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Herbage responses and animal performance of nitrogen-fertilized grass and grass-legume grazing systems

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Abstract

The study evaluated forage and livestock performance in different grazing systems over two years. Treatments were three contrasting grazing systems: (I) N-fertilized bahiagrass (*Paspalum notatum* Flüggé) in the summer overseeded during the winter by N-fertilized ryegrass (*Lolium multiflorum*) and oat (*Avena sativa* L.) (Grass + N); (II) unfertilized bahiagrass during the summer overseeded with ryegrass + oat and a blend of clovers (*Trifolium* spp.) in the winter (Grass + Clover); (III) unfertilized bahiagrass and rhizoma peanut (RP; *Arachis glabrata* Benth.) mixture during summer, overseeded during winter by ryegrass + oat + clovers mixture (Grass + Clover + RP). Average daily gain (ADG), gain per area (GPA), and stocking rate (SR) in the winter did not differ across treatments and averaged 0.87 kg/d ($P = 0.940$), 303 kg/ha, and 2.72 AU/ha. In the summer, Grass + Clover + RP had greater ADG than Grass + N (0.34 *vs.* 0.17 kg/d, respectively). During the summer, the GPA of Grass + Clover + RP was superior to Grass + N (257 *vs.* 129 kg/ha, respectively), with no difference in SR among treatments at 3.19 AU/ha. Over the entire year, ADG and GPA tended to be greater for Grass + Clover + RP. Annual SR differed between treatments, where Grass + N was greater (3.37 AU/ha) than the other treatments, which averaged 2.76 AU/ha. Integration of legumes into pasture systems in the summer and winter contributes to developing a sustainable grazing system, reducing N fertilizer use by 85% while tending to increase livestock productivity even though SR was decreased by 18%.

Introduction

Nitrogen fertilizer is crucial to promote forage productivity in grazing systems, especially grass monocultures. The increasing costs of commercial N fertilizers and environmental issues associated with improper fertilization management are raising awareness to improve N use efficiency to mitigate N losses from grassland ecosystems (Silveira *et al.*, 2015). Excess of N fertilizer application might lead to environmental pollution (Hill *et al.*, 2019; Dimkpa *et al.*, 2020) by volatilization of ammonia (NH_3), nitrous oxide (N_2O) emissions, and nitrate (NO_3^-) leaching (Dubeux and Sollenberger, 2020; Woodley *et al.*, 2020; Corrêa *et al.*, 2021). Therefore, improving N management can increase profitability and reduce N losses (Smith *et al.*, 2018). Moreover, unexpected market oscillations in commercial N can affect farmers' profitability (Santos *et al.*, 2018; Randive *et al.*, 2021; Yang *et al.*, 2022).

Legumes can fix atmospheric N in a process known as biological N_2 fixation (BNF) (Soumare *et al.*, 2020). Biological N_2 fixation is the symbiotic interaction between N-fixing bacteria broadly known as 'Rhizobia' and legume plants, and due to this symbiosis, BNF is a feasible alternative to commercial N fertilizers (de Bruijn and Hungria, 2022). The association of N-fixing legumes and grasses is desirable. It might help enhance grazing systems' productivity and profitability by increasing forage nutritive value and animal performance due to greater N supply. (Dubeux and Sollenberger, 2020). Thus, adding legumes as a component in mixed pastures has been used as an alternative to reduce off-farm N inputs (Jaramillo *et al.*, 2021a, 2021b).

Incorporating legumes into grass-based livestock systems yields numerous benefits. These include enhanced diet digestibility and increased forage intake (Muir *et al.*, 2011), elevated

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concentration of forage crude protein (CP), leading to improved animal performance (Pereira *et al*., 2020), and nitrogen (N) supply to grass-based grazing systems (Mullenix *et al*., 2016) with potential transfer rates ranging from 10 to 75 kg of N/ha/yr (Nyfeler *et al*., 2011). Additionally, this integration facilitates carbon (C) and N sequestration, contributing to environmental sustainability (Wright *et al*., 2004). Therefore, including legumes in a grass monoculture might enhance the sustainability of grass-legume mixtures compared with grass monocultures (Ball *et al*., 2015).

Livestock operations in Southeast U.S. are primarily based on warm-season grasses such as bermudagrass (*Cynodon dactylon* L.) and bahiagrass (*Paspalum notatum* Flügge), along with cool-season annual legumes and grasses, including clover (*Trifolium spp.)* and annual ryegrass (*Lolium multiflorum* Lam.), to provide forage during autumn and winter (Sanderson *et al*., 2012). The most used bahiagrass cultivars in Florida are 'Argentine' and 'Pensacola' (Vendramini and Moriel, 2020). The popularity of bahiagrass among producers could be related to its adaption to low soil fertility and basic input management needs (Newman *et al*., 2010), as well as its grazing tolerance (Rouquette *et al*., 2020). Including rhizoma peanut (RP; *Arachis glabrata* Benth.), which is well adapted to Florida conditions and used as a forage in grazing systems, might be a sound strategy to diversify livestock systems (Vendramini and Moriel, 2020). This forage legume is characterized by its rhizome propagation and slow time of establishment (Aryal *et al*., 2021), nutritive value, production, persistence, adaptation to a wide range of grazing management (Ortega-s *et al*., 1992), and great BNF potential (Dubeux *et al*., 2017).

During the cool season, when the warm-season grasses are dormant (Rouquette *et al*., 2020), cool-season annual forages can be established during the fall by broadcasting onto warm-season pastures (Dillard *et al*., 2018). Cool-season annual forages can provide excellent forage nutritive value (i.e., high crude protein concentration and digestibility) for later winter to early spring (Han *et al*., 2018) when herbage from perennial forages is scarce, optimizing the use of stored feed during the winter period (Dillard *et al*., 2018). Clovers are the most common cool-season legumes present in southern U.S. pastures, which are used as a component to integrate a grass-legume mixture with annual ryegrass during the cool season (Vendramini and Moriel, 2020). Annual ryegrass is a critical component of forage-based livestock production in the southern U.S. (Lemus *et al*., 2021). Beyond that, annual ryegrass and clover mixtures can be grown within swards of dormant warm-season perennial grasses and promote great livestock performance during the winter and spring (Rouquette *et al*., 1997). In mixture systems, Ryegrass also provides earlier grazing than clover alone and decreases the risk of bloating (Evers *et al*., 1997).

The escalating cost of N fertilizer poses a potential threat to the profitability of livestock operations. Hence, considering the integration of legumes into grazing systems as a promising approach to decreasing N inputs from industrial fertilizers is under investigation (Lemus *et al*., 2021). While the integration of legumes into grass pastures poses challenges, including factors such as establishment time (Mullenix *et al*., 2016; Jaramillo *et al*., 2018), management difficulties (Beran *et al*., 1999), and the associated costs of implantation (Castillo *et al*., 2013), there is a discernible trend of improvement in this practice worldwide. Livestock operations actively seek elevated levels of forage production, superior forage quality, and optimal animal performance within grazing systems, promoting sustainable agricultural practices. We hypothesized that including legumes in grazing systems may reduce N fertilizer input in grass-legume pastures without affecting herbage and cattle growth performance. This study assessed the herbage responses and animal performance in three contrasting grazing systems over two years.

Material and methods

Experimental site, animal management, and treatments

The current study was approved by the University of Florida, Institutional Animal Care & Use Committee (Protocol IACUC201709924). The experiment was performed at the University of Florida, North Florida Research and Education Center in Marianna, Florida (30°52′N, 85°11′ W, 35 m asl), Southeast USA, during the cool and warm seasons of 2020 and 2021. The cool season occurred from January to mid-May, and the warm season from mid-May to October for both experimental years. The soil in the experimental area was classified as Orangeburg loamy sand (fine-loamy kaolinitic, thermic Typic Kandiudults; USDA, 2014). At the establishment of bahiagrass (cv. Argentine) in these pastures in 2013, soil samples were taken to a depth of 0–15 cm at the experimental site. Laboratory analysis reported a $pH_{(water)}$ of 5.7 and average Mehlich-I extractable soil P, K, Mg and Ca concentrations of 26, 99, 43 and 224 mg/kg, respectively. Soil organic matter was 15.4 g/kg, and the estimated cation-exchange capacity was 3.8 meq/100 g.

The experiment was carried out in a randomized complete block design with three treatments and three replicates. Treatments were three grazing systems, including warm-season perennial forages and cool-season annuals (Fig. 1). The pastures were considered experimental units, and each pasture measured 0.85 ha. The treatments were (I) Grass + N, (II) Grass + Clover, and (III) Grass + Clover + RP. In the Grass + N treatment, bahiagrass was fertilized with 112 kg N/ha split equally in two applications in the summer using urea as the N source. Pastures were overseeded with a mixture of annual ryegrass (89.6 kg/ha; cv. Prine) and Oat (16.8 kg/ha; cv. RAM) fertilized with 112 kg N/ha. Total annual N fertilization for Grass + N was 224 kg N/ha. The treatment Grass + Clover consisted of bahiagrass receiving no N during the summer that was overseeded in the fall with ryegrass-oat-clover mixture, consisting of crimson clover (*Trifolium incarnatum* L. [16.8 kg/ha; cv. Dixie]), red clover (*Trifolium pratense* L. [6.7 kg/ha; cv. Southern Belle]), and ball clover (3.4 kg/ha). This treatment received an N-fertilizer application (34 kg N/ha) only in the Fall, three weeks after planting. Grass + Clover + RP consisted of the incorporation of RP (cv. Ecoturf) in consortium with bahiagrass in the summer. The RP was strip-planted and established simultaneously with bahiagrass in June 2014 as part of a previous study performed by Jaramillo *et al*. (2021b). The purpose of planting RP by strips was to reduce the labour and costs of the legume establishment because RP has stoloniferous growth and high spread capacity. The Grass + Clover + RP did not receive N-fertilizer during the summer, but pastures were overseeded with a similar ryegrass, oat, and clover mixture in the winter. N-fertilizer application was the same as for Grass + Clover (34 kg N/ha). During the cool season, the grass and clover mixtures were used to extend the grazing season with greater distribution of the forage production. The contrasting grazing systems are illustrated in Fig. 1.

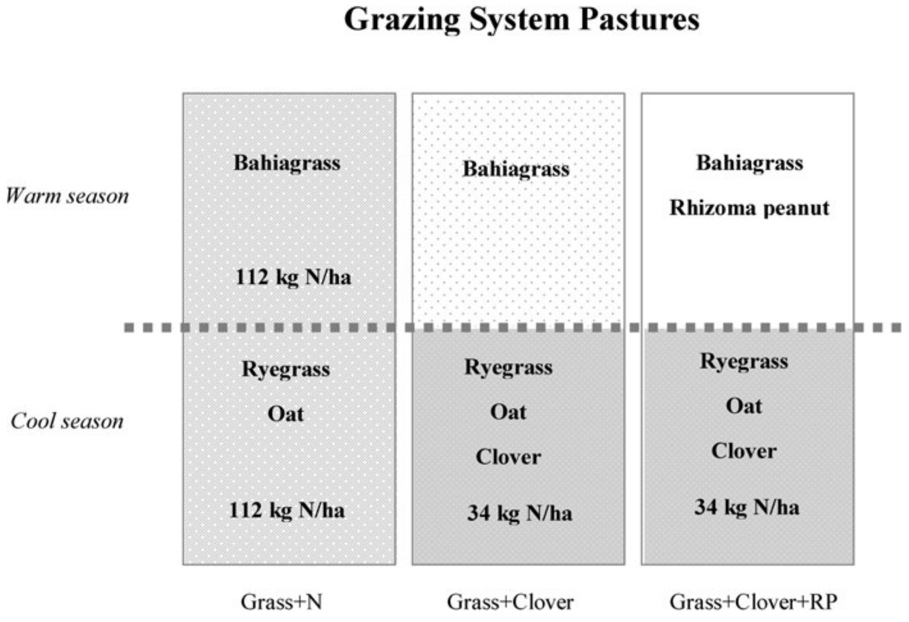

Figure 1. Grass + N, N-fertilized bahiagrass during the warm season overseed with ryegrass + oat during the cool season; Grass+Clover, bahiagrass during the warm season overseeded with ryegrass + oat plus a mixture of clovers during the cool season; Grass+Clover + RP, bahiagrass plus a mixture of rhizoma peanut during the warm season overseeded with ryegrass-oat-clover mixture during the cool season. In the summer, the fertilization was split into two equal portions (56 kg N/ha). In the winter, N fertilizer was applied at 34 and 78 kg N/ha (first and second application, respectively).

The cool-season forages were seeded in early November of each year, right after the end of the warm season. All the pastures were fertilized with 34 kg N, 45 kg P, 56 kg K and 13.5 kg S/ha after four weeks of seeding in both years. These pastures were initially established in 2014. Treatment design, seeding rate, N fertilization application, and forage species varied slightly over the years and are also described by Garcia *et al.* (2021) and Jaramillo *et al.* (2021a, 2021b). The planting and fertilization dates are presented in Table 1.

In April 2020, 3.5 l/ha of pendimethalin ($C_{13}H_{19}N_{3}O_{4}$) was applied on RP strips in all Grass + Clover + RP treatment pastures. In June 2020, 3.5 l/ha of pendimethalin plus 0.27 l/ha of imazapic ($C_{14}H_{17}N_{3}O_{3}$) was used on RP strips in all Grass + Clover + RP treatment pastures. In July 2020, 9.3 l of aminopyralid ($C_{6}H_{4}C_{12}N_{2}O_{2}$) was used for all Grass + Clover and Grass + N treatment pastures. In March and May 2021, 9.3 l per ha of pendimethalin was applied in all pastures. In August 2021, 1.48 l of rinskor ($C_{20}H_{14}C_{12}F_{2}N_{2}O_{3}$) active with ¼ of non-ionic surfactant (NIS) per 378 l was applied on Grass + Clover and Grass + N treatment pastures. The chemicals were employed for weed management, mainly during the establishment of RP.

Table 1. Nitrogen fertilization and planting period for the grazing systems in the cool and warm seasons

	2020	2021		
Cool season	Months		kg N/ha	Systems
Overseeding	November	October		All
1st fertilization	December	December	34	All
2nd fertilization	February	February	78	Grass + N
Warm season				
1st fertilization	May	June	56	Grass + N
2nd fertilization	July	August	56	Grass + N

All = Grass + N, N-fertilized bahiagrass during the warm season overseed with ryegrass and oat during the cool; Grass + Clover overs, bahiagrass during the warm season overseeded with ryegrass + oat plus a mixture of clovers during the cool season; Grass + Clover overs, rhizoma peanut and bahiagrass during the warm season overseeded with ryegrass-oat-clover mixture during the cool season. In the summer, the fertilization was split into two equal portions (56 kg N/ha). In the winter, N fertilizer was applied at 34 and 78 kg N/ha (first and second application). Periods were early February, late May, early June, early July, early August, late October, early November, and early December.

Herbage responses

Herbage mass, herbage allowance, and herbage accumulation rate

Herbage mass (HM) was determined using the double sampling method (Wilm *et al.*, 1944; Haydock and Shaw, 1975). Every 14 days, aluminium disc settling heights (cm) were taken at 30 random points in each pasture, except for the treatment Grass + Clover + RP in the warm season, where 60 disc settling heights were taken, with 30 points on each strip type (bahiagrass or rhizoma peanut). The disc settling heights were the indirect measurements with harvested samples every 28 d. Forage samples were clipped at a 5-cm stubble height using a 0.25 m^2 metallic ring, dried at 55°C for 72 h, and the dry weight was recorded. Regression equations were developed for each pasture using 18 paired samples (settling disc heights and their respective harvested sample). The equations are included in the Supplementary Table. Each prediction equation had 18-paired points for grass only and 18-paired points for legume strips. Regression equations were developed for grass-only pastures or grass-clover pastures in the cool season. After developing the equations, the average disc heights of each pasture were used as the independent variable to estimate herbage mass.

Pastures were continuously stocked using an adjustable SR, selected to match a target herbage allowance (HA; kg DM/kg BW) every 14 days using the method described by Sollenberger *et al.* (2005). Put-and-take animals were used to adjust the SR during the entire study period to maintain a similar HA among treatments in each block. The target HA was 1.0 kg DM/kg BW for the cool season and 1.5 kg DM/kg BW for the warm season.

The herbage accumulation rate (HAR) was determined using four exclusion cages placed at the initial sampling date per experimental unit. After 14 days, the cages were moved to a new location in the pasture, and the previous and new canopy heights were taken from the aluminium disc settling heights (Stewart *et al.*, 2007; Vendramini *et al.*, 2012). The same equation used to obtain the HM was used to calculate the pre-HM and post-HM inside the cage. In the warm season, because of the strips, eight cages were placed at random sites in the Grass + Clover + RP pasture, four in each strip type (bahiagrass or rhizoma peanut).

Total HAR in the pastures with legume was calculated for each component in the sward by multiplying the HAR by the proportion of grass or legume (only in the legume-containing treatments) in each pasture obtained from the botanical composition (BC, % of dry weight). In the warm season, the HAR of the RP was estimated by multiplying the proportion of RP in the BC by the HAR of RP from the evaluation period considering the RP strip area. The RP area was assessed by measuring the average width of 10 strips per experimental unit (pasture) during the warm season in the pastures with RP and bahiagrass. The strip width (m) was measured once in 2015, 2016, 2018, 2020, 2021, and 2022. The strips were measured using a measuring tape, where ten different points per Grass + Clover + RP pasture were used to obtain the width of both grass and legume and to estimate the RP strip width.

The botanical composition (BC) of each pasture was estimated using the dry weight rank method described by t'Mannetje and Haydock (1963). A metallic ring (0.25-m^2) was randomly placed on the pasture, followed by a visual estimation (% of dry-weight, DW) where all species presented were classified and recorded as either grass (ryegrass, oat), legume (clovers), or weeds for evaluation in the cool season. In the warm season, the components were grass (bahiagrass and other grass) or legumes (rhizoma peanut). This was estimated by ranks whereby the most abundant species took the first place, followed by the second and third, respectively, on a dry weight basis. This procedure was repeated 60 times in each pasture; for pastures with RP, the evaluation was performed in each strip (bahiagrass and RP), resulting in 120 observations. The BC in the cool season of the first year was estimated in March and the second year in April, while for the warm season, it was done in August and September for the first and second years, respectively.

Nutritive value

Hand-plucked samples were obtained in both seasons to analyse herbage CP and in vitro digestible organic matter (IVDOM). Grass and legume samples were collected every 14 days at each pasture at different points to represent the entire pasture and simulate grazing behaviour. All the samples were dried at 55 °C for 72 h and ground to pass a 2-mm screen using a Wiley Mill (Model 4, Thomas-Wiley laboratory Mill, Thomas Scientific, Swedesboro, NJ). The two-stage technique that Moore and Mott (1974) described was used to determine the IVDOM of herbage material. Subsamples were taken and ball-milled in a Retsch Mixer Mill MM400 (Retsch, Haan, Germany) at 25 Hz for 9 min to reduce the particle size to under 100 µm. Samples of approximately 5 mg were analysed for total N and δ^{15}N through the Dumas dry combustion method using a CHNS analyser vario MICRO Cube (Elementar, Frankfurt, Germany) coupled to an isotope ratio mass spectrometer (IRMS) using an IsoPrime100 (Elementar, Frankfurt, Germany). The IRMS provides the δ^{15}N and the concentration of elements in the sample. Once the concentration of N was obtained, the CP of all samples could be estimated by multiplying the total N concentration by 6.25 factor.

Table 2. Reference plants and average ^{15}N‰ collected every 28 days during the cool and warm seasons of 2020 and 2021

Season	Common name	Scientific name	^{15}N ‰
2020			
Cool	Pineapple cudweed	*Matricaria discoidea*	0.69
Cool	Chickweed	*Stellaria media*	−0.80
Cool	Common Bermuda	*Cynodon dactylon*	3.54
Cool	Red Sorrel	*Rumex acetosella*	1.86
Cool	Dandelion	*Taraxacum officinale*	5.62
Warm	Brachiaria	*Urochloa plantaginea*	2.28
Warm	Teaweed	*Sida rhombifolia*	3.18
Warm	Morning glory	*Ipomoea tricolor, I. purpurea*	2.51
Warm	Pigweed	*Amaranthus spinosus*	6.35
Warm	Crabgrass	*Digitaria sanguinalis*	3.00
2021			
Cool	Henbit	*Lamium amplexicaule*	0.88
Cool	Dandelion	*Taraxacum officinale*	1.73
Cool	Geranium	*Pelargonium spp.*	0.77
Cool	Red sorrel	*Rumex acetosella*	−0.89
Cool	American burnweed	*Erechtites hieraciifolius*	1.72
Warm	Pigweed	*Amaranthus spinosus*	6.24
Warm	Teaweed	*Sida rhombifolia*	4.37
Warm	Brachiaria	*Urochloa plantaginea*	3.78
Warm	Tropical soda apple	*Solanum viarum Dunal*	7.2
Warm	Dogfennel	*Eupatorium capillifolium*	3.07

All the reference plants were collected outside of the experimental units.

Biological N_2 fixation

The BNF was determined for clovers and RP using the natural abundance technique (Freitas *et al.*, 2010). Reference plants ($n = 5$) were collected every 28 days and are presented in Table 2. The reference plants were classified to the species level, dried at 55°C for 72 h, ground to pass a 2-mm screen, and ball-milled. The proportion of plant N derived from the atmosphere (Ndfa) was estimated using Eqn (1) described by (Shearer and Kohl, 1986):

$$Ndfa = (\delta^{15}N_{\text{ reference plant}} - \delta^{15}N_{\text{ fixing legume}})/(\delta^{15}N_{\text{ reference plant}} - B)\ 100 \qquad (1)$$

where the $\delta^{15}N_{\text{ reference plant}}$ is the $\delta^{15}N$ value for the non-N_2 – fixing reference plant, $\delta^{15}N_{\text{ – fixing legume}}$ is the $\delta^{15}N$ value for the N_2 – fixing (Clover and RP), and B is the δ15N value for N2 – fixing plant grown in the absence of inorganic N. In the cool season, the B value used was −1.96 ‰ and was the lowest value of clover obtained in this study. In the warm season, the B value used was −1.41‰, as reported by Okito *et al.* (2004) for *Arachis hypogea L.* The shoot N accumulation was estimated by multiplying herbage accumulation by legume N concentration. Herbage BNF was calculated by multiplying shoot N accumulation by the %Ndfa. The seasonal BNF was then assessed by multiplying the herbage BNF by the number of days (28 days) within both seasons for each year. The reference plants used throughout the study and their respective ^{15}N value are listed in Table 2.

Animal performance

Average daily gain, gain per area, and stocking rate

Each pasture had two testers crossbred (Angus × Brahman) yearling steers that remained on the pastures during the experimental period. The initial body weight (BW) of tester steers was 293 ± 23 kg and 291 ± 21 kg for 2020 and 2021, respectively. The same tester animals remained in their corresponding pasture during the months of grazing within each year for both seasons. The second year counted with new steers for each treatment. Water, shade, and a mineral supplement mixture (Ca = min. 150 and max. 190 g/kg, P = min. 30 g/kg, NaCl = min. 150 and max. 180 g/kg, Mg = min. 100 g/kg, Zn = min. 2800 mg/kg, Cu = min. 1200 mg/kg, I = min 68 mg/kg, Se = 30 mg/kg, Vitamin A = 308 370 units per kg, Vitamin D3 = 99 119 units per kg; Special Mag, W.B. Fleming Company) were available for cattle in each pasture.

Determining animal performance in cool and warm seasons followed a similar methodology. To obtain the BW, all the tester steers were weighed at the initiation of the experiment and every 28 days after that. Weights were taken at 0800 h following a 16-h fasting period. Average daily gain (ADG) was calculated for each 28-d period by dividing the average weight gain of the two tester animals in each pasture during that specific period by the number of days (kg/head/d). The ADG over the entire year, both seasons, was determined as a weighted average based on ADG per given season and year and the length of the season per given year. Grazing days were calculated by multiplying the total number of animal units (1 AU = 350 kg BW) in each pasture (both testers and put-and-takes) by the number of days within each period and subsequently summing all the grazing days at the end of the season. Gain per area (GPA) (kg/ha) was calculated by multiplying ADG by the number of grazing days per hectare within each period. The SR was calculated by dividing the grazing days by the total number of days within each season.

Statistical analyses

All response variables were analysed using mixed model procedures implemented in PROC GLIMMIX from SAS (SAS/STAT 15.1, SAS Institute). Pastures were treated as experimental units for all output variables, organized into three blocks with three replications per treatment, resulting in nine experimental units. For herbage responses, the variables HM, HAR, nutritive value, %Ndfa, and BNF were considered repeated measures. For animal responses, including ADG, GPA and SR, the model included treatment, sampling dates and their interaction as fixed effects. Block, year, and block × treatment were considered random effects. Differences were considered significant at $P \leq 0.05$. P values between 0.06 and 0.1 were considered tendencies for annual animal performance.

Results

Herbage responses

Cool season

Throughout the cool season, there was a discernible tendency ($P = 0.055$) for the N-fertilized treatment (Grass + N) to diverge from the incorporation of clovers into mixed pastures of annual ryegrass and oats in the HM measurements, although it was not significant. The HM for Grass + N, Grass + Clover and Grass + Clover + RP was 1388, 1141 and 1180 kg DM/ha, respectively. The HAR showed a treatment × sampling dates interaction ($P = 0.030$; Fig. 2). The proportion of grass in the botanical composition differed from the legume proportion among treatments in the cool season ($P = 0.0001$). The proportions of grass and legumes of Grass + Clover were 64 and 28%, respectively, and Grass + Clover + RP was 60 and 31%, respectively.

Warm season

In the warm season, the HM did not differ among treatments ($P = 0.477$) and averaged 1680 kg DM/ha. The HM for Grass + N, Grass + Clover and Grass + Clover + RP was 1736, 1686 and 1625 kg DM/ha, respectively. However, there was a sampling dates effect ($P < 0.0001$; Fig. 3a) on HM. There were 11 sampling dates, one in mid-May and two in each subsequent month (June to October). The HM in the first two sampling dates was 1000 kg DM/ha, which increased with time and peaked at 2500 kg DM/ha in August and September, decreasing in October (1600 kg DM/ha). There was no treatment effect for HAR ($P = 0.170$; Fig. 3b),

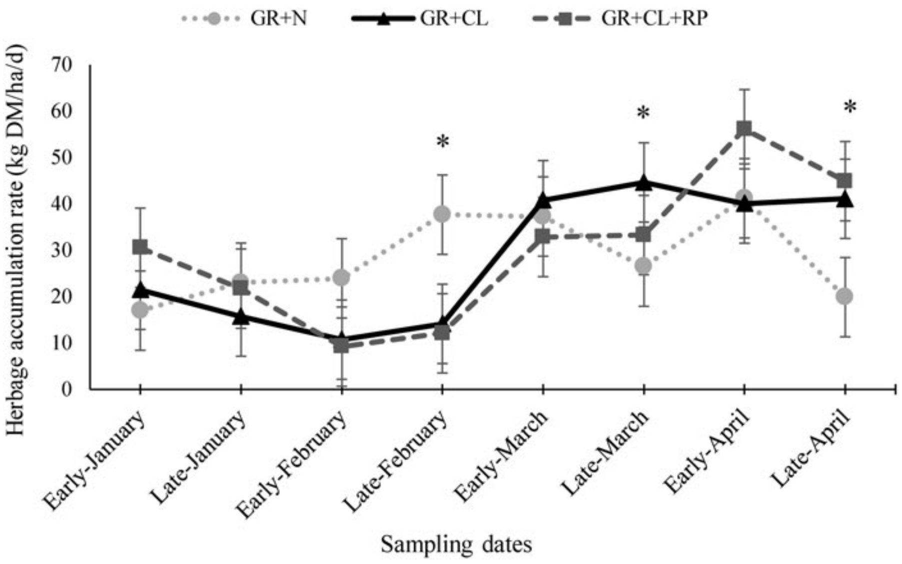

Figure 2. Treatment × sampling date interaction ($P = 0.030$; s.e. 0.055) for total herbage accumulation rate (HAR) during cool season. Error bars denote standard errors. *Significant at the 0.05 probability level according to least significant difference.

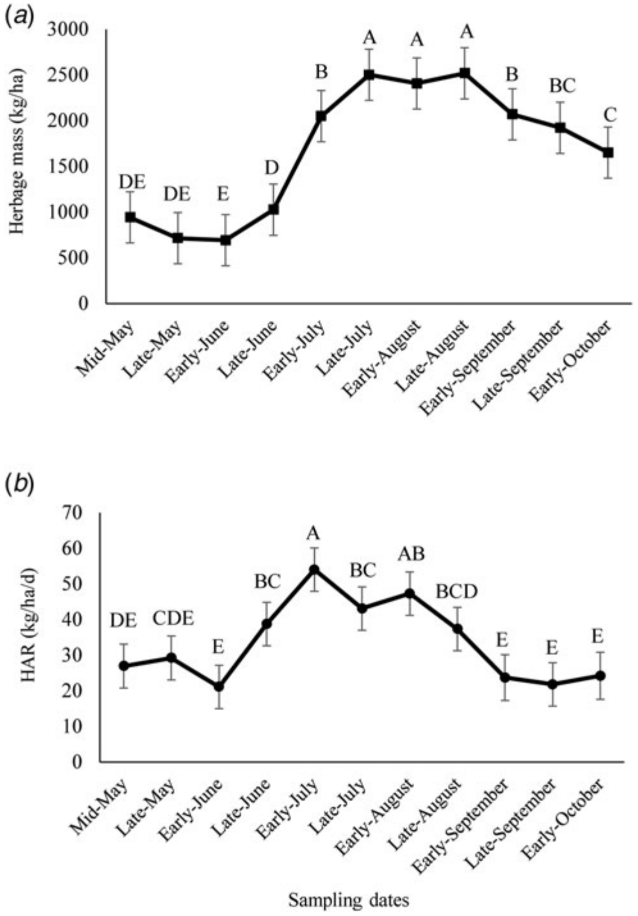

Figure 3. Warm season sampling date effect ($P < 0.0001$; s.e. 280) on herbage mass (a) and herbage accumulation rate (HAR) (b; $P < 0.0001$; s.e. 12.8). Grass + N, N-fertilized bahiagrass during the warm season overseed with ryegrass + oat during the cool season; Grass+Clover, bahiagrass during the warm season overseeded with ryegrass + oat plus a mixture of clovers during the cool season; Grass + Clover + RP, bahiagrass plus a mixture of rhizoma peanut during the warm season overseeded with ryegrass-oat-clover mixture during the cool season. DM, dry matter. Error bars denote standard errors.

and the means were 34, 29 and 37 kg/ha/d for Grass + N, Grass + Clover and Grass + Clover + RP, respectively. However, there was a sampling date effect ($P < 0.0001$) on HAR. In late June, the least HAR was observed, following an increase until reaching the peak in late July (54 kg/ha/d), decreasing after that as the season advanced.

The grass proportion differed among treatments ($P = 0.0001$). The proportion of bahiagrass in the botanical composition in the Grass + N and Grass + Clover averaged 76%. In the Grass + Clover + RP, the proportions of bahiagrass and rhizoma peanuts were 48% and 38%, respectively. The spread of RP was observed over the pasture, where the strips enlarged from 2.5 m to an average of 3.7 m from 2015 to 2022 (Fig. 4).

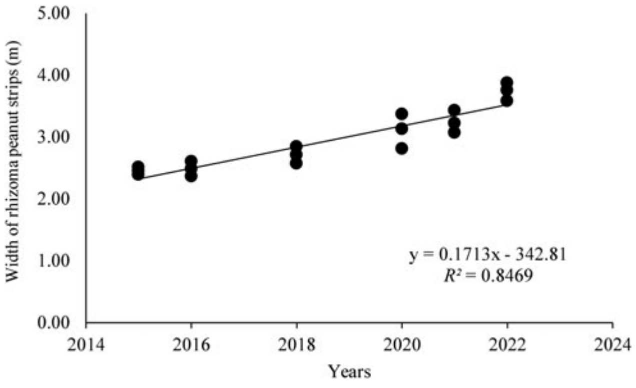

Figure 4. Width measurements of Ecoturf rhizoma peanut strips demonstrating the lateral spread in grass-legume mixture pasture over the years (2015 to 2022).

Nutritive value

Cool season

During the cool season, the CP concentration in the grass component did not differ among treatments ($P = 0.617$). However, CP differed across sampling dates ($P = 0.001$; Table 3). The CP values exhibited fluctuations throughout the season, reaching a peak concentration of 263 g/kg in late February and hitting the lowest point at 188 g/kg in late April. The IVDOM of cool-season grasses in Grass + N was greater ($P = 0.010$) at 0.72, with Grass + Clover and Grass + Clover + RP not differing at 0.69 and 0.67, respectively. There was a sampling date difference ($P < 0.0001$), and the values varied during the season (Table 3). The greatest value was 0.74 in late January and remained around 0.70 during the four sampling dates, then dropped and reached 0.60 in early May. The clover CP concentration differed across sampling dates ($P = 0.0001$; Table 3). The CP concentration was constant during the entire cool season, averaging 264 g/kg from January to early April, then dropping to 198 g/kg in late April to early May, with the end of the cool season. The IVDOM of clovers differed across sampling dates during the cool season ($P = 0.010$). The means were not different until March. In early March, the IVDOM reached the most outstanding value, 0.74; in April, it decreased and reached 0.62 in early May, the last sampling date (Table 3).

Warm season

There was a treatment × sampling date interaction ($P = 0.016$) for the CP concentration of grass in the warm season (Fig. 5). The

Table 3. Nutritive value of grass and legume during cool season in three grazing systems from 2020 to 2021

	Grass		Clover	
Evaluation date	CP g/kg	IVDOM	CP g/kg	IVDOM
Early January	240	0.70	267	0.73
Late January	214	0.74	267	0.74
Early February	241	0.71	278	0.73
Late February	263	0.73	267	0.72
Early March	246	0.72	272	0.74
Late March	208	0.73	254	0.72
Early April	225	0.69	257	0.68
Late April	188	0.66	252	0.70
Early May	192	0.6	196	0.62
P value	0.001	< 0.001	< 0.001	< 0.001
s.e.	12.8	0.012	27.6	0.017

CP, crude protein; IVDOM, *in vitro* digestible organic matter; Grass, ryegrass–oat; Legume, mixture of clovers. s.e.; standard error.

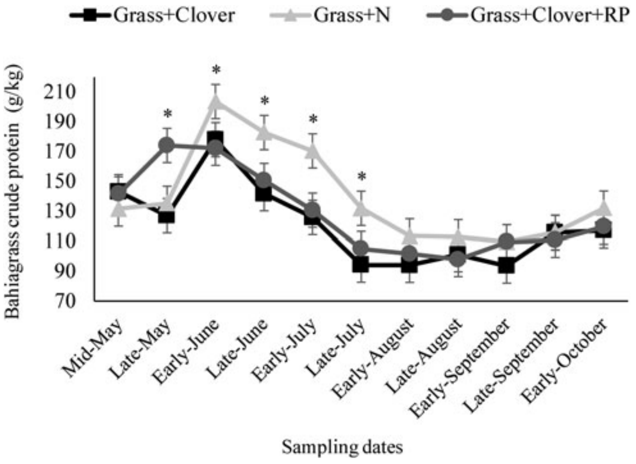

Figure 5. Warm season treatment × sampling date interactions ($P = 0.016$; S.E. 12.14) on crude protein concentrations and evaluation effect on grass ($P < 0.001$). DM, dry matter. Error bars denote standard errors. *Significant at the 0.05 probability level according to least significant difference.

differences were observed from early June to late August. In early June, Grass + Clover + RP had the greatest CP concentration (174 g/kg). The Grass + N treatment had the greatest CP concentration from late June to early August. In contrast, the most outstanding value for this treatment was 203 g/kg (Late June), the greatest CP concentration until early August. In late June, there was a CP peak for Grass + Clover (172 g/kg). As expected, with the advance of the season, from early July until the end, the CP was decreasing with the progress of the warm season and reaching 120 g/kg by October.

Following the same pattern of the CP concentration, the IVDOM of the grass component in the warm season presented a sampling date effect ($P < 0.0001$). The greatest value obtained was 0.59 in late May, then dropping to 0.51 in early June. A significant reduction in IVDOM throughout the warm season was observed, and the lowest value obtained was 0.38 in late October (Fig. 6).

The concentration of CP in RP varied significantly with sampling dates ($P = 0.020$), reaching its peak of 204 g/kg in late June (Table 4). The RP CP concentration remained consistently above 170 g/kg and below 200 g/kg throughout the season. Notably, the

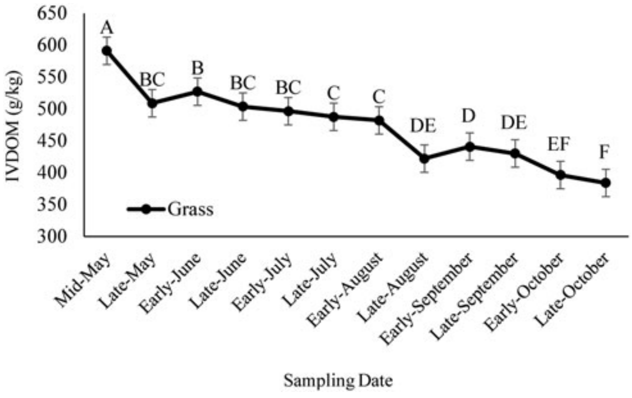

Figure 6. Warm season sampling date effect ($P < 0.0001$; S.E. 2.70) on IVDOM. Error bars denote standard errors. Averages presented with different letters are significantly different at the 0.05 probability level according to the least significant difference.

Table 4. Nutritive value of Ecoturf rhizoma peanut during warm-season in three grazing systems from 2020 to 2021

	Rhizoma peanut	
Evaluation date	CP g/kg	IVDOM
Late-May	192	0.80
Early-June	190	0.72
Late-June	221	0.69
Early-July	216	0.70
Late-July	204	0.69
Early-August	207	0.69
Late-August	167	0.68
Early-September	200	0.68
Late-September	201	0.57
Early-October	190	0.66
Late-October	196	0.63
P value	0.020	< 0.001
S.E.	8.7	0.032

CP, crude protein; IVDOM, in vitro digestible organic matter; S.E.; standard error.

IVDOM of RP exhibited a significant effect based on sampling dates ($P < 0.0001$). The highest IVDOM value, recorded in late May, was 0.8, decreasing in subsequent samplings. Throughout the season, IVDOM fluctuated between 0.6 and 0.7 (Table 4).

Nitrogen derived from the atmosphere and biological N_2 fixation

Cool season

The Ndfa differed by sampling date in the cool season ($P = 0.031$). The Ndfa increased with the progress of the season and, in early January, was observed at the lowest value (0.45), following the greatest value of 0.86 in early April (Table 5). The BNF was influenced by seasonality ($P = 0.021$) and oscillated during the cool season. The difference in sampling dates occurred especially from February to March, where in March, the BNF was 10.75 kg N/ha, and in February, it was 2.85 kg N/ha (Table 5).

Warm season

The Ndfa differed across sampling dates in the warm season ($P < 0.0001$; Table 5). The Ndfa changed during the warm season and increased with time. The least Ndfa was observed in early June, which was 0.53. The greatest value obtained was in October, the end of the season when the Ndfa was 0.79. In the warm season, the BNF did not differ among sampling dates ($P = 0.157$), and the fixation was 63 kg N/ha/season.

Animal performance

Cool season

After two consecutive grazing seasons (2020 to 2021), ADG, GPA, and HA did not differ ($P = 0.942$; $P = 0.410$; $P = 0.200$) among treatments during the cool season. The ADG, GPA and HA averaged 0.87 kg/d, 303 kg/ha and 1.06 kg DM/kg BW, respectively.

Table 5. N_2 derived from atmosphere (Ndfa) and biological N_2 fixation (BNF) of clovers and RP in the cool and warm seasons in three grazing systems from 2020 to 2021

	Ndfa	BNF (kg N/ha/period)
Cool season		
January	0.8	4
February	0.6	3
March	0.7	8
April	0.9	11
Early May	0.8	7
Season	0.7	33
P value	0.031	0.011
S.E.	0.67	2.9
Warm season		
Late May	0.9	6
June	0.5	8
July	0.6	13
August	0.6	11
September	0.7	13
October	0.8	10
Season	0.6	59
P value	< 0.001	0.157
S.E.	0.31	2.1

S.E., standard error. Season; Ndfa is the average of the season, BNF is the sum of the season.

The Grass + N treatment (Table 6) tended ($P = 0.087$) to have greater SR than other treatments (3.4 *vs* 2.4 AU/ha, respectively).

Warm season

After two warm seasons, the ADG and GPA differed among treatments ($P = 0.001$ and $P = 0.003$, respectively; Table 6). The incorporation of rhizoma peanut presented a two-fold increase in ADG and GPA compared to monoculture treatments. The ADG of Grass + Clover + RP was 0.34 kg/hd, whereas the average of Grass + N and Grass + Clover was 0.175 kg/hd/d. In GPA, the treatment Grass + Clover + RP presented 257 kg/ha, whereas the average of Grass + N and Grass + Clover was 128 kg/hd/d. The SR did not show differences among treatments ($P = 0.167$), with an average of 3.2 AU/ha. The HA of the warm season did not differ among treatments ($P = 0.75$) and averaged 0.99 kg DM/kg.

Annual grazing

The annual animal performance over the entire year with the combination of both the cool and warm seasons tended ($P = 0.090$) to present a significant difference among treatments in ADG, with greater ADG in Grass + Clover + RP (0.55 kg hd/d) than in the other treatments, averaging 0.46 kg hd/d. The GPA also did not differ among treatments, showing an average of 475 kg/ha. However, the tendency of greater GPA ($P = 0.070$) in the Grass + Clover + RP treatment compared to the others was also observed (550 *vs* 438 kg/ha). The SR showed differences among treatments ($P = 0.010$). The treatment Grass + N presented the most outstanding value (2.82 AU/ha), and the other treatments averaged 2.2 AU/ha.

Table 6. Average daily gain (ADG), gain per area (GPA), SR (AU/ha), and herbage allowance (kg DM/kg BW) in Grass+Clover, Grass+N, and Grass+Clover + RP pastures during cool and warm seasons from 2020 to 2021, and the year average

	Treatment				
	Grass + Clover	Grass + N	Grass + Clover + RP	S.E.	*P* value
Cool season					
ADG, kg/hd/d	0.89	0.87	0.86	0.056	0.940
GPA, kg/ha	286	331	293	32.7	0.410
SR, AU /ha	2.7	3.4	2.4	0.52	0.080
HA, kg DM/kg BW	1.1	1.0	1.1	0.021	0.200
Warm season					
ADG, kg/hd/d	0.18	0.17	0.34	0.027	0.001
GPA, kg/ha	127	131	257	22.7	0.003
SR, AU/ha	3.0	3.3	3.2	0.22	0.167
HA, kg DM/kg BW	1.00	0.98	0.99	0.040	0.750
Annual					
ADG, kg/hd/d	0.46	0.45	0.55	0.032	0.090
GPA, kg/ha	413	463	550	49.1	0.070
SR, AU/ha	2.8	3.4	2.89	0.31	0.010

Grass + N, N-fertilized bahiagrass during the warm season overseeded with annual ryegrass and oat during the cool season; Grass+Clover, bahiagrass during the warm season overseeded with ryegrass–oat plus a mixture of clovers during the cool season; Grass+Clover + RP, rhizoma peanut, and bahiagrass during the warm season overseeded with ryegrass–oat–clover mixture during the cool season; HA, herbage allowance; S.E., standard error; hd, head; DM, dry matter; BW, body weight. AU (animal unit; 350 kg BW).

Discussion

Herbage responses

Cool season

Nitrogen fertilizer enhances annual ryegrass production in pastures (Evers *et al*., 1997), but mixed grass-clover pastures could offer comparable herbage production while potentially improving animal performance. Our findings support this statement.

Ball clover exhibits seasonal production from March to May, red clover extends from April to the end of the season, and crimson clover precedes with an earlier production spanning from January (Ball *et al*., 2015). In late April, the Grass + N exhibited the lowest HAR at 20 kg DM/ha/d, whereas the Grass + Clover and Grass + Clover + RP showed a higher 43 kg DM/ha/d. The interaction occurred as Grass + N led to an earlier peak in forage production compared to the other treatments. The presence of ball and red clover ensured forage availability during this period when the grass was experiencing a decline in production, attributed to the decrease in annual ryegrass production from March to April (Ball *et al*., 2015; Dubeux *et al*., 2016). This result corroborates Jaramillo *et al*. (2021a, 2021b), which assessed cattle performance on rye (*Secale cereale* L.) and oats pastures with the same combination of clovers and N fertilizer amount used in the current study. The grass-clover mixture in their trial peaked in late April, as observed in the current research. They found a superior HAR (70 kg DM/ha/d) compared to the present study (40 kg DM ha/d), probably because rye usually has greater forage accumulation than ryegrass. On the other hand, Dubeux *et al*. (2016) reported similar HAR (~ 31 kg DM/ha) in ryegrass-oat mixed pastures during the cool season.

The proportion of clovers observed in this study (averaged 30%) is in the range of 20–45% proposed by Thomas (1992), which legumes could provide the benefits of N requirement for a productive and sustainable pasture in temperate or tropical conditions.

Warm season

In the warm season, N fertilizer did not affect HM and HAR, and the inclusion of RP into bahiagrass pastures showed the same result as non-fertilized and fertilized bahiagrass pastures. This result indicated the possibility of reducing N inputs by replacing 112 kg N/ha by integrating RP in the grazing system. The HM and HAR were different throughout the warm season, increasing until the peak between July and August. Our data corroborates Jaramillo *et al*. (2021a, 2021b), who observed greater HAR of all treatments between late July and early September. The non-fertilized pastures probably benefited from the grass-legume mixture strategy applied during the cool season. The N remaining in the soil throughout the year can be why this treatment had no differences in forage HM and HAR compared to Grass + N or Grass + Clover + RP. Nevertheless, this remains an assumption, highlighting the significance of conducting a soil analysis for conclusive verification. Similar forage biomass among the treatments might benefit the RP treatment because of its better forage nutritive value. The proportion of RP in this study was 38% and is inside the range proposed by Thomas (1992), which is adequate to provide N benefits to the mixed pasture. The proportion of legumes in the pasture is essential in obtaining the N_2 atmospheric fixation by legumes.

Strip planting is an excellent strategy to establish and spread RP into a grass pasture (Castillo *et al*., 2013). The results obtained in this study showed that RP has spread into a grass pasture throughout the years since its establishment. This observation is essential since this approach resulted in the lateral spread of the legume into the grass and may reduce the cost of the establishment (Castillo *et al*., 2013; Mullenix *et al*., 2016). Despite the gradual spread over time, strip-planting is a successful strategy for establishing bahiagrass and RP mixtures in the long term. With the limited number of studies utilizing this species and methodology, the findings from the present study, along with previous results, could lay the groundwork for future investigations.

Nutritive value

Cool season

The nutritive value of grass and legumes across all treatments did not differ. This is a significant result, indicating that incorporating clover with annual cool season grasses can partially replace the N fertilizer since the nutritive value of the pastures was similar across treatments. This implies that combining clovers decreased nitrogen fertilizer application from 112 kg to 34 kg of N while maintaining the same herbage mass (HM) and nutritive value. The concentrations of CP and IVDOM of ryegrass-oat were close to the ones reported by Dubeux *et al*. (2016) and Jaramillo *et al*. (2021a, 2021b).

As expected, it was noted that the nutritive value of forage varied throughout the grazing season, aligning with the advancement of the maturity stage, as indicated by the sampling dates. Crude protein concentration of annual ryegrass with N or legume decreases with the advance of maturity (Lemus *et al*., 2021). In a grazing trial assessing cool-season annual forage mixtures, Mullenix *et al*. (2012) observed the reduction of CP concentration and IVDOM through the cool season. Butler *et al*. (2012) also reported decreased CP and digestibility of ryegrass-oat mixtures with maturity. The concentration of clover CP remained consistent throughout the season, showing a decline only in the last sampling date. This stability can be attributed to the distinct growth periods of various clover species, as the blend of clovers aims to ensure the presence of each species in pastures during different times in the seasons. The decrease in IVDOM is linked to advanced maturity and was anticipated due to the natural growth process (i.e., morphological changes according to the development stage).

Warm season

The CP concentration of fertilized bahiagrass treatment was greater than bahiagrass in other treatments during most of the season, and perhaps the high CP concentration observed at the beginning of the warm season is related to the presence of remaining cool-season forages in the pasture, such as oat and ryegrass. The difference among the CP concentration of the treatments during the sampling dates was from late June to early August. However, the N fertilizers did not change the IVDOM of bahiagrass and showed no difference among treatments. A high IVDOM value for bahiagrass (0.59) was observed in May but dropped significantly to 0.51 in June. This is likely related to the presence of cool-season forages in the samples, increasing the digestibility in May and reducing in June when it is more difficult to detect the presence of cool-season forages. The current study values of CP and IVDOM for bahiagrass corroborates Jaramillo *et al*. (2021a, 2021b), which also found that bahiagrass decreased nutritive value with time and the values ranged from 160 to 80 g/kg and 0.5 to 0.4, from May to October, respectively, for CP and IVDOM. As expected, the CP and IVDOM of RP presented variation with the advance of the growing period. The IVDOM declined from 0.8 in May to 0.63 in October.

Nitrogen derived from atmosphere and biological nitrogen fixation

Cool season

Determining the percentage of Ndfa allows the estimation of how much atmospheric N_2 the legume is fixing, especially in a mixture of grasses, which depends on location, forage accumulation, and management (Carlsson and Huss-Danell, 2003). In the cool season, the Ndfa of this study was similar to the values reported by Brink (1990), who found a Ndfa of crimson clover around 0.77. Kristensen *et al.* (2022) reported a Ndfa in a range of 0.65 to 0.80 for white and red clover fertilized with 100 kg N/ha. This range corroborates the clover Ndfa found in the current study, which reached 0.86 in treatment with 34 kg N/ha since N fertilization can affect N_2-fixation activity (Kristensen *et al.*, 2022). The BNF of clovers in monoculture in the Southeast U.S. was reported by Brink (1990). The author reported that clover can fix around 155 kg N/ha, which was superior to the result obtained in this current study, which presented 32.5 kg N/ha for the season. However, this result is also in the range proposed by Morris *et al.* (1990), who state that clover incorporated into pastures has the potential to fix between 20 and 60 kg N/ha/yr. The findings of the current study align with those reported by Lucas *et al.* (2010), who observed a white clover nitrogen fixation of 46 kg N/ha/yr that ranged from 18 to 90 kg N/ha/yr. Jaramillo *et al.* (2021a, 2021b) further support these observations, attributing the lower fixation levels to grazing effects on clover biomass and the proportion of legume in their results. The observed fixation is intricately connected to both fixation rates and the legume proportion within the system. Lucas *et al.* (2010) demonstrated a robust correlation ($R^2 = 0.96$) between fixed nitrogen and the clover yield. This high correlation emphasizes the pivotal role of clover biomass in influencing nitrogen fixation.

Warm season

The rhizoma peanut showed an average of 0.64 of Ndfa during the season. The value is similar to the one reported by Santos *et al.* (2018), which was up to 0.65 in two years of study with ecoturf. The average BNF of RP over the two years obtained in the current study was 53 kg N/ha/season, superior to the one reported by Santos *et al.* (2018) that presented N_2 fixation of 30 to 44 kg N/ha/yr in pure stands of RP cultivar Ecoturf. In the current study, however, RP was not in monoculture but in a mixed stand with bahiagrass. The present BNF of RP in our study was greater than the one reported by Jaramillo *et al.* (2021a, 2021b), who attributed the low BNF (16 kg N/ha/season) to the preference of cattle for RP, which negatively affected the herbage accumulation rate, decreasing the contribution via BNF. The amount of N fixed in grass-legume mixtures is directly related to the legume proportion in the mix, legume biomass, N concentration in the legume, and proportion of N that is derived from the atmosphere *v.* that from the soil (Dubeux and Sollenberger, 2020).

Animal performance

Cool season

Steers' performance may fluctuate according to pastures and species composition (Ball *et al.*, 2015). The result of ADG in ryegrass-oat pastures for the current study is less than that reported by Mullenix *et al.* (2012), who observed an ADG of 1.39 kg/hd/d during the cool season. However, the results align with what Ball *et al.* (2015) proposed, indicating that profitable stocking would anticipate ADG of around 0.681 kg/hd/d per season. Also, Beck *et al.* (2013) suggested that stocker producers use higher SR to reduce costs and expect an ADG of around 0.9 kg/hd/d, which could be a considerable result for the current study. The GPA observed in the present study was superior to that reported by Jaramillo *et al.* (2021a, 2021b). The GPA observed by Jaramillo *et al.* (2021a, 2021b) in a rye-oat + N system was 285 kg/ha compared with 303 kg/ha provided by the annual ryegrass-oat + N system in the current study. Based on this, annual ryegrass in a mixture of oats and clover had greater animal performance results in the cool season compared to the previous study of Jaramillo *et al.* (2021a, 2021b) with rye-oat-clover. The GPA results demonstrate that the Grass + Clover system can meet the needs of southeast beef producers by yielding an average of 300 kg/ha. Furthermore, the incorporation of clovers into ryegrass pastures allows for a reduction in nitrogen application. The GPA results demonstrate that the Grass + Clover system can respond to the demands of southeastern beef producers, providing an average of 300 kg/ha and reducing N application by including clovers in ryegrass pastures. Ryegrass has the potential to extend the annual grazing season when overseeding dormant perennial warm-season grasses or even extend the winter grazing season when mixed with early development small grains (e.g., rye or oat), increasing the temporal distribution of forage production (Ball *et al.*, 2015).

Warm season

Producers are usually concerned about the profitability of legume inclusion in grazing systems due to the need for more information, investment demand, and establishment difficulty. Including RP in bahiagrass showed a great result compared to the N-fertilized monoculture, which can be considered a feasible alternative to improve animal performance, reducing the need for N fertilizers. Although treatments of the current research were the same as those used by Jaramillo *et al.* (2021a, 2021b), the performance of grazing steers was lower in the current research. The lower animal performance observed in the present study may be attributed to the lower herbage mass and accumulation compared to the previous study. This disparity could be linked to the drier conditions experienced in 2020 and 2021, in contrast to the period from May to September 2016–2019. Overall, the RP-bahiagrass mixture is the best system and corroborates the previous study since the Grass + Clover + RP represents the reduction of N fertilizers application while increasing annual animal performance.

Annual grazing

In the annual grazing season (warm and cool seasons), the inclusion of legumes in the system confirmed the reduction of N inputs and the feasibility of legume incorporation into grass pastures, as ADG and GPA for treatments with legume inclusion were comparable to N fertilizer treatments. Incorporating legumes into grass pastures promoted an equal gain in the cool season compared with grass N fertilized treatment. In the warm season, incorporating legumes into grass pastures promoted the highest animal performance compared to the grass-only plus N fertilizer. This reflects the reduction of N fertilization application by 190 kg N/ha, where the annual N application of Grass + N was 224 kg N/

ha/yr. In comparison, the N application was only 34 kg/ha in Grass + Clover and Grass + Clover + RP, representing an 85% reduction in synthetic N use. The same annual animal performance observed for the Grass + Clover treatment compared to Grass + N or Grass + Clover + RP also suggests the positive effect of legume incorporation on the system during the cool season. Furthermore, when examining the warm season, the results were notably improved. Including RP in bahiagrass pastures enhanced ADG and GPA compared to the N-fertilized treatment. The greater ADG during the warm season while maintaining the same ADG during the cool season can explain the tendency for greater annual ADG and GPA in Grass + Clover + RP treatment even with lower SR compared to Grass + N treatment (Table 6). This tendency suggests greater efficiency of livestock systems based on grass-legume mixtures pastures, with possible improvements in long-term animal performance when legumes are incorporated into the system. Further studies are encouraged to evaluate long-term animal performance among these contrasting systems. In summary, the most important result for the entire year is that the system can be sustainable by incorporating legumes since the GPA in grass-legume mixtures corresponds to only grass-fertilized pastures. This is an example of sustainable intensification because obtaining the same livestock gain was possible using less nitrogen fertilizer.

Conclusions

In the cool season, annual ryegrass-oat associated with clovers and 34 kg N/ha fertilization performed the same grass-only N-fertilized treatment (112 kg N/ha) regarding the animal responses. Annual ryegrass is well cultivated in the southeast United States and shows a great herbage response into oat and clover mixtures. Including rhizoma peanut during the summer improved the animal performance compared with fertilized and non-fertilized bahiagrass pastures. The continuity of RP in the system and the strip-planting approach showed the success of RP establishment in this system in Florida.

The incorporation of legumes into grass pastures beyond improved animal performance during the warm season, warranted a satisfactory herbage production throughout seasons, with the potential to increase transference of N_2 atmospheric into the system, thus reducing dependence on off-farms inputs and avoiding N losses caused by high levels of fertilizer. Integration of legumes into grass system pastures in warm and cool seasons may contribute to developing a sustainable grazing system, reducing the N fertilizer application by 85%. Annual cattle performance under grass-legume systems showed equivalency in relation to N fertilizer grass pasture treatment, demonstrating the viability of this approach for sustainable intensification of grazing systems.

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References

Aryal P, Sollenberger LE, Kohmann MM, da Silva LS, Cooley KD and Dubeux Jr JC (2021). Plant growth habit and nitrogen fertilizer affect rhizoma peanut biomass partitioning during establishment. *Grass and Forage Science* **76**, 485–493.

Ball DM, Hoveland CS and Lacefield GD (2015) *Southern Forages: Modern Concepts for Forage Crop Management*, 5th Edn. Atlanta, GA: Int. Plant Nutr. Inst.

Beck PA, Anders M, Watkins B, Gunter SA, Hubbell D and Gadberry MS (2013) Improving the production, environmental, and economic efficiency of the stocker cattle industry in the Southeastern United States. *Journal of Animal Science* **91**, 2456–2466. https://doi.org/10.2527/jas.2012-5873

Beran DD, Masters RA and Gaussoin RE (1999) Grassland Legume Establishment with Imazethapyr and Imazapic. *Agronomy Journal* **91**, 592–596. https://doi.org/10.2134/agronj1999.914592x

Brink GE (1990) Seasonal dry matter, nitrogen, and dinitrogen fixation patterns of crimson and subterranean clovers. *Crop Science* **30**, 1115–1118. https://doi.org/10.2135/cropsci1990.0011183X003000050031x

Butler TJ, Biermacher JT, Kering MK and Interrante SM (2012) Production and economics of grazing steers on rye-annual ryegrass with legumes or fertilized with nitrogen. *Crop Science* **52**, 1931–1939. https://doi.org/10.2135/cropsci2011.11.0611

Carlsson G and Huss-Danell K (2003) Nitrogen fixation in perennial forage legumes in the field. *Plant and Soil* **253**, 353–372.

Castillo MS, Sollenberger LE, Blount AR, Ferrell JA, Williams MJ and Mackowiak CL (2013) Strip planting a legume into warm-season grass pasture: defoliation effects during the year of establishment. *Crop Science* **53**, 724–731. https://doi.org/10.2135/cropsci2012.08.0485

Corrêa DCDAC, Cardoso ADAS, Ferreira MR, Siniscalchi D, Gonçalves PHDEA, Lumasini RN, Reis RA and Ruggieri AC (2021) Ammonia volatilization, forage accumulation, and nutritive value of marandu palisade grass pastures in different N sources and doses. *Atmosphere* **12**, 1179. https://doi.org/10.3390/atmos12091179

de Bruijn FJ and Hungria M (2022) Biological nitrogen fixation. Good microbes in medicine, food production, biotechnology. *Bioremediation, and Agriculture* **1**, 466–475. https://doi.org/10.1002/9781119762621.ch37

Dillard LS, Hancock DW, Harmon DD, Kimberly Mullenix M, Beck PA and Soder KJ (2018) Animal performance and environmental efficiency of cool- and warm-season annual grazing systems. *Journal of Animal Science* **96**, 3491–3402. https://doi.org/10.1093/jas/sky025

Dimkpa CO, Fugice J, Singh U and Lewis TD (2020) Development of fertilizers for enhanced nitrogen use efficiency – trends and perspectives. *Science of The Total Environment* **731**, 139113. https://doi.org/10.1016/j.scitotenv.2020.139113

Dubeux JC and Sollenberger LE (2020) Nutrient cycling in grazed pastures. In Rouquette Jr M and Aiken G (eds), *Management Strategies for Sustainable Cattle Production in Southern Pastures* (pp. 59–75). Academic Press. https://doi.org/10.1016/B978-0-12-814474-9.00004-9

Dubeux Jr JC, DiLorenzo N, Blount A, Mackowiak C, Santos ER, Silva HM, Ruiz-Moreno M and Schulmeister T (2016) Animal performance and pasture characteristics on cool-season annual grass mixtures in north Florida. *Crop Science* **56**, 2841–2852, 1027–38. https://doi.org/10.2135/cropsci2016.03.0141

Dubeux Jr JCB, Blount ARS, Mackowiak C, Santos ERS, Pereira Neto JD, Riveros U, Garcia L, Jaramillo DM and Ruiz-Moreno M (2017) Biological N_2 fixation, belowground responses, and forage potential of rhizoma peanut cultivars. *Crop Science* **57**, 1027–1038.

Evers GW, Smith GR and Hoveland CS (1997) Ecology and production of annual ryegrass. In Rouquette FM and Nelson LR (eds), *CSSA Special Publications*. Crop Science Society of America, pp. 29–43. https://doi.org/10.2135/cssaspecpub24.c3

Freitas ADS, Sampaio EVSB, Santos CERS and Fernandes AR (2010) Biological nitrogen fixation in tree legumes of the Brazilian semi-arid caatinga. *Journal of Arid Environments* **74**, 344–349. https://doi.org/10.1016/j.jaridenv.2009.09.018

Garcia L, Dubeux Jr JC, Sollenberger LE, Vendramini JM, DiLorenzo N, Santos ER, Jaramillo DM and Ruiz-Moreno M (2021) Nutrient excretion from cattle grazing nitrogen-fertilized grass or grass–legume pastures. *Agronomy Journal* **113**, 3110–3123. https://doi.org/10.1002/agj2.20675

Han KJ, Smith DJ and Pitman WD (2018) Potential of cool-season species as cover crops and forage in the Southeastern United States. *Crop, Forage & Turfgrass Management* **4**, 1–7. https://doi.org/10.2134/cftm2017.05.0038

Haydock K and Shaw N (1975) The comparative yield method for estimating dry matter yield of pasture. *Australian Journal of Experimental Agriculture* **15**, 663–70.

Hill J, Goodkind A, Tessum C, Thakrar S, Tilman D, Polasky S, Smith T, Hunt N, Mullins K, Clark M and Marshall J (2019) Air-quality-related health damages of maize. *Nature Sustainability* **2**, 397–403. https://doi.org/10.1038/s41893-019-0261-y

Jaramillo DM, Dubeux Jr JC, Mackowiak C, Sollenberger LE, DiLorenzo N, Rowland DL, Blount ARS, Santos ERS, Garcia L and Ruiz-Moreno M (2018) Annual and perennial peanut mixed with 'Pensacola'bahiagrass in North Florida. *Crop Science* **58**, 982–992. https://doi.org/10.2135/cropsci2017.09.0542

Jaramillo DM, Dubeux JCB, Sollenberger L, Mackowiak C, Vendramini JMB, DiLorenzo N, Queiroz LMD, Santos ERS, Garcia L, Ruiz-Moreno M and Santen E (2021a) Litter mass, deposition rate, and decomposition in nitrogen-fertilized or grass–legume grazing systems. *Crop Science* **61**, 2176–2189. https://doi.org/10.1002/csc2.20475

Jaramillo DM, Dubeux JC, Sollenberger LE, Vendramini JM, Mackowiak C, DiLorenzo N, Garcia L, Queiroz LMD, Santos ERS, Homem BGC, Van Cleff F and Ruiz-Moreno M (2021b) Water footprint, herbage, and livestock responses for nitrogen-fertilized grass and grass–legume grazing systems. *Crop Science* **61**, 3844–3858. https://doi.org/10.1002/csc2.20568

Kristensen RK, Fontaine D, Rasmussen J and Eriksen J (2022) Contrasting effects of slurry and mineral fertilizer on N_2-fixation in grass-clover mixtures. *European Journal of Agronomy* **133**, 126431. https://doi.org/10.1016/j.eja.2021.126431

Lemus R, White JA and Morrison JI (2021) Impact of cool-season legume interseedings or timed nitrogen applications on yield and nutritive value of annual ryegrass. *Crop, Forage & Turfgrass Management* **7**, e20090. https://doi.org/10.1002/cft2.20090

Lucas RJ, Smith MC, Jarvis P, Mills A and Moot DJ (2010) Nitrogen fixation by subterranean and white clovers in dryland cocksfoot pastures. New Zealand Grassland Association. *Proceedings of the New Zealand Grassland Association* **72**, 141–146. https://doi.org/10.33584/jnzg.2010.72.2825

Moore JE and Mott GO (1974) Recovery of residual organic matter from in vitro digestion of forages. *Journal of Dairy Science* **57**, 1258–1259. https://doi.org/10.3168/jds.S0022-0302(74)85048-4

Morris DR, Weaver RW, Smith GR and Rouquette FM (1990) Nitrogen transfer from arrowleaf clover to ryegrass in field plantings. *Plant and Soil* **128**, 293–297. https://doi.org/10.1007/BF00011122

Muir JP, Pitman WD and Foster JL (2011) Sustainable, low-input, warm-season, grass–legume grassland mixtures: mission (nearly) impossible? *Grass and Forage Science* **66**, 301–315. https://doi.org/10.1111/j.1365-2494.2011.00806.x

Mullenix MK, Bungenstab EJ, Lin JC, Gamble BE and Muntifering RB (2012) CASE STUDY: productivity, quality characteristics, and beef cattle performance from cool-season annual forage mixtures. *The Professional Animal Scientist* **28**, 379–386. https://doi.org/10.15232/S1080-7446(15)30371-5

Mullenix MK, Sollenberger LE, Wallau MO, Blount AR, Vendramini JMB and Silveira ML (2016) Herbage accumulation, nutritive value, and persistence responses of rhizoma peanut cultivars and germplasm to grazing management. *Crop Science* **56**, 907–915. https://doi.org/10.2135/cropsci2015.08.0507

Newman Y, Vendramini J and Blount A (2010) Bahiagrass (*Paspalum notatum*): overview and management: SS-AGR-332/AG342, 5/2010. *EDIS* **2010**, 1–9. https://doi.org/10.32473/edis-ag342-2010

Nyfeler D, Huguenin-Elie O, Suter M, Frossard E and Lüscher A (2011) Grass–legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. *Agriculture, Ecosystems & Environment* **140**, 155–163. https://doi.org/10.1016/j.agee.2010.11.022

Okito A, Alves BRJ, Urquiaga S and Boddey RM (2004) Isotopic fractionation during N_2 fixation by four tropical legumes. *Soil Biology and Biochemistry* **36**, 1179–1190. https://doi.org/10.1016/j.soilbio.2004.03.004

Ortega-S JA, Sollenberger LE, Quesenberry KH, Jones CS and Cornell JA (1992) Productivity and persistence of rhizoma peanut pastures under different grazing managements. *Agronomy Journal* **84**, 799–804. https://doi.org/10.2134/agronj1992.000219620

Pereira JM, Rezende CDP, Ferreira Borges AM, Homem BGC, Casagrande DR, Macedo TM, Alves BJR, Sant`Anna SAC, Urquiaga S and Boddey RM (2020) Production of beef cattle grazing on Brachiaria brizantha (Marandu grass) – Arachis pintoi (forage peanut cv. Belomonte) mixtures exceeded that on grass monocultures fertilized with 120 kg N/ha. *Grass and Forage Science* **75**, 28–36. https://doi.org/10.1111/gfs.12463

Randive K, Raut T and Jawadand S (2021) An overview of the global fertilizer trends and India's position in 2020. *Mineral Economics* **34**, 371–384. https://doi.org/10.1007/s13563-020-00246-z

Rouquette FM, Bransby DI and Riewe ME (1997) Grazing management and use of ryegrass. In Rouquette FM and Nelson LR (eds), *CSSA Special Publications*. Crop Science Society of America, pp. 79–99. https://doi.org/10.2135/cssaspecpub24.c6

Rouquette M, Corriher-Olson V and Smith GR (2020) Management strategies for pastures and beef cattle in the middle-south: the I-20 corridor. In Rouquette Jr M and Aiken G (eds), *Management Strategies for Sustainable Cattle Production in Southern Pastures*. Elsevier, pp. 123–187. https://doi.org/10.1016/B978-0-12-814474-9.00007-4

Sanderson MA, Jolley LW and Dobrowolski JP (2012) Pastureland and hayland in the USA: land resources, conservation practices, and ecosystem services. In Nelson J (ed.), *Conservation Outcomes From Pastureland and Hayland Practices: Assessment, Recommendations, and Knowledge Gaps*. Lawrence, KS: Allen Press, pp. 25–40.

Santos ERS, Dubeux JCB, Sollenberger LE, Blount ARS, Mackowiak C, DiLorenzo N, Jaramillo DM, Garcia L, Pereira TP and Ruiz-Moreno M (2018) Herbage responses and biological N $_2$ fixation of bahiagrass and rhizoma peanut monocultures compared with their binary mixtures. *Crop Science* **58**, 2149–2163. https://doi.org/10.2135/cropsci2018.02.0128

Shearer G and Kohl DH (1986) N_2-fixation in field settings: estimations based on natural 15n abundance. *Functional Plant Biology* **13**, 699–756. https://doi.org/10.1071/PP9860699

Silveira ML, Vendramini JMB, Sellers B, Monteiro FA, Artur AG and Dupas E (2015) Bahiagrass response and N loss from selected N fertilizer sources. *Grass and Forage Science* **70**, 154–160. https://doi.org/10.1111/gfs.12078

Smith AP, Christie KM, Rawnsley RP and Eckard RJ (2018) Fertiliser strategies for improving nitrogen use efficiency in grazed dairy pastures. *Agricultural Systems* **165**, 274–282. https://doi.org/10.1016/j.agsy.2018.06.017

Sollenberger LE, Moore JE, Allen VG and Pedreira CGS (2005) Reporting forage allowance in grazing experiments. *Crop Science* **45**, 896–900. https://doi.org/10.2135/cropsci2004.0216

Soumare A, Diedhiou AG, Thuita M, Hafidi M, Ouhdouch Y, Gopalakrishnan S and Kouisni L (2020) Exploiting biological nitrogen fixation: a route towards a sustainable agriculture. *Plants* **9**, 1011. https://doi.org/10.3390/plants9081011

Stewart Jr, RL, Sollenberger LE, Dubeux Jr JCB, Vendramini JMB, Interrante SM and Newman YC (2007) Herbage and animal responses to management intensity of continuously stocked bahiagrass pastures. *Agronomy Journal* **99**, 107–112. https://doi.org/10.2134/agronj2006.0167

Thomas RJ (1992) The role of the legume in the nitrogen cycle of productive and sustainable pastures. *Grass and Forage Science* **47**, 133–142. https://doi.org/10.1111/j.1365-2494.1992.tb02256.x

t'Mannetje LT and Haydock KP (1963) The dry-weight-rank method for the botanical analysis of pasture. *Grass and Forage Science* **18**, 268–275. https://doi.org/10.1111/j.1365-2494.1963.tb00362.x

USDA, U. S. Department of Agriculture. National Cooperative Soil Survey (2014) Available at https://soilseries.sc.egov.usda.gov/OSD_Docs/O/ORANGEBURG.html (accessed by December, 12/18/2023).

Vendramini J and Moriel P (2020) Management of forages and pastures in lower-south: i-10 corridor. In Rouquette Jr M and Aiken Glen (eds), *Management Strategies for Sustainable Cattle Production in Southern Pastures*. Elsevier, pp. 101–122. https://doi.org/10.1016/B978-0-12-814474-9.00006-2

Vendramini JMB, Sollenberger LE, Lamb GC, Foster JL, Liu K and Maddox MK (2012) Forage accumulation, nutritive value, and persistence of 'Mulato II' brachiariagrass in northern Florida. *Crop Science* **52**, 914–922. https://doi.org/10.2135/cropsci2011.06.0338

Wilm HG, Costello DF and Klipple GE (1944) Estimating forage yield by the double-sampling method. *Agronomy Journal* **36**, 194–203. https://doi.org/10.2134/agronj1944.00021962003600030003x

Woodley AL, Drury CF, Yang XY, Phillips LA, Reynolds DW, Calder W and Oloya TO (2020) Ammonia volatilization, nitrous oxide emissions, and corn yields as influenced by nitrogen placement and enhanced efficiency fertilizers. *Soil Science Society of America Journal* **84**, 1327–1341. https://doi.org/10.1002/saj2.20079

Wright AL, Hons FM and Rouquette Jr FM (2004) Long-term management impacts on soil carbon and nitrogen dynamics of grazed bermudagrass pastures. *Soil Biology and Biochemistry* **36**, 1809–1816. https://doi.org/10.1016/j.soilbio.2004.05.004

Yang Z, Du X, Lu L and Tejeda H (2022) Price and volatility transmissions among natural gas, fertilizer, and corn markets: a revisit. *Journal of Risk and Financial Management* **15**, 1–14.