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Forage and macronutrient accumulation in grass-legume intercropping in a warm climate

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ABSTRACT: Diversifying pastures with forage legumes may reduce nitrogen fertilization due to biological N fixation. This study aimed to quantify forage accumulation rate and macronutrients extraction and to identify the best intercropping combination between butterfly pea (*Clitoria ternatea*) – a legume, and three warm-season forage grasses of different growth habits (signalgrass – *Urochloa decumbunes*, Guinea grass - *Mega thyrsus maximus*, and bermudagrass *Cynodon dactylon*). Treatments consisted of mixes of perennial herbaceous legume, butterfly pea (twining stem), with grasses, signalgrass (decumbent stem) and Guinea grass (erect stem), and bermudagrass (stoloniferous/rhizomatous). The experiment was arranged in a randomized complete block design, with three treatments and three replications. There was interaction between the intercropping combinations and cutting cycles for forage accumulation rate (FAR) and N, P, K, Ca, Mg, and S uptake. Phosphorus and K uptake was reduced from the second cycle onwards, except for the signalgrass-butterfly pea intercropping, whose reduction was only from the third cycle. Conversely, the signalgrass-butterfly pea and bermudagrass-butterfly pea intercropping did not differ from each other in relation to total N concentration in soil, but the signalgrass-butterfly pea intercropping showed total N concentration in soil higher than that of Guinea grass-butterfly pea intercropping. A higher predominance of the N-NH_4^+ form was observed in the soil. Grass-legume intercropping increased the demand for nutrients, which makes it indispensable to verify the export of macronutrients to know when to supply these nutrients removed from the soil solution.

Keywords: forage intercropping, *Clitoria ternatea* L., warm-season, legume, forage production.

INTRODUCTION

The high demand for animal feed has led to the intensification of pastures using increasingly productive and nutritionally demanding forage. One of the factors that contributed to the productivity of forages was N fertilization, which increased the productive response of these forages in quantity and quality. Another alternative to improve pastures is the intercropping between legumes and grasses to achieve better results in herd productivity while being more sustainable (Shonieski et al., 2011; Pereira et al., 2015).

Diversification of pastures with forage legumes in traditional production systems has numerous advantages, including lower costs with N fertilization due to biological N fixation, forage availability, and N transfer to grasses in intercropping (Pirhofer-Walzl et al., 2011; Lindström et al., 2014). Thus, grasses and legumes with similar characteristics (growth habits) should be selected (Høgh-Jensen and Søegaard, 2012), supporting approximate rest periods, because frequent cuts can lead to the disappearance of legumes in the system (Silva et al., 2010a). The plants should also be comparable in terms of canopy structure, thereby minimizing competition for nutrients (Tambara et al., 2017).

Identifying a successful grass-legume intercropping relies on evaluating species adaptable to edaphoclimatic conditions that support intercropping. Stargrass (*Cynodon nlemfluensis*)-forage peanut (*Arachis pintoi* Krapovickas & W.C. Gregory cv. Belmonte) intercropping was implanted in Acre State and recommended for its compatibility (method of establishment, perennity, demand of soil fertility) and nutritional value because forage peanut has 20.4 % crude protein, therefore, this grass-legume intercropping has potential for the production of animal feed (Muir et al., 2011; Sollenberger and Dubeux Junior, 2022).

Inclusion of forage legumes in intercropping consisting of low-input grassland mixtures improves forage quantity, quality and soil fertility by adding nitrogen (N) from N_2- fixation. Intercropping is a multiple cropping practice, which involves growing two or more crops in proximity. Legumes also improve the nutritional value of the surrounding low-quality native pastures and are an important component of farming system because of their high nutritional value and ability to restore depleted nutrients in the soil (Gulwa et al., 2018). Higher yields in intercropping are due to the effective utilization of environmental resources. Intercropping under low N fertilization conditions has been shown to use resources more efficiently for plant growth, such as light, water, and nitrogen (Ton, 2021). Pereira et al. (2015) reported yields in a palisade grass [*Urochloa brizantha* (Hochst. Ex A. Rich.) R. Webster cv. Marandu]-forage peanut intercropping was 23 % higher than in the single cultivation of palisade grass under the N rate of 120 kg ha^{-1} yr^{-1}.

With the hypothesis the grass-legume intercropping with different forage growth habits could increase the demand for macronutrients and modify the concentrations of inorganic nitrogen in the soil, this study aimed to evaluate how the presence of an intercropping between the legume butterfly pea and forage grasses with different growth habits affects nitrogen levels in soil, and their response for forage accumulation rate and macronutrient accumulation.

MATERIALS AND METHODS

The experiment was conducted at the Center for Teaching and Studies in Forage, located in the Department of Animal Science of the Federal University of Ceará, in Fortaleza, located in the coastal region of Ceará State, Brazil, with 15.49 m of altitude, 30° 43′ 02″ S, and 38° 32′ 35″ W, in 2017. Climate in the area is classified as tropical rainy climate Aw′ (Köppen, 1936). Climatic data over the experimental period were obtained at the Meteorological Station of the Federal University of Ceará, 0.5 km from the experimental area (Figure 1).

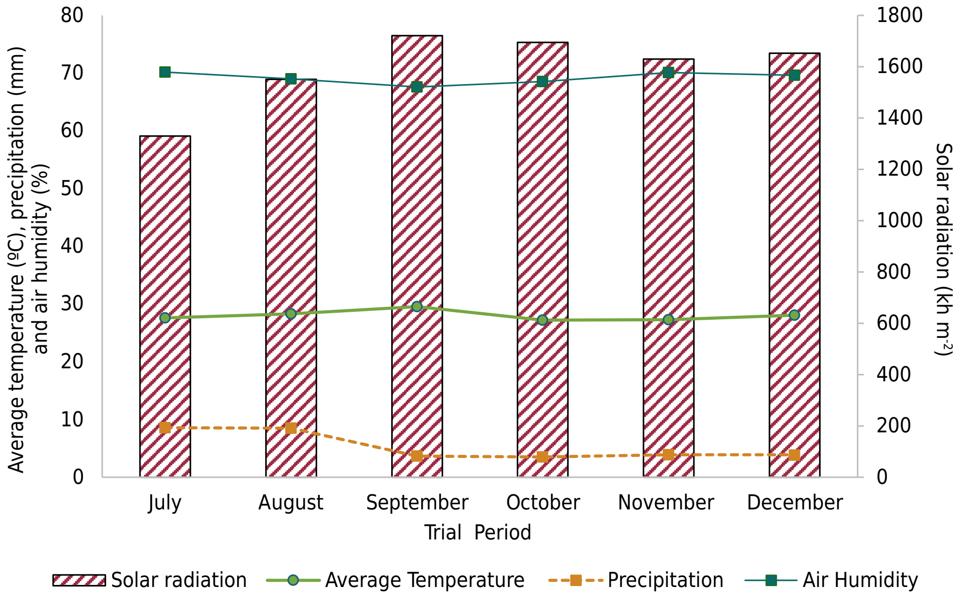

Figure 1. Climatic data over the experimental period obtained at the Meteorological Station of the Federal University of Ceará (0.5 km from the experimental area), 2017. Fortaleza, Ceará, Brazil.

The soil of the area was classified as a typical *Argissolo Amarelo Eutrófico* (Santos et al., 2018), which corresponds to an Acrisol (IUSS Working Group WRB, 2015). Samples were collected for soil fertility analyses at the layers of 0.00-0.20 and 0.20-0.40 m (Table 1). Based on the soil analysis results, recommendations were made according to Alvarez et al. (1999) for fertility levels suggested to meet production systems using the forage and legume grasses (high-level technology – for forage grasses). The recommended rates were: 50 kg ha^{-1} of P_2O_5 (single superphosphate) and 50 kg ha^{-1} of micronutrients FTE BR-12 (B – 0.8 %, Mn – 2.0 %, Zn – 9.0 %, S – 1.0 %), which were applied at sowing time and 60 kg ha^{-1} of K_2O (potassium chloride) split into five applications during the experimental period for system maintenance.

Treatments consisted of a mix of perennial herbaceous legume of twining stem, butterfly pea (*Clitoria ternatea* L.) and three warm-season grasses of different growth habits: two tufted grasses, signalgrass (*[Urochloa decumbens (Stapf.) R. Webster* cv. Basilisk with

Table 1. Chemical and granulometric properties of typical *Argissolo Amarelo Eutrófico* from the experimental area (Fortaleza, Ceará, Brazil) at the layers of 0.00-0.20 and 0.20-0.40 m.

Layer	pH($CaCl_2$)	OM	P	K	Ca^{2+}	Mg^{2+}	H+Al	Al^{3+}	SB	CEC	BS	M
m		g dm^{-3}	mg dm^{-3}		cmol$_c$dm^{-3}						%	
0.00-0.20	6.3	5	15	39	1. 0	0.9	1.5	0	2.0	3.5	57	0
0.20-0.40	6.6	5	8	23	0.6	0.6	1.3	0	1.2	2.5	49	0

	S	Na	B	Cu	Fe	Zn	Mn
	mg dm^{-3}		mg dm^{-3}				
0.00-0.20	7	5	0.15	0.3	3	11	5.6
0.20-0.40	6	7	0.14	0.2	4	4.5	2.5

	Clay	Silt	Total Sand	Coarse Sand	Fine Sand
	g kg^{-1}				
0.00-0.20	92	8	900	710	190
0.20-0.40	101	9	890	670	220

pH: hydrogenionic potential (soil:solution ratio of 1:2.5); OM: organic matter; P: phosphorus; K: potassium; Ca: calcium; Mg: magnesium; H+Al: potential acidity; Al: aluminum; SB: sum of exchangeable bases; CEC: cation exchange capacity; BS: base saturation; m: exchangeable aluminum saturation; S: sulfur; Na: sodium; Cu: copper; Fe: iron; Zn: zinc; Mn: manganese; B: boron.

decumbent stem and Guinea grass [*Megathyrsus maximus* (Jacq.) B. K. Simon & S. W.L. Jacobs cv. BRS Tamani) with erect stem, and a stoloniferous/rhizomatous bermudagrass [*Cynodon dactylon* (L.) Pers. cv. Tierra Verde]), without N fertilization. The experiment was arranged as a randomized complete block design in split-plots, with three replications. Plots were a mix of forages and the subplots consisted of cycles (sampling times).

Due to the difference in establishment time between the species, the strategy of early sowing of the legume was used. Legume was sown on April 21, and the grasses on May 23, 2017. Sowing was carried manually in the row, with spacing between rows of 0.25 m for plots with a total area of 12.5 m² (2.5 × 5.0 m). To begin the experiment, a uniform cut was made to 0.10 m from the ground level using a motorized coastal brush cutter, on August 14, 2017 (experiment data collecting period began after the first cut – August, until December). Rates of sowing were 4.8 kg ha^{-1} of seeds.

Cuts were performed when the canopy in each intercropping intercepted 95 % of PAR (photosynthetically active radiation) measured with AccuPAR LP-80 ceptometer. The stubble height in each mixed sward was determined as the height at which stubble leaf area index was equal to one (LAIs = 1.0) (Parsons et al., 1988). This corresponded to a stubble height of 18 cm from the ground for both signalgrass-butterfly pea and guinea grass-butterfly pea intercropping combinations, and 16 cm for bermudagrass-butterfly pea. The time of each cut (harvest) was between 20 to 30 days. The variation was due to the photosynthetically active radiation of each treatment/plot.

Pasture irrigation was operated under low-pressure fixed sprinkler irrigation (operating pressure <2.0 kg cm^{-2}) for 40 min in the morning (6:00 a.m.) with an irrigation level of 6.8 mm day^{-1}. The irrigation system was evaluated using rain gauges (Fabrimar®) at a height of 0.50 m, throughout the experimental area with a spacing of 3.0 × 3.0 m to ensure all plots received the same water level.

Forage was clipped through a metal frame (0.5 × 1.0 m) positioned in the center of each plot to the level of the target stubble height for each mixed pasture in all growth cycles. Forage samples were taken to the Animal Nutrition Laboratory of the Department of Animal Science of the Federal University of Ceará to measure the botanical composition using hand-sorting forage sampling in grass and legume. Grass and legume mass were weighed and washed in running water with neutral detergent, and distilled water containing 0.03 mL of dilute hydrochloric acid to remove impurities from the field (Miyazawa et al., 2009). Subsequently, they were dried, and the resulting dry matter was weighed on a precision scale and ground in a Willey mill with a 1-mm sieve. Forage accumulation rate was calculated by dividing the forage dry matter of each mixed pasture (DM) by the number of days of each growth cycle (FAR, kg DM^{-1} day^{-1}).

Plant tissue analyses were determined using forage dry matter digested by nitric-perchloric acid for two hours to determine the concentrations of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S); P was determined by spectrophotometry, K by flame photometry, S by turbidity, and the others were determined by atomic mass spectrophotometry (Malavolta et al., 1997). For N determination, samples underwent digestion with sulfuric acid, and determination by Kjeldahl's method, according to Malavolta et al. (1997) and AOAC (1995).

Nutrient accumulation was calculated for the different periods using the harvested forage mass and macronutrient concentrations in the forage. Macronutrient exportation was determined for all experimental treatments by calculating the accumulation (Equation 1).

$$\text{Nutrient accumulation and exportation} = \text{Total forage mass accumulation (kg ha}^{-1}) \times \text{nutrient content (g kg}^{-1}) \qquad \text{Eq. 1}$$

The daily accumulation rate of nutrients in the forage mass was estimated by the sum of each nutrient, divided by the total growth cycles, and later divided by the rest period of the treatments.

Three simple subsamples were collected to form a composite sample per plot, in the 0.00-0.10 m soil layer to determine ammonium ($N\text{-}NH_4^+$) and nitrate ($N\text{-}NO_3^-$) soil concentrations. Samples were stored in plastic and frozen bags to determine $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ concentrations. Determinations of $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ were performed using a Kjeldahl's method and mineral N extracted by potassium chloride solution according to Silva et al. (2010b).

Data were evaluated using analysis of variance (F-Test), comparing means, in which the interactions of the factors (intercropping × cycle) were further studied only when significant ($p<0.05$). The computer program SISVAR (Ferreira, 2011) was used for statistical analysis.

RESULTS

There was an interaction ($p<0.05$) between intercropping and growth cycle for FAR and macronutrient accumulation (Tables 2 and 3). A higher FAR was observed in the signalgrass-butterfly pea intercropping in relation to other intercropping combinations and in all cycles; however, this did not differ statistically from the bermudagrass-butterfly pea intercropping in the first growth cycle. For signalgrass-butterfly pea and Guinea grass-butterfly pea intercropping combinations, the 3rd and 4th cycles promoted the higher FAR value, and for bermudagrass-butterfly pea, the 1st cycle promoted the higher FAR value, but it did not differ statistically from the 4th cycle.

For N accumulation rate, signalgrass-butterfly pea and bermudagrass-butterfly pea intercropping combinations were superior to Guinea grass-butterfly pea intercropping in the 1st and 2nd cycles, while in the 3rd cycle, the signalgrass-buterfly pea intercropping stood out. The opposite was observed in the 4th cycle, in which signalgrass-butterfly pea intercropping showed lower N accumulation rate. Yet, for all intercropping combinations, the 2nd cycle revealed higher values of N accumulation rate in relation to the other cycles (Table 3).

For P accumulation rate, the bermudagrass-butterfly pea intercropping showed a lower value in the 1st and 3rd cycle, whereas in the 2nd cycle, the lowest P accumulation rate was observed in guinea grass-butterfly pea intercropping. Similar to N, the 2nd cycle of every intercropping combination showed higher values of P accumulation rate in relation the other cycles (Table 3).

Table 2. Forage accumulation rate (FAR) of forage grasses and butterfly pea intercropping in the warm-season, 2017, in the municipality of Fortaleza, Ceará, Brazil

Intercropping	FAR					
	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Mean	CV
	kg ha^{-1} day^{-1}					%
Signalgrass- butterfly pea	61.30 Ac[(1)]	75.80 Ab	86.90 Aa	86.20 Aa	77.60	7.10
Guinea grass- butterfly pea	48.20 Bb	52.20 Bb	69.10 Ba	68.70 Ba	59.60	
Bermudagrass- butterfly pea	68.20 Aa	55.30 Bb	54.30 Cb	63.90 Bab	60.40	
Mean	59.20	61.10	70.10	72.90		

[(1)] Averages followed by lowercase letters in the row and uppercase in the column do not differ from each other by the Tukey test (5 %). Signalgrass – *Urochloa decumbunes*, Guinea grass – *Megathyrsus maximus,* Bermudagrass – *Cynodon dactylon* and Butterfly pea – *Clitoria ternatea*,

Table 3. Nutrient accumulation rate in forage grasses and butterfly pea intercropping in the warm season, 2017. Fortaleza, Ceará, Brazil

Intercropping	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Mean	CV
	g ha^{-1} day^{-1}					%
			N			
Signalgrass-butterfly pea	1,422.50 Ab[1]	2,075.40 Aa	2,005.30 Aa	914.70 Bc	1,604.50	
Guinea grass-butterfly pea	1,004.50 Bab	1,745.60 Ba	1,419.60 Bb	1,125.30 Ac	1,323.70	6.00
Bermudagrass-butterfly pea	1,562.40 Ac	1,977.50 Aa	901.80 Cc	1,261.90 Ab	1,425.90	
Mean	1,329.80	1,932.80	1,442.30	1,100.60		
			P			
Signalgrass-butterfly pea	172.00 Ab	292.50 Aa	287.10 Aa	149.10 Ab	225.20	
Guinea grass-butterfly pea	186.60 Ab	243.60 Ba	179.50 Bb	143.70 Ab	188.30	9.20
Bermudagrass-butterfly pea	132.80 Bb	276.00 ABa	129.40 Cb	159.70 Ab	174.50	
Mean	163.80	270.70	198.60	150.80		
			K			
Signalgrass-butterfly pea	1193.10 Ac	1943.70 Ab	2215.10 Aa	728.00 Bd	1520.00	
Guinea grass-butterfly pea	1214.40 Ab	1651.90 Ba	1018.70 Bbc	779.60 Abc	1166.20	8.40
Bermudagrass-butterfly pea	864.60 Bbc	2061.70 Aa	678.40 Cc	999.00 Ab	1150.90	
Mean	1090.70	1885.80	1304.10	1069.80		
			Ca			
Signalgrass-butterfly pea	121.20 Bb	249.00 Aa	234.80 Aa	137.00 Bb	185.50	
Guinea grass-butterfly pea	170.00 Aab	197.00 Ba	177.20 Bab	153.70 ABb	174.50	7.80
Bermudagrass-butterfly pea	123.70 Bc	229.10 Aa	127.10 Cc	170.80 Ab	162.70	
Mean	138.30	225.00	179.70	153.80		
			Mg			
Signalgrass-butterfly pea	185.80 Bb	328.80 Aa	351.10 Aa	129.30 Bc	248.80	
Guinea grass-butterfly pea	233.90 Aab	257.50 Ba	202.00 Bb	151.00 Abc	208.60	6.90
Bermudagrass-butterfly pea	168.50 Bb	317.70 Aa	130.00 Ac	163.10 Abc	194.80	
Mean	196.10	301.30	227.70	147.80		
			S			
Signalgrass-butterfly pea	88.20 Aab	168.10 Aab	187.80 Aa	63.80 Bb	126.90	
Guinea grass-butterfly pea	107.80 Ab	145.30 Ba	87.50 Bb	80.20 Bb	105.20	10.70
Bermudagrass-butterfly pea	76.50 Bc	171.10 Aa	45.80 Cd	129.60 Ab	105.70	
Mean	90.80	161.50	107.00	91.20		

[1] Averages followed by lowercase letters in the row and uppercase in the column do not differ from each other by the Tukey test (5 %). Signalgrass – *Urochloa decumbunes*, Guinea grass – *Megathyrsus maximus,* Bermudagrass – *Cynodon dactylon* and Butterfly pea – *Clitoria ternatea*.

For K accumulation rate in the 1st and 3rd cycles, the bermudagrass-butterfly pea intercropping was inferior to other intercropping (guinea grass-butterfly pea and bermudagrass-butterfly pea), in the 2nd and 4th cycles the lower rates were associated to Guinea grass-butterfly pea and signalgrass-butterfly pea intercropping, respectively. Guinea grass-butterfly pea and bermudagrass-butterfly pea intercropping combinations showed higher values of P accumulation rate in relation to the other cycles (Table 3), whereas signalgrass-butterfly pea performed better in the 3rd cycle in terms of P accumulation (Table 3).

Calcium and Mg accumulation rates showed similar results in the 1st and 2nd cycles. In the 1st cycle, guinea grass-butterfly pea intercropping performed better. In the 2nd cycle, signalgrass-butterfly and bermudagrass-butterfly pea intercropping stood out. In the 3rd cycle, signalgrass-butterfly intercropping accumulated more Ca, and signalgrass-butterfly and bermudagrass-butterfly pea intercropping accumulated more Mg. In the 4th cycle, guinea grass-butterfly pea intercropping stood out for Ca accumulation, and guinea grass-butterfly pea and bermudagrass-butterfly pea intercropping accumulated more Mg. As in N accumulation, the 2nd cycle revealed the highest Ca and Mg accumulation rate (Table 3).

Sulfur accumulation rates were lower in bermudagrass-butterfly pea intercropping for the 1st and 3rd cycles, whereas in the 2nd cycle, Guinea grass-butterfly pea intercropping showed a lower value, and in the 4th cycle, Guinea grass-butterfly pea and signalgrass-butterfly pea intercropping had the lowest S accumulation rates. Again, the 2nd cycle showed the highest S accumulation rates (Table 3).

For signalgrass-butterfly pea and guinea grass-butterfly pea intercropping, grass proportions were higher than legume in all cycles (Figure 2). However, the legume was superior to the grass for bermudagrass-butterfly pea intercropping.

As for soil N concentration, signalgrass-butterfly pea and bermudagrass-butterfly pea intercropping combinations did not differ from each other as for total soil N concentration; however, signalgrass-butterfly pea intercropping showed higher total N concentration in soil than that of the bermudagrass-butterfly pea intercropping (Table 4). Conversely, Gginea grass-butterfly pea and bermudagrass-butterfly pea intercropping showed higher concentration of NH_4^+ and inorganic nitrogen in soil in relation to signalgrass-butterfly pea intercropping.

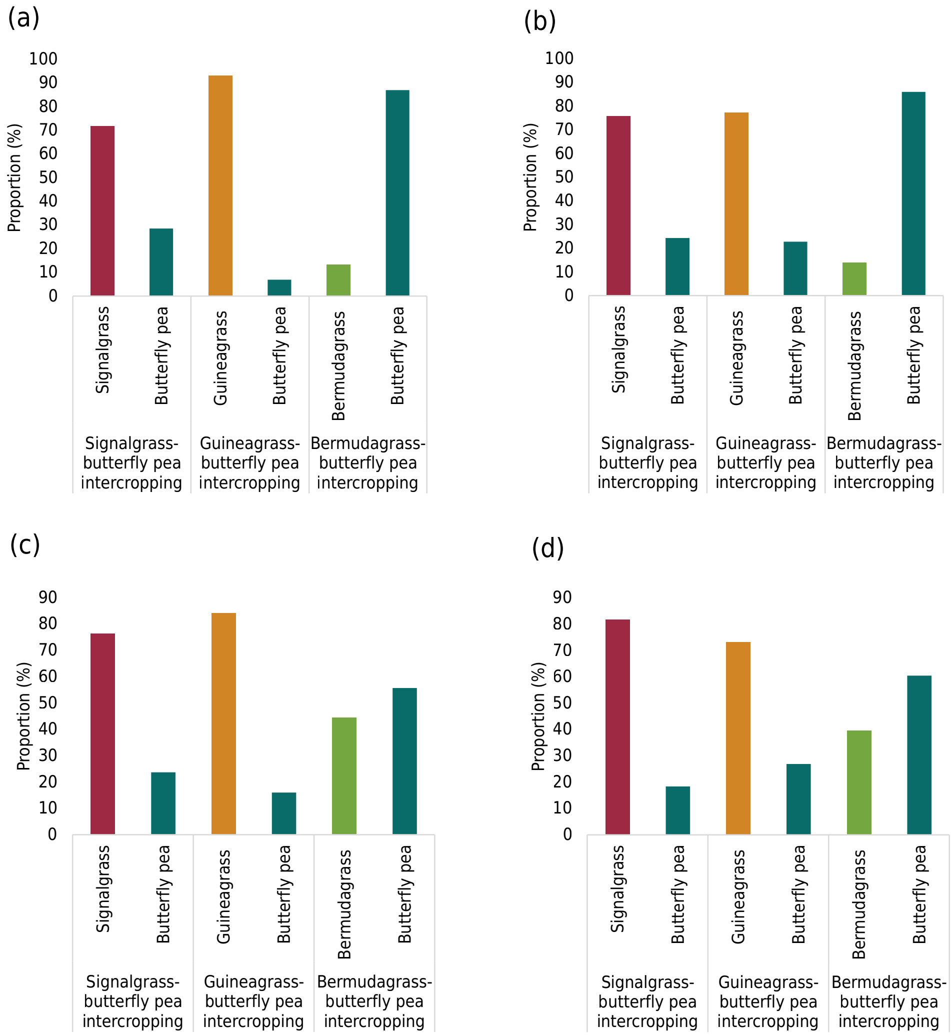

Figure 2. Proportion of forage grasses and butterfly pea intercropping in relation to the first growth cycle (a), second growth cycle (b), third growth cycle (c) and fourth growth cycle (d), 2017. Fortaleza, Ceará, Brazil. Signalgrass – *Urochloa decumbunes*, Guinea grass - *Megathyrsus maximus*, Bermudagrass - *Cynodon dactylon* and Butterfly pea - *Clitoria ternatea*.

Table 4. Soil content of total N (N-total), ammonium ($N-NH_4^+$), nitrate ($N-NO_3^-$) and inorganic N (IN) of forage grasses and butterfly pea intercropping in the warm-season, 2017, in the municipality of Fortaleza, Ceará, Brazil

Intercropping	N-total	$N-NH_4^+$	$N-NO_3^-$	IN
	g kg^{-1}		mg kg^{-1}	
Signalgrass-butterfly pea	0.87a	3.69b	0.06	3.75b
Guinea grass-butterfly pea	0.72b	4.64a	0.11	4.76a
Bermudagrass-butterfly pea	0.85ab	4.59a	0.26	4.85a
CV (%)	5.60	4.30	37.7	5.60
P-Value	9.54*	25.08*	1.72^{NS}	17.42*

N-total: total N. $N-NH_4^+$: ammonium. $N-NO_3^-$: nitrate (#-variable had its data transformed into square root). IN: inorganic N. *: significant at 5 % of probability. NS: not significant. Signalgrass – *Urochloa decumbunes*, Guinea grass – *Megathyrsus maximus,* Bermudagrass – *Cynodon dactylon* and Butterfly pea – *Clitoria ternatea*.

DISCUSSION

The increase in FAR of signalgrass-butterfly pea intercropping can be attributed to the higher proportion of signalgrass in the forage mass as well as to the capacity of signalgrass to adapt to low soil fertility (van Raij et al., 1997; Pereira et al., 2018), which, in this study, is associated with the absence of N fertilization. The sequence of nutrient accumulation in aboveground forage mass of signalgrass was K> N> Mg> P> Ca> S. The lower demand for soil fertility by signalgrass is due to its association with arbuscular mycorrhiza fungi, which increases the ability of roots to explore a greater soil volume (George et al., 1995; Clark and Zeto, 2000). Furthermore, the N fixed by the butterfly pea may have been sufficient to favor grass productivity as well as less competition for nutrients because the sequence of nutrients accumulated in the legume was N > K > Mg > Ca > P > S, different from signalgrass, thereby playing a complementary role in the intercropping.

Regarding N accumulation rate of forage, the interaction between intercropping and growth cycle revealed signalgrass-butterfly pea and bermudagrass-butterfly pea intercropping combinations did not differ in the first two growth cycles, however, when analyzing the 3rd cycle, signalgrass-butterfly pea intercropping had a higher N accumulation rate than those of the other intercropping combinations (Table 3). In the signalgrass-butterfly pea and bermudagrass-butterfly pea intercropping, the proportions of legume in the first three cycles (Figure 2) may have favored N accumulation rate in forage, and greater photosynthesis, providing protein and enzyme formation in plant metabolism (Taiz et al., 2017). Moreover, the higher proportion of butterfly pea may have led to a greater biological N fixation, thereby favoring the accumulation of N by cycling this nutrient (Tambara et al., 2017).

Nitrogen accumulation rate of the signalgrass-butterfly pea in the 4th cycle was reduced by 119.2 % in relation to the other three cycles (Table 3), without a reduction in FAR (Table 2). This fact may be related to reducing the proportion of legumes in the pasture. On the other hand, Guinea grass-butterfly pea intercropping has also been shown to reduce N accumulation in the biomass by 26.1 % between cycles three and four, although it did not reduce FAR. This fact was not expected since the proportion of legumes in the total biomass of cycle four increased by 10.8 % and, therefore, it was expected there would be an increase in N accumulation in the canopy.

However, it is possible that the reduction in the proportion of legumes between cycles two and three in 6.7 % reflected in N accumulation only in the 4th cycle and may be related to the balance of nutrients as a function of the dilution effect – improved forage biomass, but without improved nutrient concentration (Figure 2). Legumes associated with grasses in intercropping form complexes that benefit N transfers by indirect relationship in soil C:N ratio, enabling an increase in the organic matter mineralization rate (Barbosa et al., 2017).

Regarding P and K accumulation rates in the forage's dry matter, there was a reduction from the 2nd cycle, except for the signalgrass-butterfly pea intercropping, whose reduction was only from the 3rd cycle, probably due to the decrease in the uptake of these nutrients from soil. Although P was applied as base fertilization and K for four applications, it was not sufficient to meet the nutritional requirement. Nevertheless, this response did not compromise the forage accumulation during the cycles, nor did the plants show deficiency symptoms for these nutrients. However, because they are essential nutrients in plant metabolism, monitoring and replenishing nutrients through fertilization in production systems is of fundamental importance to ensure yields and persistence of mixed pasture.

By making an inference between the annual K accumulation rate in the plant tissue of the signalgrass-butterfly pea, guinea grass-butterfly pea and bermudagrass-butterfly pea intercropping combinations have accumulated 419.64, 547.08, and 368.30 kg ha^{-1}, respectively, much higher than the amount of K_2O recommended by Alvarez et al. (1999), 200 kg ha^{-1} yr^{-1} containing 166 kg ha^{-1} yr^{-1} of K for fertilization maintenance in intensively managed pastures, which confirms the importance of monitoring the nutritional status of forages to maintain their productivity.

Values of K accumulation are below those presented by Galindo et al. (2017), who studying Mombaça grass found a K accumulation of 663 kg ha^{-1} yr^{-1} with N fertilization of 300 kg ha^{-1} yr^{-1}. The superiority of K export reported by the authors is related to the higher nutritional requirement of Mombaça guinea grass because it is a large forage grass with high biomass production potential (Gomide et al., 2007).

A higher Ca accumulation rate in the forage mass was observed in the signalgrass-butterfly pea intercropping in the 2nd and 3rd cycles and may be related to higher stem production (665.9 kg DM ha^{-1} cycle^{-1}) in relation to the others, with an average of 342.1 kg DM ha^{-1} cycle^{-1}. The highest Ca accumulation rate is associated with stem production because Ca plays a major role in the constitution of the plant cell wall, increasing the mechanical resistance of tissues and support (Thor, 2019).

The higher Mg accumulation rate was observed in the 2nd cycle, except for the signalgrass-butterfly pea intercropping, in which the Mg accumulation rate persisted until cycle three, with a reduction in the export of this nutrient in cycle four. Higher Mg deposition occurred in the signalgrass-butterfly pea intercropping, which exported 29.8 kg Mg during the experimental period, while the other intercropping exported an average of 25.1 kg of Mg. This is due to the highest forage accumulation rate of signalgrass-butterfly pea intercropping (Table 2). Magnesium has several important functions in plants, such as activating numerous enzymes, including carbon fixation for photosynthesis, such as rubisco and phosphoenolpyruvate carboxylase, as well as being part of the chlorophyll molecule (Guo et al., 2016).

Sulfur accumulation rate was higher in the signalgrass-butterfly pea intercropping, especially in cycle three. This superiority can be attributed to the increase in N accumulation rate in this intercropping, which consequently increased the uptake and accumulation of S in the harvested forage. Both N and S have synergism in plant metabolism for protein and sulfur amino acid production and occurs by the junction of metabolic pathways of S assimilation by the incorporation of sulfide in O-acetylserine by the enzyme OAS-thiolylysis for cysteine formation (Taiz et al., 2017).

When observing the botanical composition, a higher proportion of butterfly pea was observed in the bermudagrass-butterfly pea intercropping (72.3 %) and the lowest proportion in Guinea grass-butterfly pea intercropping (18.1 %), considering an average of the four growth cycles (Figure 2). The low proportion of bermudagrass in the first two growth cycles may be related to lower grass stability in relation to butterfly pea, which possibly reduced the regrowth vigor due to low accumulation and mobilization of organic reserves, since the intercropping was maintained without mineral N fertilization.

In relation to the bermudagrass-butterfly pea intercropping, a lower proportion of legume was observed in the system, which may be associated with growth habit with higher photosynthetic efficiency of Tamani grass (Tambara et al., 2017). Interactions between intercropping and growth cycle show the good performance of butterfly pea in the grass-legume intercropping, and this legume is adapted to tropical conditions. Moreover, the butterfly pea exhibits persistence in pasture, high forage production, and high nutritional value, with crude protein around 22 % (Nunes et al., 2017; Souza et al., 2017).

The highest total N content can be attributed to the higher amount of accumulated biomass that, consequently, produced higher dead forage biomass, contributing to material decomposition. This can also increase the amount of organic matter available in soil, which mobilizes N, increasing the content of total N in roots (Pirhofer-Walzl et al., 2011).

These results can be attributed to the intense addition of organic carbon through the renewal of the root system of grasses and legume (Oliveira et al., 2016). Also, according to authors, some plant residues from pastures could be poor in lignin, a chemical precursor of recalcitrant compounds, thus soils under pastures have less recalcitrant organic matter and, therefore, are more sensitive to climatic, chemical, and microbiological variations that result in a higher speed of soil organic matter mineralization. In the bermudagrass-butterfly pea intercropping, the higher proportion of legumes in the canopy may have contributed to greater mineralization of organic matter, resulting in greater availability of total N in soil (Table 4).

Regarding the concentrations of $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ in soil, a higher predominance of the ammoniacal form was observed and may be an indicator of nitrate ion loss by nitrate leaching, given the daily application of irrigation in soils with a sandy texture. Under pasture conditions, the $N\text{-}NH_4^+$ form is favored by substances excreted by roots of grass, which promote nitrification (Oliveira et al., 2016). On the other hand, the low concentrations of $N\text{-}NO_3^-$ in soil may indicate a greater demand for soil microbiota and roots, in addition to leaching losses, because this ion is rapidly leached due to its negative charge, or even to the low levels of $N\text{-}NO_3^-$ in soil, common in soils of tropical ecosystems (Gonzalez-Meler et al., 2017).

CONCLUSIONS

Signalgrass-butterfly pea intercropping has a higher rate of accumulation of forage and macronutrients. Productive characteristics of the *Urochloa* and butterfly pea intercroppings are favored by decumbent grass growth, allowing compatibility and increased biomass production. Guinea grass-butterfly pea and bermudagrass-butterfly pea intercropping combinations showed an increase in the remaining ammoniacal N in soil. Both grass-legume pastures increased the demand for nutrients, making it essential to verify the export of macronutrients and, when necessary, to replenish these nutrients removed from the soil solution.

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