduction of cactus biomass, combined with weekly replacement of cactus with fresh dung. This treatment may have created more favorable conditions for methanogenesis and improved process stability. These findings align with other studies that emphasize the importance of feedstock pre-treatment or inoculum acclimation to enhance anaerobic digestion performance [27,28]. For instance, methane yields increased when cattle dung was incubated at 37 °C for 2 weeks before use [29]. The authors attributed this improvement to microbial acclimatization to mesophilic conditions. Similarly, swine manure stored in a pit for two months had more efficient inoculum, supporting sustained biogas production [30]. These studies highlight the value of using aged or pre-treated manure as a microbial starter with enhanced functional capacity. Weekly replacement of cactus with fresh cattle dung further improved methane production. This likely supplied fresh microbial inoculum, alkalinity, and nitrogen, thereby enhancing buffering capacity and maintaining favorable conditions for methanogenesis. According to Zeng et al. [27], inoculum acclimation is an important operational step in bioreactor setups, promoting microbial adaptation and system stability during. Likewise, anaerobic acclimation of inoculants over 72 days has been shown to enrich specific microbial populations, thereby enhancing the stability and efficiency of methane production [28]. Although microbial populations were not evaluated in this study, differences in methane production among treatments were likely influenced by shifts in microbial community structure and functional activity during digestion. Further research involving microbial characterization is needed to better elucidate these mechanisms.

A notable observation across all treatments was the delayed onset of significant methane production, with appreciable yields emerging after approximately six weeks. It has been reported that biogas production increases with retention time, reaching pH stability after around 40 days in continuous digesters [31]. The 40-day timeline observed in this study aligns closely with the observed 6-week lag phase in our study. One possible explanation for the delayed onset of methane production is the initial drop in pH caused by the introduction of cactus biomass, which inhibits methanogens that thrive optimally within a narrow pH range [12]. The extended lag phase observed in this study likely reflects the time required for system stabilization and favorable conditions for methanogenesis [32,33]. Similar patterns of delayed methane production have been observed in other studies involving lignocellulosic substrates [34,35]. According to Espinosa-Slares et al. [26], the initial degradation of complex organic matter by hydrolytic microorganisms is typically slow and may be associated with the accumulation of acidic intermediates during the early stages of digestion. Although lag phases remained relatively long across treatments, the greater methane production observed after this adaptation period suggests efficient methanogenesis once microbial communities stabilized. The longer lag phase compared with short-term batch studies [28,36] likely reflects microbial acclimation and pH stabilization under semi-continuous feeding conditions.

The modified Gompertz model further supported these observations, with high R^2 values indicating that methane production kinetics were adequately described across productive treatments. Similar findings were reported by Zhang et al. [36], who showed that sigmoidal models effectively captured cumulative methane production trends during anaerobic co-digestion of agricultural wastes. Higher methane production potential (A) and maximum methane production rates (R_{max}) in the day-42 treatments indicate greater methane generation capacity and more efficient substrate conversion.

In this study, treatments involving pre-fermented cattle dung and weekly cactus replacement consistently maintained pH levels above 7.0 throughout the 20 weeks. This aligns with optimal conditions for methanogenesis reported by Liu et al. [12]. Anaerobic pre-incubation of cattle dung before cactus addition likely enhanced a stable microbial population before the addition of cactus. The observed changes in pH following longer pre-fermentation have been reported in other studies and likely reflect improved microbial acclimatization [34] and shifts in microbial community structure [35,37], which develop over time in anaerobic systems. For example, Espinosa-Solares et al. [26] demonstrated that manure pre-fermentation enhanced microbial adaptation and improved pH stability during cactus co-digestion. The weekly recharge with fresh dung may have improved system buffering capacity and reduced acidification [18,38].

In contrast, treatments with cactus only or with unprimed dung exhibited progressive acidification, with pH declining to values near or below 5.0. The low pH observed in this study has been reported to inhibit methanogenic activity [39]. Cactus cladodes are typically acidic, with pH values ranging from 3.5 to 5.5, likely due to the accumulation of organic acids produced through their CAM pathway [40]. When used as a sole feedstock, these characteristics often lead to rapid acidification during anaerobic digestion, as seen here, resulting in system instability [10]. This results in low methane production since methanogens are sensitive to changes in operating parameters [41].

Changes in biogas composition and CH_4:CO_2 ratios likely reflected shifts in microbial activity and digestion efficiency. Higher CO_2 concentrations and lower CH_4:CO_2 ratios during the early phase likely indicate active hydrolysis and acidogenesis, during which readily degradable organic matter was converted into volatile fatty acids and other intermediates [35,42]. As digestion progressed, the increase in CH_4 concentration and CH_4:CO_2 ratios in pre-fermented cattle dung treatments suggests more efficient methanogenesis and improved conversion of these intermediates into methane [42]. In contrast, persistently low CH_4 concentrations and low CH_4:CO_2 ratios in day-0 and cactus-only treatments likely reflect prolonged acidogenic conditions and suppressed methanogenesis due to rapid acidification driven by cactus-derived soluble carbohydrates and organic acids at low pH [10, 41].

Cactus is rich in readily fermentable, water-soluble carbohydrates that rapidly release carbon during anaerobic digestion [43]. The steady increase in CH4-C observed in treatments with pre-fermented cattle dung could be attributed to microbial efficiency in the highly fermentable carbon in cactus. According to Madamwar and Mithal [44], pectin-rich feedstocks exhibit higher gas production and methane concentrations. Since cactus is naturally rich in pectin [45], this likely contributed to the efficient methane conversion observed in this study. Typically, pectin acts similarly to activated carbon, providing adsorption sites that create localized zones of increased substrate concentration, thereby supporting microbial growth [44]. This may explain the greater CH_4-C contributions observed when cactus was co-digested with pre-fermented dung.

Significant differences in macronutrient concentrations were observed among effluents from different treatments, consistent with previous findings showing that digestate nutrient composition varies with feedstock type and digestion conditions [46]. All macronutrient concentrations measured in this study were within the ranges reported by Santos et al. [45] for typical anaerobic digestates, indicating values consistent with those of conventional digestates. The greater concentrations of N, phosphorus, potassium, and sulfur in the cattle dung-only treatment can be attributed to the inherently nutrient-rich composition of dung [25,46]. The low total N concentration observed in the cactus feedstock likely resulted in a digesta with lower nutrient availability [10,12]. This nutrient limitation may have reduced microbial activity and consequently the release of mineral nutrients in the cactus-only treatment. Notably, the results from treatments where cactus was introduced after 42 days of cattle dung pre-fermentation suggest that co-digestion with pre-incubated cattle dung can enhance nutrient recovery compared with cactus digestion alone. This is likely due to improved digestive efficiency and greater substrate conversion [26,47]. These treatments yielded macronutrient concentrations approaching those of the cattle dung treatment, suggesting improved digestion efficiency and nutrient recovery under these treatment conditions.

Applying effluent from the biodigester increased aboveground and root biomass in annual ryegrass, suggesting the ability to supply plant nutrients. Similar effluent responses have been observed in hybrid giant napiergrass (*Pennisetum hydridum*) [47], winter rye (*Secale cereale* L., var. Turbogreen) [48], and switchgrass (*Panicum virgatum* L.) [49,50]. In these studies, digestate application enhanced biomass yield and nutrient uptake, highlighting its potential as a bio-fertilizer. In this study, differences in ryegrass biomass among treatments were likely driven by variation in digestate nutrient composition, particularly the concentrations of N, P, and K which are critical for plant growth [48]. Such responses can be significant, particularly in low-fertility soils, such as those found in drylands. Future research should conduct field experiments to evaluate these effects on drought-tolerant and locally relevant crops, thereby enhancing practical field application.

5. Conclusions

Results from this study suggest that the timing and management of feedstock inclusion influenced CH_4 production during co-digestion of cactus and cattle dung. Anaerobic pre-incubation of dung likely improved the stability of digestion by maintaining more favorable pH conditions and reducing acidification during digestion. Similarly, the weekly addition of dung likely improved nutrient balance and supported sustained biogas production. A delayed peak in methane production between treatments demonstrated the need for microbial multiplication and adaptation when using lignocellulosic or acidogenic substrates. Future studies should also investigate microbial community dynamics to better understand the mechanisms driving treatment differences in methane production. There is also a need to evaluate harmful gases such as hydrogen sulfide. The macronutrient concentrations in effluents from co-digestion treatments supported increased ryegrass biomass production, confirming the potential of these effluents as a biofertilizer for small-scale farming. A co-digestion strategy that incorporates pre-fermentation of cattle dung for about 6 weeks before cactus, and replaces cactus with cattle dung once a week, was associated with greater methane yield and improved digestate nutrient value under the conditions evaluated in this study.

The evaluated feedstock management strategies show promise as practical approaches to improving renewable energy generation and nutrient recycling in small-scale rural systems. Importantly, this approach does not advocate for the spread of invasive cactus species but rather seeks to place communities at the center of management efforts. In the long term, the introduction of spineless cactus varieties, such as those widely cultivated in Brazil and Mexico, could provide a sustainable alternative should invasive populations be successfully eradicated. Subsequent research should focus on field-testing low-cost biodigester models over extended periods to monitor system performance, costs, and ecological implications. Future studies should also evaluate the relative gains in methane yield, biofertilizer value, and economic benefits associated with the inclusion of cactus in co-digestion systems.

CRediT authorship contribution statement

Kenneth T. Oduor: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **Jose C.B. Dubeux:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Willis Owino:** Investigation, Project administration, Resources, Supervision, Writing – review & editing. **Lynn E. Sollenberger:** Supervision, Writing – review & editing. **Nicolas DiLorenzo:** Supervision, Writing – review & editing. **Adegbola Adesogan:** Supervision, Writing – review & editing. **Aurel Siebert:** Funding acquisition, Writing – review & editing. **Igor L. Bretas:** Data curation, Writing – review & editing. **Martin Ruiz-Moreno:** Formal analysis, Writing – review & editing. **Marilia A. Bernardini:** Investigation, Writing – review & editing. **Luana M.D. Queiroz:** Investigation, Writing – review & editing. **Kevin R. Trumpp:** Investigation, Writing – review & editing. **Javier P. Acuña:** Investigation, Writing – review & editing. **Mario A. Lira Junior:** Investigation, Writing – review & editing. **Beatriz E. Bizzuti:** Investigation, Writing – review & editing. **Flavia F. Simili:** Investigation, Writing – review & editing.

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.

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Data availability

Data will be made available on request.

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