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legume or N fertiliser application on N cycling**

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Abstract The absence of grazing management, coupled with limited N replacement,
significantly promotes grassland degradation. Nitrogen input via fertiliser or biological
fixation is critical for achieving productive and sustainable grassland systems. This two-

year study assessed the N cycling of three pasture types (PTs): 1) mixed Marandu palisadegrass (*Urochloa brizantha*) and perennial horse gram (*Macrotyloma axillare*) without N fertiliser (Mixed); 2) monoculture Marandu palisadegrass fertilised with 138 kg N ha⁻¹ yr⁻¹ (N-fertilised); and 3) monoculture Marandu palisadegrass without N fertiliser (Unfertilised). Litter responses, forage and N intake, N livestock excretion and N cycling were measured. There were no differences between PTs for the existing litter (an average of 3,485 kg ha⁻¹; $P = 0.204$). More N was cycled via litter in the Mixed pasture than N-fertilised and Unfertilised pastures (95.7 vs. 63.6 and 51.8 kg N ha⁻¹ season⁻¹; $P < 0.001$). There was no difference between PTs for the total N excretion per animal (an average of 79.5 g animal d⁻¹; $P = 0.727$). However, per hectare, total N excretion in the N-fertilised pasture increased 20.4 and 22.5% than Mixed and Unfertilised pastures, respectively ($P = 0.010$). In the N-fertilised and Mixed pastures, there was a positive overall change of N in the soil-plant-animal system of 83.5 and 28 kg N ha⁻¹ year⁻¹, respectively. In the Unfertilised pasture, there was an overall negative change of N in the soil-plant-animal system of -27 kg N ha⁻¹ year⁻¹. Nitrogen application or perennial horse gram integration in a grass pasture increased the conservation of soil N reserves.

Keywords: Litter deposition and decomposition, livestock excretion, *Macrotyloma axillare*, N-fertilised pasture, *Urochloa*, warm-season legume.

Introduction

Most Brazilian grass-fed beef operations are characterized by low productivity and economic indices, mostly due to poor pasture management and low investment (Martha et al. 2012). Historically, pasture establishment in tropical regions has been based on

grasses of African origin, mainly of the *Urochloa* genus (Jank et al. 2014). Low soil nutrient availability, especially nitrogen (N), is the main limiting factor for pasture productivity in tropical regions (Boddey et al. 2004; Jank et al. 2014). Owing to the expanding global population and growing demand for food, there is a need to intensify livestock systems. In view of this scenario, the increase in productivity of livestock production on pasture in a sustainable manner becomes a necessity in Brazil (Muir et al. 2011).

Nitrogen application is the fastest and easiest way to produce more meat in smaller areas (Delevatti et al., 2019; Pereira et al., 2020). However, N fertiliser applied to pastures is susceptible to ammonia volatilization and nitrification/denitrification that can favour its loss, increase the greenhouse gas (GHG) emissions and reduce the efficiency of N use (Vendramini et al. 2014). Furthermore, it is also difficult for farmers with little financial resources to employ fertilisers due to their high cost on the agricultural market (Dubeux et al. 2007; Muir et al. 2011; Bernardino de Carvalho and De Zen 2017). Therefore, the development of sustainable strategies for replacing nutrients in the soil is essential for reducing environmental impacts related to N fertiliser application (Barcellos et al. 2005).

Grass-legume mixed pastures are a promising alternative within sustainable strategies for the recovery of degraded pastures. There are several benefits associated with the introduction of legumes into pastures. By fixing atmospheric N₂ (BNF), these plants can increase N input into the soil (Cadisch et al. 1994; Cantarutti et al. 2002; Homem et al. 2021b). The mineralization of the deposited material derived from surface litter and from roots and nodules of legumes enables the transfer of N from legumes to grasses (Thilakarathna et al. 2016). In addition to a notable improvement in the nutritive value of the diet of the animals, and consequently, an increase in animal weight gain (Pereira et al. 2020; Homem et al. 2021a; dos Santos et al. 2023), secondary metabolites present in

the legumes, such as saponins and tannins, can reduce the amount of N excreted in the urine, and consequently, reducing the nitrous oxide emissions (N₂O) from excreta (Mueller-Harvey et al. 2019). The presence of these secondary metabolites may also lead to reductions in the emissions of enteric methane (Furtado et al. 2023; Homem et al. 2024).

Through grazing, animals convert nutrients stored in the herbage mass of the pasture into carcass and remainder is excreted by the animals, changing the routes and efficiency with which N is returned to the soil (Homem et al. 2021a). Nitrogen losses associated with animal excreta are important. The high concentration of highly labile forms, mainly in urine, associated with the heterogeneous distribution of excreta in the pasture, favours N loss from the soil (Lessa et al. 2014). On the other hand, the deposition of senescent material from plant tissues acts in a more homogeneous and gradual manner, reducing reactions that lead to N loss. As a result, greater efficiency in N cycling in pastures is attributed to litter deposition of forage residues (litter - Boddey et al. 2020). Understanding the different N sources used to intensify productivity in pastures contributes to the development of more efficient and sustainable management techniques and strategies, favouring the use of N recycled in the system (Dubeux et al. 2007). We hypothesize that the introduction of the legume perennial horse gram (*Macrotyloma axillare*) in pastures of Marandu palisade grass contributes to N cycling through fixation of atmospheric N₂. Therefore, the study assessed the effects of N input via BNF in Marandu palisade grass/perennial horse gram mixed pasture or Marandu palisade grass fertilised or not with 138 kg of N ha⁻¹ year⁻¹ on N cycling via litter and excreta, N intake and N balance in the soil-plant-animal system under continuous stocking.

Materials and Methods

Experimental site and treatments

The study was carried out at the Experimental Farm of the Federal Rural University of Rio de Janeiro, Brazil (22°47'S, 43°41'W; 20 m above sea level). According to the Köppen classification, the climate of experimental area is “Aw”, characterized by a wet season in summer, from November to April, and a dry season in winter, from May to October. The trial was carried out for two years, during the years 2021 and 2022. The wet season was from October to May, and the dry season June to September. Meteorological data were obtained from a station located 2.94 km from the experimental area (Supplementary Fig. 1).

The soil in the area is a Red Yellow Acrisol with transition to Planosols (WRB/FAO classification 2014). The relief is classified as gently rolling. Soil analyses were conducted according to standard Embrapa techniques (Claessen et al. 1997). The soil (0–0.20 m) had the following properties at the beginning of the experiment: pH (H₂O) = 5.6; exchangeable Al, Ca, Mg, 0.04, 1.5, and 0.6 cmolc dm⁻³, respectively; available P (Mehlich-I method) 6.1 mg dm⁻³, exchangeable K 31.4 mg dm⁻³, and soil carbon concentration 6.5 g kg⁻¹.

The experimental area was divided into four blocks, with each block divided into three paddocks of approximately one hectare. In each block, three pasture types (PT) were randomly distributed: 1) Marandu palisade grass [*Urochloa brizantha* (Hochst. ex A. Rich.) R.D. Webster cv. Marandu] fertilised with 138 kg of N ha⁻¹ year⁻¹ (**N-Fertilised**); 2) Marandu palisade grass mixed with perennial horse gram [*Macrotyloma axillare* (E. Mey.) Verdc. cv. Java] (**Mixed**); 3) Marandu palisade grass without N fertiliser application (**Unfertilised**).

Establishment of pasture treatments

In 2015, the area was ploughed and harrowed, and dolomitic limestone was incorporated at a dose of 1.5 Mg ha⁻¹ to correct acidity and neutralize toxic aluminium. Subsequently, 33 kg ha⁻¹ of P in the form of monoammonium phosphate was applied together with 5 kg of Marandu palisade grass seeds with the aid of a PHS3 type planter (Tatu Marchesan, Matão, SP). In the respective paddocks of the Mixed treatment, perennial horse gram was broadcast together with Marandu grass seed also at 5 kg of pure viable seeds ha⁻¹. Due to the low persistence of this legume in the system, it was necessary to carry out reseeded during the 2021 rainy season, in addition to supplying perennial horse gram seeds mixed with salt supplied to the animals *ad libitum* (salt lick) for ingestion and propagation in their dung (endozoochoric dispersal).

In September 2020, another 1 Mg ha⁻¹ of dolomitic limestone was applied to all paddocks. In November of the same year, 15 kg of P ha⁻¹ were applied in the form of single superphosphate (SSP) along with 50 kg of K ha⁻¹ in the form of potassium chloride. Annually, in the N-Fert treatment 138 kg of N ha⁻¹ was added as urea divided into two equal doses, one at the beginning and another at the end of the wet season. Manual weeding was carried out annually to control spontaneous plants in the experimental area.

In May 2020, the animals (Nellore steers) were introduced into the experimental area to adapt to management and subsequently the paddocks were maintained under continuous stocking with a variable stocking rate during the whole experimental period. In each experimental year, two Nellore steers (278 ± 11 kg of initial BW and 12 ± 1.3 months of age) were used as tester animals in each paddock. At the end of the first experimental year, the animals were replaced. Herbage allowance was adopted as a grazing management criterion. The animal stocking rate was regulated monthly to intended target of to 3 kg of green forage kg body weight⁻¹ for each treatment based on the animal body weights and green herbage mass. When it was necessary, “put and take”

animals were added or removed from pastures (Allen et al. 2011). Water and mineral supplementation were supplied *ad libitum*.

Evaluations

Herbage mass and N stock

Harvests to quantify herbage mass were sampled every 28 days. Six square frames (1 x 1m) were thrown randomly in each paddock, and all material contained within the frame was cut to a height of 0.10 m using a sickle. Subsequently, the samples were taken to the laboratory, for the manual separation of botanical and morphological components. Green grass mass (kg ha⁻¹) and the legume mass (kg ha⁻¹) were considered as leaf + stem. Grass mass (kg ha⁻¹) was considered green grass mass plus dead material mass. Herbage mass was considered to be the aboveground biomass of herbaceous plants (grass and grass plus legume mass, according to treatment). After separating the material, subsamples were removed, weighed, and taken to an oven for 72 h at 65 °C.

After drying, forage samples were ground in a Cyclotec mill (Tecator, Herndon, VA) through 2 mm sieves. After grinding, the DM of each sample was obtained by oven drying at 105 °C for 18 h (method 934.01; AOAC 2000). Further grinding of the samples was achieved using a roller mill similar to that described by Arnold and Schepers (2004). The C and N concentration were determined of these finely ground samples using an automated C-H-N elemental analyser (method 972.43; AOAC, 2000). Grass and legume N stock was assessed by the concentration of N in the grass or legume multiplied by their respective mass. The total N stock was the sum of N stocks in grass and legumes, according to the treatment. The results were presented as average per season.

Litter dynamics

Litter deposition, decomposition, and chemical composition were measured throughout the two years. The decomposition rate was estimated by collecting existing and deposited litter (Bruce and Ebersohn 1982; Rezende et al. 1999). Every 28 days, six points were randomly marked on each paddock using rectangular frames measuring 0.5 m² (1 x 0.5 m). The collection of all existing litter (EL) on the soil surface in an area of 0.5 m² was carried out. Litter was considered the dead material detached from the plant on the soil surface (Allen et al., 2011). After 14 days, all deposited material was collected from the same areas - deposited litter (DL). The total deposited litter was the sum of all the litter harvested during each season of the year (Rezende et al. 1999). After collection, litter samples were oven-dried at 55 °C for 72 h to constant weight. After drying, all litter samples were ground in a Cyclotec mill (Tecator, Herndon, VA) to pass a 2-mm screen. After grinding, DM of each sample was obtained by oven drying at 105 °C for 18 h (method 934.01; AOAC 2000), and organic matter (OM) was obtained by ashing at 500 °C for 4 h (Moore and Mott 1974). The EL and DL were used to calculate the decomposition constant 'k' using the equation described in Rezende et al. (1999):

$$k = [\ln (EL_{(n-1)} + DL_n) - \ln (EL_n)] / t$$

where $EL_{(n-1)}$ is the amount of existing litter in the previous cycle, DL_n is the amount of deposited litter in the current cycle, EL_n is existing litter in the current cycle, and t is the interval time (14 d). The half-life ($t^{1/2}$) was estimated according to the following equation Rezende et al. (1999):

$$t^{1/2} = \ln(2) / k$$

where k is the decomposition rate ($\text{g g}^{-1} \text{d}^{-1}$). These sample of litter were also finely ground using the roller mill and the N e C concentration were also determined using the C-H-N elemental analyser (method 972.43; AOAC, 2000).

The samples of litter (both EL and DL) containing 300 to 500 $\mu\text{g C}$ were also analysed for ^{13}C natural abundance using a Finnigan Delta Plus continuous-flow isotope-ratio mass spectrometer in the John Day Stable Isotope laboratory of Embrapa Agrobiologia. The proportion of grass-C and legume-C in the litter was estimated through the isotopic ratio of ^{13}C and ^{12}C using the equation:

$$\% \text{Legume} = \frac{100 (\delta^{13}\text{C}_G - \delta^{13}\text{C}_M)}{(\delta^{13}\text{C}_G - \delta^{13}\text{C}_L)}$$

where %legume is the proportion of carbon originating from the legume in the litter, $\delta^{13}\text{C}_G$ is the value of the abundance of $\delta^{13}\text{C}$ in the senescent material of the grass (-13.53‰), $\delta^{13}\text{C}_L$ is the value of the abundance of $\delta^{13}\text{C}$ in the senescent material of the legume (-28.37‰) and $\delta^{13}\text{C}_M$ is the value of $\delta^{13}\text{C}$ abundance in the litter of the Mixed treatment.

Forage intake

Hand-plucked forage (simulated grazing) samples were collected during the forage intake evaluation for nutritional value of the forage (Vries 1995). In the Mixed treatment, grass and legume were collected and separated in the laboratory. Forage samples were oven-dried at 55 °C for 72 h to a constant weight, subsequently, a composite sample of each species was made for each experimental unit. These samples were ground in a Cyclotec mill (Tecator, Herndon, VA) to pass a 1-mm screen. The DM of each sample was obtained by oven drying at 105 °C for 18 h (method 934.01; AOAC 2000).

Forage intake was estimated from faecal production and indigestible neutral detergent fibre (iNDF). Faecal production was estimated using the titanium dioxide

methodology as an external marker (Titgemeyer et al. 2001). For nine consecutive days, 10 g of titanium dioxide (TiO₂) was supplied to each animal. An adaptation period of six days was necessary so that the amount of marker excreted in the faeces reached equilibrium (constant concentration). Subsequently, faecal samples were collected for three days directly from the rectum, always at the same time of day. The faecal samples were oven-dried at 55 °C for 72 h to determine DM concentration, air equilibrated, weighed, and ground in a Cyclotec mill (Tecator, Herndon, VA) to pass a 1-mm screen. The faecal samples were analysed for titanium dioxide concentration, according to Myers et al. (2004).

Faecal and hand-plucked forage samples were incubated in the rumen for 288 hours to determine iNDF (Huhtanen et al. 1994). Two cannulated heifers fed with a diet that consisted of Marandu palisadegrass and forage peanut mixed pasture or Marandu palisadegrass monoculture were used in the iNDF estimate, according to treatment. Faecal production was used to calculate the amount of iNDF excreted in faeces, in this way, it was possible to obtain the iNDF intake. After that, the iNDF contained in the hand-plucked samples was used to estimate forage intake.

In the Mixed pasture treatment, the proportion of grass and legume in the forage intake was estimated through the ratio of natural ¹³C and ¹²C isotopes, as described previously. Thus, the proportion of legume-derived carbon in the residual iNDF from faecal samples (Lopes de Sá, 2017) was estimated using the natural abundance value of ¹³C from the residual iNDF in hand-plucked samples of the grass (-14.54‰), the value of ¹³C abundance of residual iNDF in hand-plucked samples of the legume (-26.91‰), and the value of ¹³C abundance in residual iNDF of faecal samples.

Nitrogen concentration in hand-plucked forage samples was determined by the C-H-N elemental analyser (method 972.43; AOAC, 2000) and used to determine the total

N intake by the forage. The N intake per area was obtained by multiplying daily N intake per animal by the stocking rate for each treatment.

Biological N₂ fixation from the Mixed pasture

The estimation of the N derived from biological fixation (BNF) was determined using the ¹⁵N natural abundance method (Shearer and Kohl 1986). This technique is based on the knowledge that the soil is slightly enriched with ¹⁵N in relation to the atmosphere. Therefore, plants that fix atmospheric N₂ have a lower abundance of ¹⁵N than plants that depend on N available in the soil. Non-N₂-fixing plants obtain all their N from the soil, so they are slightly enriched with ¹⁵N.

This plant material was oven-dried at 55 °C for 72 h to a constant weight and ground to a fine powder using the roller mill (Arnold and Schepers, 2004). The ¹⁵N abundance of the samples containing between 30 and 50 µg N was determined using the same isotope-ratio mass spectrometer used for the estimation of ¹³C abundance. Nitrogen derived from the atmosphere (Nd_{fa}) was determined using the equation developed by Shearer and Kohl (1986):

$$\%Nd_{fa} = \frac{\delta^{15}N_{\text{reference}} - \delta^{15}N_{\text{legume}}}{\delta^{15}N_{\text{reference}} - "B"}$$

Perennial horse gram was the legume evaluated. The non-fixing species used (reference plants) were collected monthly. The species evaluated were *Panicum maximum* and the “weeds” as *Sporolobus indicos*, *Sida glaziovii*, *Waltheria indica* and *Solanum palinacanthum*. The ¹⁵N abundance value of the plant completely dependent on BNF is known as the *B* value, and the *B* value used in this experiment was -1.83‰, reported by Unkovich et al (2008).

The amount of N fixed in the shoot tissue of perennial horse gram (kg N ha⁻¹) was calculated from the total legume N intake (kg N ha⁻¹) of the steers plus the total legume N deposited in the litter (kg N ha⁻¹) multiplied by the %Ndfa.

Nitrogen excretion

The N concentration in the faeces was determined by the C-H-N elemental analyser (method 972.43; AOAC, 2000). The amount of N deposited in faeces (g N d⁻¹) was calculated by multiplying the N concentration in faeces by faecal production determined using the TiO₂ marker. The volume of urine produced by the steers was estimated from urine spot samples, which were collected at the time of faeces collection. A 12 ml aliquot of urine was taken from each sample by adding 48 ml of a 0.02 N sulfuric acid (H₂SO₄) solution (Chen and Gomes 1992). A composite sample was made from the collections from the three days of evaluation and stored in a plastic bottle at -20 °C. The concentration of creatinine in urine was determined using a commercial kit (Creatinine K, Labtest, Lagoa Santa, Brazil). This parameter was used as a marker to calculate the volume of urine produced, assuming that the animals excrete a known amount of creatinine daily (Silva et al., 2012). Urinary volume was determined using the following equation:

$$VU = (0.0345 \times BW^{0.9491}) \div CC_U$$

where, VU (L d⁻¹) is the volume of urine produced daily, BW (kg) is the empty body weight of the steers and CC_U (g L⁻¹) is the concentration of creatine in the urine.

The amount of N excreted in urine (g of N d⁻¹) was determined by multiplying the N concentration (method 920.87; AOAC 2000) by the volume of urine produced. The amount of N in faeces and urine per season (kg ha⁻¹ season⁻¹) was calculated by multiplying the average total N excreted daily as urine and faeces by each animal by the

stocking rate for each treatment. The N retained in each animal was obtained through the difference between the N ingested and the N excreted in the form of faeces and urine.

Nitrogen cycling balance.

Nitrogen cycling balance was measured according to the values of N cycled via litter and livestock excretion (Figs. 2, 3 and 4). Values in rectangular boxes are pool sizes (kg N ha⁻¹), and other values are annual fluxes of N in kg N ha⁻¹ year⁻¹. Plant uptake or N in annual net aerial primary productivity was considered as deposited litter N + total N intake + change in standing N biomass. Nitrogen output was considered the N retained by animal (product) and losses. Nitrogen retained was measured by difference between N intake and N livestock excretion. Nitrogen losses were estimated based on a 5% loss of N in faeces and 50% of N in urine (Boddey et al. 2004). Nitrogen recycled in faeces and urine was calculated by difference between livestock N excretion and N losses. Nitrogen stocked in the animal carcass was calculated as live weight x 0.025 (Scholefield et al. 1991). For the N-Fertilised pasture, N lost from the fertiliser were considered as 1.1 and 14.5% for the N₂O and NH₃, respectively, as described in the 2019 Refinement of the 2006 IPCC Guidelines for National Greenhouse Gas Inventories (Klein et al. 2019). Nitrogen inputs from rainfall and in atmospheric deposition was not quantified (Boddey et al. 2004).

Statistical analyses

The experimental design used was randomized blocks with three treatments (N-fertilised, Mixed and Control) distributed in four blocks with repeated measurements throughout the seasons. The data were analysed using the “MIXED” procedure of the SAS 9.4 software (SAS institute Cary, NC). The effects of the pasture type and season variables

were considered as fixed, and the effect of block and year were considered random. Akaike's criterion was used to select the (co)variance structure with the best fit to the data (Akaike 1974). All variance components were estimated using the SAS “LSMEANS” function and comparisons were performed using Fisher’s protected least significant difference (LSD) test at 10% significance. When the data did not meet the normality assumptions, the logarithmic transformation was applied. The statistical model used was:

$$Y_{ijkz} = \mu + B_i + PT_j + \gamma_{ij} + Y_k + S_z + (PT \times S)_{jz} + \varepsilon_{ijkz}$$

where Y_{ijkz} = value observed in the i th block of the j th PT of the k th year of the z th season; μ = overall average; B_i = random effect associated with the i th block, $i = 1, 2, 3, 4$; PT_j = fixed effect associated with j th pasture type, $j = 1, 2, 3$; γ_{ij} = random error associated with the i th block in the j th PT. Y_k = random effect associated with k th year, $k = 1, 2$; S_z = fixed effect associated with z th season, $z = 1, 2, \dots, 4$; $(PT \times S)_{jz}$ = fixed effect of interaction j th PT with the z th season. ε_{ijkz} = random error associated with the i th block, the j th PT, the k th year, and the z th season.

Results

Herbage mass and N stock

The herbage, grass and green grass mass were greatest for the N-fertilised pasture treatment ($P < 0.001$; Table 1). In relation to the seasons, herbage and grass mass had higher values in dry season compared to wet season ($P \leq 0.003$). No difference was recorded for the green grass mass between seasons ($P = 0.376$), with an average of 1,338 kg ha⁻¹. In the Mixed pasture, the legume mass was 480 and 331 kg DM ha⁻¹ for the wet and dry seasons, respectively. The botanical composition (legume proportion in the herbage mass) in the Mixed treatment was on average of 23.9 % with greater proportion during the wet season (Table 1).

The Marandu palisade grass in the Mixed pasture was 22.2 % and 17.1 % greater in N concentration compared to N-fertilised and Unfertilised treatments, respectively ($P = 0.010$). The N concentration in the Marandu palisade grass was greater in the wet season ($P = 0.014$). Total N stock was greatest for the N-Fertilised and Mixed pasture compared to the Unfertilised pasture ($P = 0.010$). However, when only grass mass was evaluated, the greatest N stock was recorded in the N-Fertilised pasture ($P < 0.001$). There were no differences between seasons for the total and grass N stocks ($P \geq 0.397$). Regarding the amount of N stored in the perennial horse gram, the N concentration was 2.2 times greater than that of the Marandu palisade grass. The mean N stock in the legume mass was of 6.2 kg of N ha⁻¹, ranging from 8.1 to 4.3 kg of N ha⁻¹ in the wet and dry seasons, respectively.

Litter dynamics

There was no difference between PTs for the existing litter ($P = 0.204$; Table 2), with a mean of 3,485 kg OM ha⁻¹; however, the lowest amount of grass existing litter was recorded for the Mixed pasture ($P = 0.001$). More total and grass existing litter were recorded for the dry season ($P < 0.001$). The legume proportion in the existing litter was on average 13.3%, which corresponds to 388 kg of OM ha⁻¹ (Table 3). The amount of legume existing litter was affected by climatic factors, being higher in the dry season. However, the proportion of legume in the litter (14%) was obtained in the wet season, as determined by the $\delta^{13}\text{C}$ abundance (-15.61‰).

The greatest C and N concentrations and the lowest C/N ration in the existing litter were recorded for the N-Fertilised and Mixed pastures treatments ($P \leq 0.047$). The seasons did not show a significant effect on N concentration or C/N ratio ($P \geq 0.128$). However, the C concentration in the existing litter was greatest in the dry season ($P = 0.075$). The N stock in existing litter (kg ha⁻¹ season⁻¹) did not differ between PTs ($P =$

0.204). The greatest N stock in existing litter was observed during the dry season, being 29.1% higher than the wet season.

The litter deposition rate ($\text{kg OM ha}^{-1} \text{ day}^{-1}$) was greatest for the N-fertilised pasture treatment ($P = 0.003$; Table 3). In this treatment, the deposition rate of senescent tissues was 10.4 and 9.3% higher compared to Unfertilised and Mixed pastures, respectively. The Mixed pasture had the lowest value of grass litter deposition ($P < 0.001$). The greatest total and grass deposition rate were recorded for the dry season ($P < 0.001$). The ^{13}C abundance in the deposited litter for the Mixed pasture was -15.42‰, showing more negative values in the wet season. However, the legume deposited litter was 10.4 $\text{kg MO ha}^{-1} \text{ day}^{-1}$ in the wet and 13.4 $\text{kg MO ha}^{-1} \text{ day}^{-1}$ in the dry season, showing a greater legume litter fall in the period of reduced rainfall. In the Mixed pasture, the legume deposited litter averaged to 12.7%, which corresponded to a deposition rate of 11.9 $\text{kg ha}^{-1} \text{ day}^{-1}$.

The decomposition rate and half-life showed no difference between treatments ($P \geq 0.640$), although the greatest litter decomposition rate and hence the lowest half-life time were recorded for the wet season ($P \leq 0.009$). The N concentration for the grass deposited litter was greater in the N-Fertilised and Mixed pastures than Unfertilised pasture ($P = 0.070$). There was no difference between treatments for the C concentration in the grass deposited litter (an average of 376 g kg^{-1} ; $P = 0.384$); however, the lowest C/N ratio for the grass legume deposited litter was recorded in the Mixed pasture, but with no significant difference when compared to N-Fertilised pasture ($P = 0.016$). There were no differences between seasons for the N and C concentration and C/N ratio in the grass deposited litter ($P \geq 0.140$). The perennial horse gram legume had more N concentration and lower C/N ratio when compared to Marandu palisade grass (Table 3). The Mixed pasture had the greatest N cycled via litter ($P < 0.001$), but no difference was

recorded for the N cycled through the grass between N-Fertilised and Mixed pasture. In the Mixed pasture, the perennial horse gram legume cycled 0.21 kg of N ha⁻¹ day⁻¹, which accounted for 37.4% of the N amount cycled in this treatment. The dry season had the greatest total and grass deposited N per day ($P < 0.001$).

Forage and N intake

There was no difference between the PTs for the total DM intake ($P = 0.330$; Table 4). On average, the animals ingested 7.2 kg of DM animal⁻¹ day⁻¹. The lowest grass DM intake was recorded for the Mixed pasture ($P = 0.005$). In the Mixed pasture treatment, perennial horse gram represented 12.7% of the total DM intake, which was equivalent to 0.87 kg DM animal day⁻¹. The greatest total and grass DM intakes were registered in the wet season ($P < 0.001$). The N concentration of grass hand-plucked (simulated grazing) samples was greatest in the Mixed pasture ($P = 0.001$), but with no difference between seasons ($P = 0.716$). There was no difference between PTs for the total N intake, which on average was 91.9 g animal d⁻¹ ($P = 0.718$); however, the grass-derived N intake was greatest for the N-Fertilised and Unfertilised pastures ($P = 0.023$). More total and grass N intakes were recorded for the wet season. The N concentration in the perennial horse gram was twice that of the Marandu palisade grass, and the legume corresponded to 24.6% of N intake by steers in the Mixed pasture.

Biological N₂ fixation in the Mixed pasture

The ¹⁵N abundance of perennial horse gram was on average $-1.31 \pm 0.3\text{‰}$, ranging between -0.60 and -1.92‰ over the experimental period (Fig. 1). The mean $\delta^{15}\text{N}$ content of the reference plants was significantly greater ($P < 0.001$) than those of the perennial horse gram on all sampling dates, indicating a large proportional contribution of fixed N to the

legume. The mean ^{15}N abundances of the reference plants were: *Sida rhombifolia* $3.09 \pm 0.87\text{‰}$, *Conyza bonariensis* $3.46 \pm 0.44\text{‰}$, *Phyllanthus niruri* L. $3.74 \pm 0.1\text{‰}$, *Solanum sisymbriifolium* $2.34 \pm 1.81\text{‰}$, *Sida acuta* $1.69 \pm 0.03\text{‰}$, *Emilia sonchifolia* $3.39 \pm 0.41\text{‰}$ and for *Eupatorium maximilianii* $2.34 \pm 0.42\text{‰}$. The perennial horse gram showed great potential for using atmospheric N_2 , with mean values of %Ndfa ranging of 82.0% in the wet season and 85.4% in the dry season (Supplementary Table 1). Annually, the N content of the net primary productivity of the legume in the Mixed pasture amounted to $90 \text{ kg of N ha}^{-1}$, which corresponded to an input of $75.3 \text{ kg of N ha}^{-1}$ through BNF (Supplementary Table 1).

Nitrogen excretion

There were no differences between PTs for the total, faecal and urinary N excretion per animal ($P \geq 0.570$; Table 5) or between seasons for the urinary N excretion ($P = 0.531$). On average, total, faeces and urinary N excretion were 79.5, 49.5 and $29.8 \text{ g animal day}^{-1}$, respectively. The greatest total and faecal N excretion were recorded in the wet season ($P < 0.001$).

Nitrogen cycling

The greatest stocking rate was recorded for the N-fertilised pasture ($P = 0.001$; Table 5), being 15.1 and 18.2% greater than the Mixed and Unfertilised pastures, respectively. Regarding N cycling per hectare, there was no difference between PTs for total N intake and urinary N excretion ($P \geq 0.230$), which averaged 0.26 and $0.07 \text{ kg ha}^{-1} \text{ d}^{-1}$, respectively. The greatest grass N intake, and total and faecal N excretion were recorded for the N-Fertilised pasture ($P \leq 0.010$). The stocking rate and all variables linked to N

cycling per hectare were greatest for the wet season ($P \leq 0.023$). For the mixed pasture, legume N intake accounted for 23.0% of the total N consumed by steers in this treatment.

Using data from the Tables, it was possible to create diagrams that demonstrate the N flows and compartments in the soil-plant-animal system for the PTs evaluated. Nitrogen input from rainfall or atmospheric deposition is considered to be very low ($< 5 \text{ g N ha}^{-1} \text{ yr}^{-1}$; Urquiaga et al. 2012), and it was not accounted for. In the N-fertilised pasture, N lost from the fertiliser were considered as 1.1 and 14.5% for the N_2O and NH_3 , respectively (Klein et al. 2019). Thus, N loss of the fertiliser was estimated to be 1.5 and 20 kg of N ha^{-1} for the N_2O and NH_3 , respectively. In treatments managed with N input for the system (via fertiliser or BNF), the total N balance in the system was positive, which accounted to an accumulate of 83.5 and 28.0 kg N $\text{ha}^{-1} \text{ year}^{-1}$ for the N-fertilised and Mixed pastures, respectively (Figs. 2 and 3). In the PT with no N input, there was an overall negative change in system N of $-27 \text{ kg N ha year}^{-1}$.

Discussion

How does N input, whether via fertilisation or FBN, affect canopy structure and N stock in herbage mass?

The N fertiliser application promoted a greater herbage mass than the Unfertilised pasture. Nitrogen input via fertiliser resulted in a greater amount of N stored in the herbage mass compared to the Unfertilised pasture. This effect reaffirms the significant influence of this element on the physiological processes of plants, intensifying plant growth, especially in grasses (Paiva et al. 2015).

The concentration of N in the grass of the Mixed pasture was greater than in the monoculture, even when the monoculture was fertilised with N. Thus, even after a short time after the establishment (five years), the companion grass in the Mixed pasture

increased its N concentration, which it is linked to transfer of biologically fixed N from the legume. Conversely, Homem et al. (2021b) reported that even though forage peanut was able to fix 109 kg ha⁻¹ of N, the transfer of this N to the grass was slower, as it is dependent on the biological process of mineralization (i.e., litter decomposition) or transfer from livestock excreta., and this explained the similar N concentration in the grass of Mixed and Unfertilised pastures in this previous study. However, the soil in the current experiment is characterised as low fertility and sandy texture. Thus, the possible improvements in soil health due to the legume integration on grasslands were achieved faster when compared to the study of Homem et al. (2021b), in which it was carried out on clay texture soil after three years of establishment.

The herbage mass for the N-fertilised treatment was 33.4% greater than in the Mixed pasture. However, the N stock in the herbage mass for both PTs was similar. This situation is directly related to the presence of perennial horse gram in the Mixed pasture treatment. In general, C₃ species have lower N use efficiency than C₄ plants (Sage and Pearcy 1987). Therefore, a greater quantity of ribulose-1,5-bisphosphate carboxylase/oxygenase enzymes is necessary to maintain high photosynthetic rates in C₃ plants (Taiz and Zeiger, 2006). Therefore, the N concentration per leaf area of legumes is higher than that of grasses (Gomes et al., 2018). This explains the high N stock in the Mixed pastures, which even with lower herbage mass, presented same N stock in the mass as the N-fertilised pasture.

The greatest herbage and grass mass observed during the dry season are related to the accumulation of senescent material in the canopy. The dry season in the region of current experiment is marked by a reduction in temperature, rainfall and photoperiod, negatively impacting the emergence of new leaves and tillers with a subsequent reduction in pasture production (Fagundes et al. 2005). From this perspective, the renewal of plant

tissues is reduced, increasing the time interval in which senescent leaves, stems and tillers remain attached to the plant (Lara and Pedreira 2011).

In the Mixed pasture, there was a reduction in the proportion of the legume in the herbage mass during dry season. Although perennial horse gram presents some drought tolerance mechanisms, there was a noticeable lower legume mass due to the limiting climatic conditions of the dry season (Morris 2008). To reduce the transpiration surface, legumes reduce the leaf and stomatal area, impairing gas exchange and consequently carbon fixation (Black et al. 2009). Reductions in plant development occur because cell expansion and division are associated with cell turgor pressure, which is substantially reduced under water deficit conditions (Da Silva et al. 2015). In this scenario, the senescence of legume tissues is favoured.

How does N input, whether via fertilization or FBN, affect the N cycling dynamics via litter?

The amount of existing litter in the grassland is the result of the difference between the rate of deposition and decomposition (Dubeux et al. 2007). In the current study, the discrepancy between wet and dry seasons for the existing litter showed that climatic conditions had a great influence on the litter stock in the pasture. The higher deposition rate and lower decomposition in the dry season resulted in a greater mass of existing litter in the pasture in this season of the year.

There was a noticeable increase in litter deposition in response to N fertilisation. The litter deposition is directly associated with the grazing efficiency by animals and the senescence rate of plant tissues (Homem et al., 2021b). Nitrogen influences the breaking of bud dormancy and accelerates the tissues flow in the plant. In this way, the turnover of leaves and tillers increases, resulting in a higher rate of appearance and senescence (Paiva

et al. 2012). Therefore, the grazing manager needs to increase harvest efficiency in response to the increase in the rate of plant growth. Thus, grazing management criteria based on high green herbage allowance result in an increase in the proportion of dead material in the canopy of N-fertilised pasture. These factors appear to be the reason for the increased deposition of senescent tissues in the N-fertilised treatment.

Qualitative aspects of the legume seem to have contributed to the increase in N concentration together with the reduction in C concentration and the C/N ratio in existing litter. This effect will favour the mineralization of the litter and, subsequently, the N release (Jaramillo et al. 2021). Thus, the effects of the application of N fertiliser and the integration of the legume on the chemical characteristics of the litter were similar. The higher growth rate driven by N fertilisation, or the presence of the legume leads to the deposition of tissues with a lower physiological age and a more labile nutrient concentration (Pereira et al. 2015). Furthermore, pastures managed under continuous stocking, the plant tissue deposition is gradual and with continuous addition of labile plant fractions will stimulate the decomposition of the existing litter (Rezende et al. 1999; Ramalho et al. 2019).

Climatic conditions regulated the growth and senescence of perennial horse gram tissues. Since the high temperatures and precipitation in the wet season favoured the legume growth in the pasture, in the winter, the limiting climatic conditions stimulated the senescence rate. As a result, the litter deposition from legume in the dry season was 22.2% higher compared to the wet season. However, greater legume proportion in the existing and deposited litter as witnessed by the more negative $\delta^{13}\text{C}$ values were observed in the wet season. Due to the greater amount of grass deposited during the dry season, the legume proportion in the total existing and deposited litter mass was reduced. Thus, the

legume influence on the litter decomposition and mineralization was more marked during the wet season.

Although PTs managed with N input showed a higher N concentration in the deposited material derived from Marandu palisade grass, the magnitude of this difference was not enough to increase the decomposition rate. The high C/N ratio of the material deposited in this experiment from grass suggests a high potential for N immobilization in microbial biomass (Gama-Rodrigues et al. 2007). The speed of decomposition and mineralization capacity is directly linked to the decomposers activity that are stimulated by the gradual release of N in the litter (Apolinário et al. 2014). Generally, grasses that have a high C concentration and C/N ratio over 30. They have more recalcitrant compounds such as lignin and polyphenols (Gerdes et al. 2000). Therefore, N fertiliser application with 138 kg of N ha⁻¹ year⁻¹, or the presence of the legume in the Mixed pasture, did not influence the decomposition rate.

However, in this study, environmental factors such as temperature, rainfall and soil moisture acted with greater intensity in modifying the decomposition rate compared to the chemical characteristics of the litter (Sanches et al. 2009). Therefore, the '*k*' value varied between seasons, showing greater mass rate of decomposition in the wet season. Temperature is directly related to microbial activity. Experiments evaluating the litter mass decay from two species, *Calluna vulgaris* and *Pteridium aquilinum*, showed that microbial respiration increased exponentially as the temperature increased (Anderson and Hetherington 1999). Secondly, the water availability in the soil can limit the fungi and bacteria activity (Kohmann et al. 2018). In general, temperature is considered the most influential environmental factor in decomposition. However, in tropical conditions, soil moisture assume greater importance in maintaining high microbial activity rates (Krishna and Mohan 2017). It is known that there is a synergistic effect between these two

variables, meaning that decomposition is favoured in situations of high temperatures and precipitation (Kohmann et al. 2019).

The BNF potential of perennial horse gram

Perennial horse gram showed great potential for N₂ fixation, exhibiting Ndfa values above 80%. This legume is considered as promiscuous in nodulation in that it does not require the inoculation of specific bacteria to achieve high N₂ fixation rates (Morris 2008). Thus, this condition the compatibility of the host plant with the indigenous N₂-fixing bacteria seems to have favoured the high efficiency of N₂ fixation.

The Ndfa proportion between the seasons did not show major variations, being 82.0 and 85.4% for wet and dry seasons, respectively. However, a greater contribution from BNF was observed in the wet season. Since there were no major differences in the symbiotic efficiency of N₂ fixation between seasons, the variation in the N amount originating from BNF was associated with climatic conditions favourable to legume plant development during the wet season.

How does N input, whether via fertilization or FBN, affect N intake and excretion by beef steers?

Canopy structure was not uniform throughout the year, which directly affected the animal forage intake (Rouquette 2015). In this study, there was no difference in the herbage mass between seasons. Therefore, the intake depletion by steers in the dry season seems to occur due to the change in the canopy structure leading to the accumulation of senescent material of the plant during this season. In this scenario, forage harvesting by animals is more difficult due to a reduction in the proportion of leaves and an increase in the amount

of dead material in the canopy, negatively influencing the animal ingestive behaviour in grazing (Da Silva et al., 2013).

The N ingested per animal is defined by the relationship between the forage intake and its nutritive value (Homem et al., 2021a). In view of the similarity of the forage intake between treatments, the change in the forage nutritive value was not sufficient to alter N intake per animal. Thus, the same N intake between treatments resulted in no differences for the total, faecal and urinary N excretion per beef steers. However, greater stocking rate in the N-fertilised pasture led to more total and faecal N excretion per hectare. The differences in harvest efficiency between PTs changed the routes of N cycling. Nitrogen cycling via livestock excreta corresponded to 59.3, 37.8, and 40.7% of the total N cycling in the N-fertilised, Mixed, and Unfertilised pastures, respectively. Therefore, the greater harvest efficiency with more animals per ha, as observed in N-fertilised pasture, led to proportionally greater N cycling via excreta and lower N cycling via litter, compared to Mixed and Unfertilised pastures.

Nitrogen use efficiency (NUE) and overall change of N in soil-plant-animal system

The introduction of a species richer in crude protein, such as perennial horse gram, directly influences the N supply to the ruminal microbiota (Rotz et al. 2005). The legume provided an increase in the NUE (kg N ingested/kg N in plant uptake) by animals, presenting values of 10.1, 10.0 and 17.8% for N-fertilised, Unfertilised and Mixed pasture, respectively. The differences in NUE may be associated with the forage nutritive value in the animal diet. In this regard, the potentially digestible fibre of legumes presents a lower rate of degradation in the rumen, favouring synergism with the carbohydrate source in the diet (Detmann et al. 2009). Therefore, in the Mixed treatment, greater microbial protein synthesis and lower N excretion via urine will be observed, along with

an increase in the recalcitrant N content in animal faeces (Guimarães et al. 2022). This explains why the increase in N provided by legumes in the diet did not increase N excretion per animal.

Litter and livestock excreta are the most important nutrient cycling pathways in the soil-plant-animal system (Haynes and Williams 1993). The increase in the renewal of plant tissues in the wet season associated with greater grazing efficiency favoured the increase in leaves of reduced physiological age in the animal diet (Bahmani et al. 2003). As a result, high levels of digestibility and crude protein content of the forage ingested are observed, providing greater N excretion per animal in this season (Da Silva et al., 2013).

In pastures without N application, there was a noticeable negative balance of this element. This type of management, in the long term, reduces the availability of N in the soil, leading the system to degradation (Homem et al., 2021b). Otherwise, the N input, through N fertiliser application or legume integration favoured the increase in N stock in the soil. In this experiment, the N return through the litter was greater for the Mixed pasture in relation to the Unfertilised and N-fertilised pastures, highlighting the favourable impact that the use of legumes has on the N cycling. In view of the direct relationship between the use of N fertilisers and the increase in the GHGs concentration in the atmosphere, the use of mixed pastures represents a more appropriate possibility for recovering pastures and reducing environmental impacts associated with global warming (Lessa et al. 2014; Homem et al. 2024).

Conclusions

Over the years, grasslands without N replacement can degrade. Applying N fertiliser or integrating perennial horse gram into grass pastures promotes a positive N balance in the soil-plant-animal system by enhancing total N recycling through litter and livestock

excretion, increasing the likelihood of achieving productive and sustainable grassland systems. The pasture with N fertiliser showed higher harvest efficiency, resulting in lower N cycling through litter and higher N cycling through livestock excreta.

Although the legume proportion in the Mixed pasture was reduced over the two years of the experiment, the 75 kg N ha⁻¹ year⁻¹ fixed biologically by perennial horse gram is comparable to a moderate nitrogen fertiliser application in tropical pastures. Furthermore, the legume favoured the increase in the N concentration of the grass companion, which is directly linked to transference capacity of N from the legume to the grass companion. Thus, the Marandu palisade grass/perennial horse gram mixed pastures showed the capacity to maintain the soil reserves of N preventing pasture degradation. Therefore, legumes represent a promising alternative for recovering pastures without use of nitrogen fertiliser.

Conflict of Interest

The authors declare that there is no conflict of interest.

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Figure captions

Fig. 1 $\delta^{15}\text{N}\%$ of the reference plants and the legume in the Mixed pasture from November 2020 to October 2022. Values are means of five replicates. Error bars represent $\pm$ standard errors of the means.

Fig. 2 – Schematic of the annual nitrogen cycle in a Marandu palisadegrass and perennial horse gram mixed pasture continuously stocked with beef steers. Values in rectangular boxes are pool sizes (kg N ha^{-1}), and other values are annual fluxes of N in $\text{kg N ha}^{-1} \text{ year}^{-1}$. N losses are estimated based on a 5% loss of N from faeces and 50% of N from urine (Boddey et al. 2004).

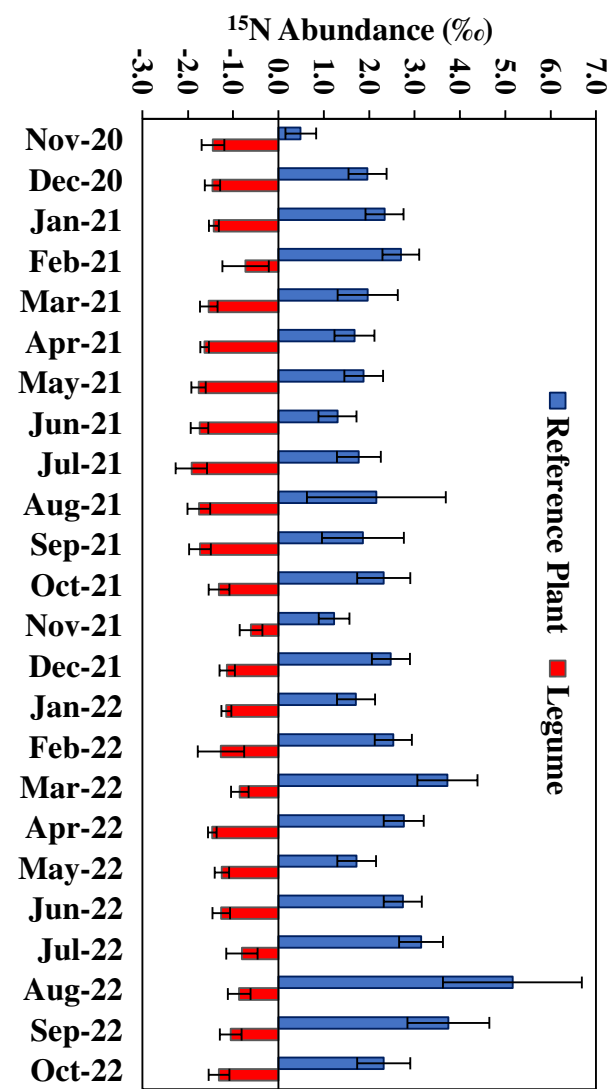
SOM: soil organic matter; BNF: biological N_2 fixation

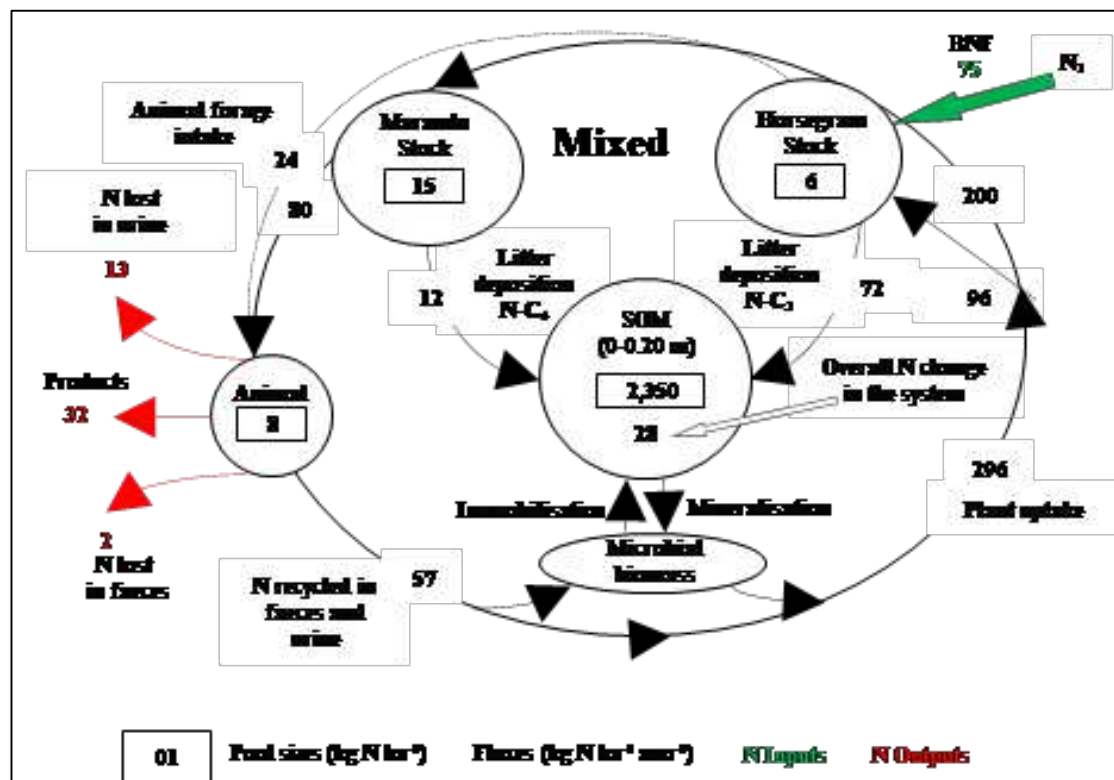
Fig. 3 Schematic of the annual nitrogen cycle in a Marandu palisadegrass pasture fertilised with 138 kg N ha^{-1} and continuously stocked with beef steers. Values in rectangular boxes are pool sizes (kg N ha^{-1}), and other values are annual fluxes of N in $\text{kg N ha}^{-1} \text{ year}^{-1}$. N losses are estimated based on a 5% loss of N from faeces and 50% of N from urine (Boddey et al. 2004).

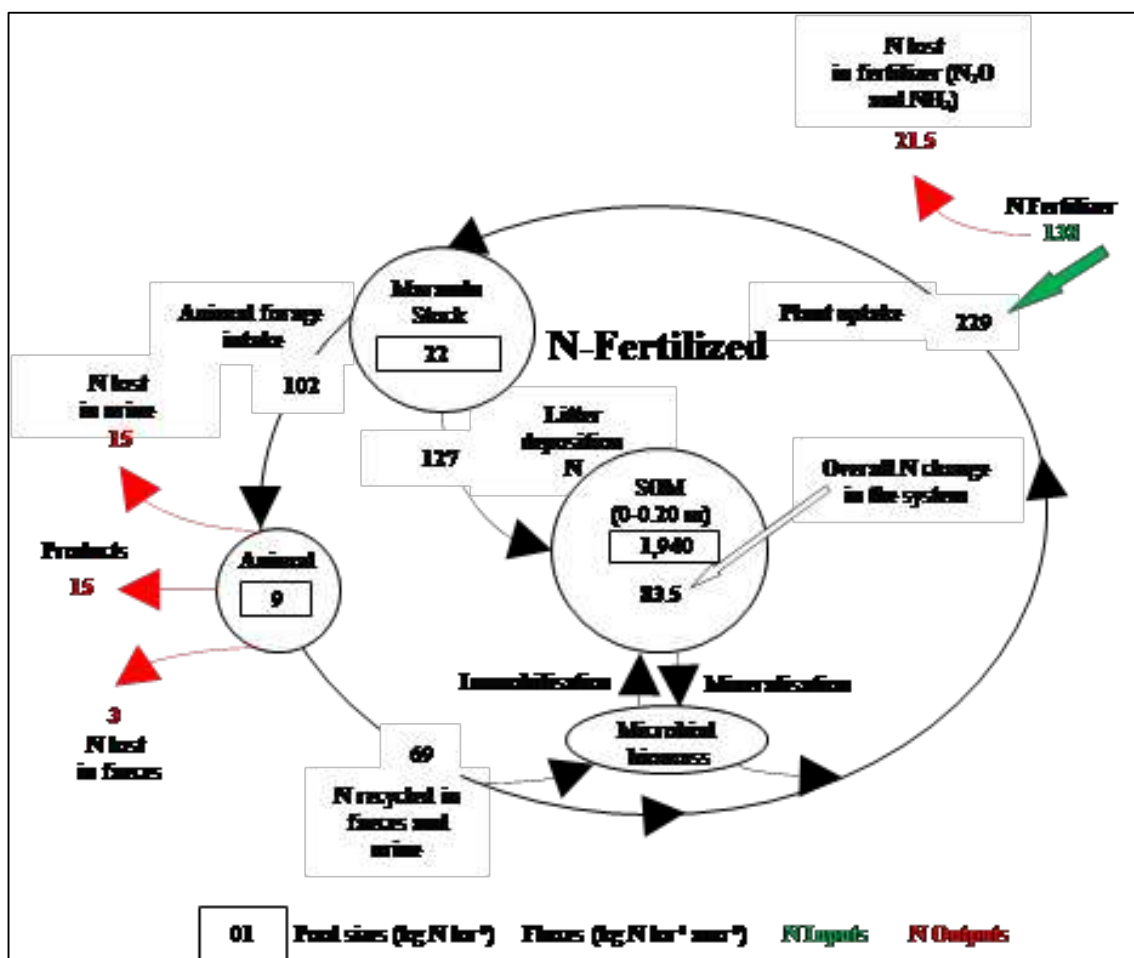
SOM: soil organic matter

Fig. 4 Schematic of the annual nitrogen cycle in a Marandu palisadegrass without N fertilizer application continuously stocked with beef steers. Values in rectangular boxes are pool sizes (kg N ha^{-1}), and other values are annual fluxes of N in $\text{kg N ha}^{-1} \text{ year}^{-1}$. N losses are estimated based on a 5% loss of N from faeces and 50% of N from urine (Boddey et al. 2004).

SOM: soil organic matter







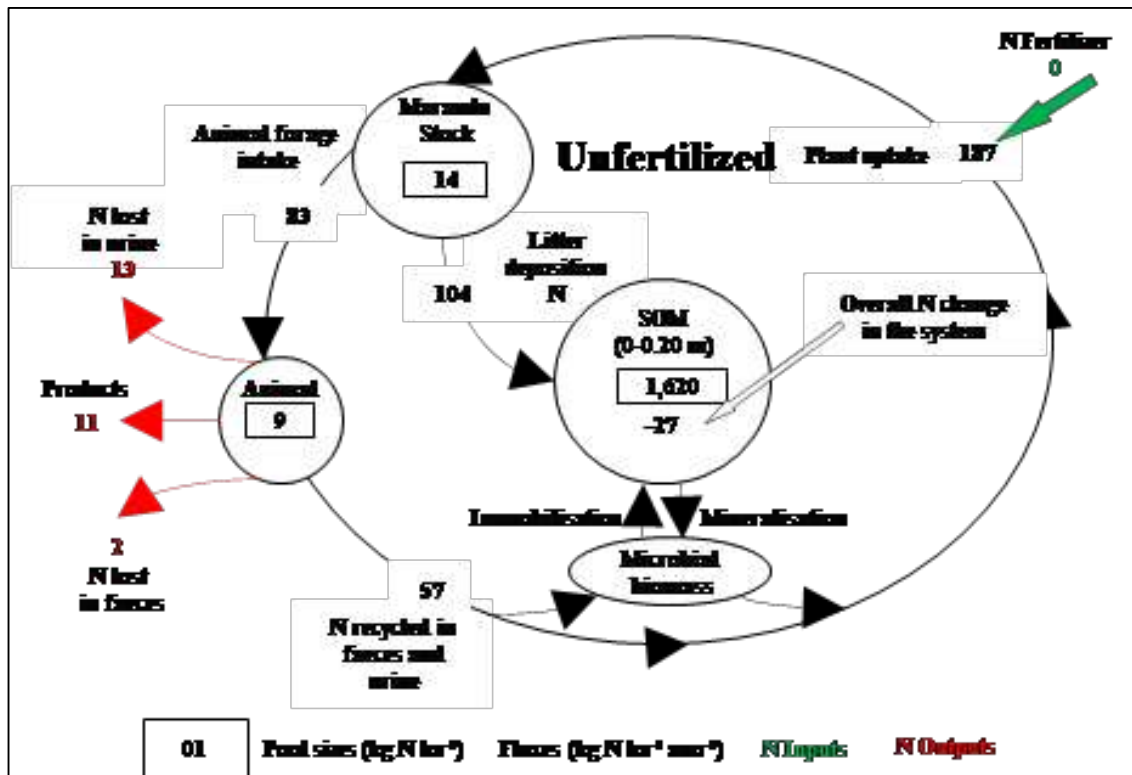


Table 1 Herbage mass characteristics, and N concentration and stock in Marandu palisade grass pastures fertilized or not with 138 kg of N ha⁻¹ year⁻¹ or mixed with perennial horse gram during the seasons of the experimental period.

Item	Pasture type (PT)			Seasons (S)		SEM ^a	<i>P value</i>		
	N-fertilised	Mixed	Unfertilised	Wet	Dry		PT	S	PT*S
Herbage mass characteristics									
Herbage mass, kg ha ⁻¹	4,103a	2,822b	2,433b	2,825B	3,352A	511	<0.001	0.003	0.691
Grass mass, kg ha ⁻¹	4,103a	2,416b	2,433b	2,726B	3,241A	511	<0.001	0.001	0.632
Green grass mass, kg ha ⁻¹	1,903a	1,105b	1,006b	1,379	1,297	171	<0.001	0.376	0.916
Legume mass, kg ha ⁻¹	-	405	-	480	331	-	-	-	-
Botanical composition (% legume in herbage mass)	-	24.0	-	26.9	21.1	-	-	-	-
N concentration and stock in herbage mass									
Grass N concentration, g kg ⁻¹	5.4b	6.9a	5.7b	6.4A	5.6B	0.5	0.010	0.014	0.18
Legume N concentration, g kg ⁻¹	-	15.2	-	17.2	13.1	-	-	-	-
Total N stock, kg ha ⁻¹	21.8a	21.2a	13.9b	19.2	18.7	2.6	0.010	0.705	0.334
Grass N stock, kg ha ⁻¹	21.8a	15.0b	13.9b	16.5	17.2	2.6	<0.001	0.397	0.546
Legume N stock, kg ha ⁻¹	-	6.2	-	8.1	4.3	-	-	-	-

Means within a row followed by different lowercase, or uppercase letters differ at $P \leq 0.10$.

^aStandard error of the means.

Table 2. Dynamics of existing litter in Marandu palisade grass pastures fertilized or not with 138 kg of N ha⁻¹ year⁻¹ or mixed with perennial horse gram during the seasons of the experimental period.

Item	Pasture types (PT)			Seasons (S)		SEM ^a	<i>P value</i>		
	N-fertilised	Mixed	Unfertilised	Wet	Dry		PT	S	PT*S
Total existing litter, kg OM ^b ha ⁻¹	3,658	3,262	3,535	2,944B	4,019A	469	0.204	<0.001	0.382
Grass existing litter, kg OM ha ⁻¹	3658a	2874b	3535a	2841B	3870A	480	0.001	<0.001	0.480
Legume existing litter, kg OM ha ⁻¹	-	388	-	330	446	-	-	-	-
δ ¹³ C existing litter in Mixed pasture, ‰	-	-15.5	-	-15.6	-15.4	-	-	-	-
% legume in existing litter ^c	-	13.3	-	14.0	12.5	-	-	-	-
Existing litter N concentration, g kg ⁻¹	6.1a	6.6a	5.4b	5.9	6.2	0.6	0.001	0.128	0.230
Existing litter C concentration, g kg ⁻¹	308a	311a	295b	295B	315A	51	<0.001	0.075	0.929
Existing litter C/N ratio	50.5b	48.b	55.1a	51.2	51.6	5.4	0.047	0.840	0.296
Existing litter N stock, kg ha ⁻¹	22.7	21.4	19.1	17.5B	24.7A	3.9	0.204	<0.001	0.156

Means within a row followed by different lowercase, or uppercase letters differ at $P \leq 0.10$.

^aStandard error of the means.

^bOrganic matter.

^cCalculated from ¹³C abundance of the grass (- 13.53‰) and legume litter (- 28.37 ‰) and of the existing litter in the Mixed and Unfertilised pastures.

Table 3 Dynamics of litter deposition, decomposition, and N cycling of Marandu palisade grass pastures fertilized or not with 138 kg of N ha⁻¹ year⁻¹ or mixed with perennial horse gram during the seasons of the experimental period.

Item	Pasture types (PT)			Seasons (S)		SEM ^a	<i>P value</i>		
	N-fert	Mixed	Unfertilised	Wet	Dry		PT	S	PT*S
Deposition rate, kg OM ^b ha ⁻¹ d ⁻¹	108.3a	100.7b	97.0b	84.9B	117.5A	11.7	0.003	<0.001	0.430
Grass deposition rate, kg OM ha ⁻¹ d ⁻¹	108.3a	88.8c	97.0b	82.1B	114.0A	11.9	<0.001	<0.001	0.430
Legume deposition rate, kg OM ha ⁻¹ d ⁻¹	-	11.9	-	10.4	13.4	-	-	-	-
δ ¹³ C deposited litter, ‰	-	-15.4	-	-15.6	-15.3	-	-	-	-
<i>k</i> , g OM g ⁻¹ day ⁻¹	0.0196	0.0203	0.0195	0.0214A	0.0183B	0.0058	0.970	0.009	0.490
Half-life, d	40.7	40.3	40.8	35.8B	45.5A	10.8	0.640	0.001	0.860
Deposited grass litter, N concentration, g kg ⁻¹	3.4a	3.5a	3.0b	3.4	3.5	0.2	0.070	0.200	0.380
Deposited grass litter, C concentration, g kg ⁻¹	380	374	375	376	377	6.2	0.384	0.855	0.975
C/N ratio of deposited grass litter	116ab	107b	128a	116	108	11	0.016	0.140	0.875
Deposited legume litter N concentration, g kg ⁻¹	-	17.9	-	17.9	17.9	-	-	-	-
Deposited legume litter C concentration, g kg ⁻¹	-	400	-	400	400	-	-	-	-
C/N ratio of legume deposited litter	-	22.3	-	22.3	22.3	-	-	-	-
Deposited litter N, kg N ha ⁻¹ d ⁻¹	0.37b	0.55a	0.29b	0.33B	0.48A	0.05	<0.001	<0.001	0.361
Grass deposited litter N, kg N ha ⁻¹ d ⁻¹	0.37a	0.34ab	0.29b	0.27B	0.40A	0.03	0.054	<0.001	0.266
Legume deposited litter N, kg N ha ⁻¹ d ⁻¹	-	0.21	-	0.18	0.24	-	-	-	-

Means within a row followed by different lowercase, or uppercase letters differ at $P \leq 0.10$.

^aStandard error of the means.

^bOrganic matter.

Table 4 Forage and N intake of animals grazing Marandu palisade grass pastures fertilized or not with 138 kg of N ha⁻¹ year⁻¹ or mixed with perennial horse gram during the seasons of the experimental period.

Item	Pasture types (PT)			Seasons (S)		SEM ^a	<i>P value</i>		
	N-fertilised	Mixed	Unfertilised	Wet	Dry		PT	S	PT*S
Total DM ^b intake, kg animal d ⁻¹	7.5	6.8	7.4	8.9A	5.5B	2.1	0.330	<0.001	0.879
Grass DM intake, kg animal d ⁻¹	7.5a	5.9b	7.4a	8.6A	5.3B	1.1	0.005	<0.001	0.819
Legume DM intake, kg animal d ⁻¹	-	0.87	-	1.1	0.69	-	-	-	-
Grass N concentration, g kg ⁻¹	12.6b	13.6a	12.6b	12.8	12.7	0.90	0.001	0.716	0.916
Legume N concentration, g kg ⁻¹	-	27.9	-	30.8	25.1	-	-	-	-
Total N intake, g animal d ⁻¹	90.4	95.2	90.0	109.9A	73.9B	19.8	0.718	<0.001	0.985
Grass N intake, g animal d ⁻¹	90.4a	73.0b	90.0a	101.2A	67.4B	15.7	0.023	<0.001	0.788
Legume N intake, g animal d ⁻¹	-	23.4	-	29.5	17.2	-	-	-	-

Means within a row followed by different lowercase, or uppercase letters differ at $P \leq 0.10$.

^aStandard error of the means.

^bDry matter.

Table 5. Nitrogen excretion by beef steers grazing Marandu palisade grass pastures fertilized or not with 138 kg of N ha⁻¹ year⁻¹ or mixed with perennial horse gram during the seasons of the experimental period.

Item	Pasture types (PT)			Season (S)		SEM ^a	<i>P value</i>		
	N-fertilised	Mixed	Unfertilised	Wet	Dry		PT	S	PT*S
Total N excretion, g animal d ⁻¹	81.3	77.4	79.9	89.1A	69.9B	24.2	0.727	<0.001	0.350
Faecal N excretion, g animal d ⁻¹	51.4	47.7	49.2	59.9A	38.9B	13.5	0.570	<0.001	0.470
Urinary N excretion, g animal d ⁻¹	29.4	29.4	30.5	29.1	30.5	10.9	0.894	0.531	0.917
Stocking rate, AU ^b ha ⁻¹	2.92a	2.47b	2.39b	2.81A	2.38B	0.18	0.001	0.013	0.674
Total N intake, kg ha ⁻¹ d ⁻¹	0.27	0.28	0.22	0.34A	0.18B	0.06	0.209	<0.001	0.846
Grass N intake, kg ha ⁻¹ d ⁻¹	0.27a	0.21b	0.22ab	0.31A	0.16B	0.05	0.050	<0.001	0.971
Legume N intake, kg ha ⁻¹ d ⁻¹	-	0.07	-	0.09	0.04	-	-	-	-
Total N excretion, kg ha ⁻¹ d ⁻¹	0.23a	0.20b	0.19b	0.26A	0.16B	0.06	0.061	<0.001	0.469
Faecal N excretion, kg ha ⁻¹ d ⁻¹	0.15a	0.12b	0.12b	0.17A	0.09B	0.03	0.016	<0.001	0.491
Urinary N excretion, kg ha ⁻¹ d ⁻¹	0.08	0.07	0.07	0.08A	0.06B	0.02	0.562	0.023	0.534

Means within a row followed by different lowercase, or uppercase letters differ at $P \leq 0.10$.

^aStandard error of the means.

^bAnimal unit: 500 kg body weight (Allen et al., 2011).

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