Optimizing nitrogen use efficiency of six forage grasses to reduce nitrogen loss from intensification of tropical pastures

Journal Article

Author(s):
Bastidas, Mike; Vázquez, Eduardo; Villegas Salazar, Daniel; Rao, Idupulapati M.; Gutierrez, Jhon F.; Vivas-Quila, Nelson J.; Amado, Miguel; Berdugo, Carlos; Arango, Jacobo

Publication date:
2024-06-15

Permanent link:
https://doi.org/10.3929/ethz-b-000666644

Rights / license:
Creative Commons Attribution 4.0 International

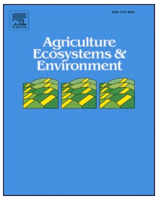
Originally published in:
Agriculture, Ecosystems & Environment 367, https://doi.org/10.1016/j.agee.2024.108970

Agriculture, Ecosystems and Environment 367 (2024) 108970

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee



Optimizing nitrogen use efficiency of six forage grasses to reduce nitrogen loss from intensification of tropical pastures

Mike Bastidas [a,b,c], Eduardo Vázquez [b], Daniel M. Villegas [a], Idupulapati M. Rao [a], Jhon F. Gutierrez [a], Nelson J. Vivas-Quila [c], Miguel Amado [d], Carlos Berdugo [d], Jacobo Arango [a,*]

[a] *International Center for Tropical Agriculture (CIAT), Cali, Colombia*
[b] *Departamento de Producción Agraria, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas (ETSIAAB), Universidad Politécnica de Madrid (UPM), Madrid, Spain*
[c] *Universidad del Cauca, Popayán, Colombia*
[d] *YARA, Barranquilla, Colombia*

ARTICLE INFO

Keywords:
N application
forage production
N_2O emissions
tropical livestock systems

ABSTRACT

We aimed to evaluate the effect of different types and rates of nitrogen (N) fertilizers on plant biomass production, nitrogen use efficiency (NUE), and nitrous oxide (N_2O) emissions of six tropical forage grass cultivars. This field study was conducted in Colombia under tropical conditions in two phases. Phase 1: we evaluated the effect of different types of N fertilizers (urea; calcium ammonium nitrate (CAN); and urea-ammonium sulfate (U-AS) using a single dose of application of 25 kg N ha^{-1} cut^{-1}, along with a control treatment for each grass without application of fertilizer) on forage dry matter, crude protein, N uptake, N surplus, and NUE. The study focused on six tropical forage grass cultivars: *Urochloa humidicola* cv. Tully, *U.* hybrid cv. Cobra, *U.* hybrid cv. Cayman, *Megathyrsus maximus* cv. Mombasa, *Cynodon nlemfuensis* cv. Stargrass and *U. decumbens* cv. Basilisk. We found that the biomass production and protein content of each forage grass cultivar responded differently to different N fertilizer types. In consequence, the NUE of each forage grass cultivar was strongly affected by the type of N fertilizer applied. For example, Cayman showed a NUE of 87.8% with CAN and 40.4% with urea, while the NUE of Basilisk with CAN was 8.0% and 76.4% with urea. Maximum NUE values were obtained for Stargrass and Cayman with CAN (91.7 and 87.8%, respectively) and for Mombasa with urea (89.9%). Phase 2: we selected three combinations of forage grass cultivars and type of N fertilizer, and we evaluated the impact of four rates of N application (0, 10, 20, and 30 kg N ha^{-1} cut^{-1}) on forage production and N_2O emissions upon fertilization. In general, higher N fertilization rates increased the accumulated N_2O emissions. However, we observed that the fertilization of Stargrass and Cayman grass with a rate of 20 kg N^{-1} cut^{-1} and Mombasa grass with a rate of 30 kg N^{-1} cut^{-1} were more beneficial than the other rates of N fertilization based on the biomass production and amount of N_2O emitted are considered. This study highlights the importance of optimizing NUE in tropical pasture systems using an appropriate design of N fertilization strategy. Inappropriate N fertilizer use can significantly increase the N losses (e.g., through N_2O emissions, with a potential contribution from N leaching). These findings provide valuable information for sustainable intensification of productive tropical livestock systems to spare land for other uses.

1. Introduction

Livestock production has great economic, social, and environmental importance in contributing to food security and it accounts for 20–24% of agricultural gross domestic product (GDP) in developed and developing countries (ILRI, 2019). In some Latin American countries, livestock production generates between 21.8% and 46% of agricultural GDP, indicating the particular importance of the sector in the region (Arango et al., 2020). However, livestock production in Latin America is characterized by a low stocking rate due to the use of degraded pastures and soils, low productivity, inefficient use of natural resources, inadequate access to new technologies, and poorly trained human capital (Figueroa et al., 2022). For example, Colombia has an approximate livestock inventory of 28 million heads of cattle and 39 million hectares

---

* Corresponding author.
E-mail address: j.arango@cgiar.org (J. Arango).

https://doi.org/10.1016/j.agee.2024.108970
Received 5 December 2023; Received in revised form 16 February 2024; Accepted 7 March 2024

(78% of agricultural land use) are used for livestock production (ENA, 2019; ICA, 2020). This translates into a low stocking rate of 0.7 animal units per hectare and hence a lower land use efficiency when contrasted with well-managed and fertilized pasture systems that can achieve stocking rates of up to 3.8 animals per hectare (Rincón et al., 2018). The consequence of this low stocking rate is a continuous increase of land area dedicated to grazed pastures by deforestation or land use change to meet the growing demand for livestock products during the past few decades (Lerner et al., 2017). This land use change to increase the grazed area contributes up to 40% from the livestock sector to the total greenhouse gas (GHG) emissions in Latin America (Gerber et al., 2014). However, the negative environmental and social impacts of deforestation and land use change over the past few decades, together with increasing land scarcity, land price and land degradation have moved the livestock sector towards a need for sustainable intensification (Gerber et al., 2014; Herrero et al., 2016; Notenbaert et al., 2021; Rao et al., 2015). Sustainable intensification of pastures in the tropics may help to protect tropical forests and natural ecosystems by reducing the continuous need to increase the pasturelands and sparing land for nature or other uses (Matson and Vitousek, 2006; Strassburg et al., 2014; White et al., 2001). Therefore, research in the livestock sector should focus on generating strategies for large-scale sustainable intensification of production systems that are more environmentally friendly, resource-use efficient, and economically and ecologically sustainable than the inefficient current livestock and pasture management practices (Gerber et al., 2014; Matson and Vitousek, 2006; Notenbaert et al., 2021; zu Ermgassen et al., 2018).

In contrast to temperate regions where the use of nitrogen (N) fertilizers in grasslands is common (Velthof et al., 2009), the lack of an adequate and efficient use of N fertilizers in tropical pastures is one of the main constraints of livestock farming in Latin America (Barbieri et al., 2021; Delevatti et al., 2019; Gerber et al., 2014). The low N availability in tropical pasture soils leads to low productivity of forage biomass, lower stocking rates and eventual pasture degradation (Barbieri et al., 2021; Delevatti et al., 2019; Gerber et al., 2014). In this context, sustainable use of N fertilizer in tropical pastures of Latin America could contribute towards intensifying pasture productivity while reducing the total area dedicated to grazed pastures (Delevatti et al., 2019). This could improve the profitability of small and medium-size farms and contribute to improving the rural livelihoods in the region. However, the use of N fertilizer must be correctly designed and optimized to avoid the negative environmental impacts of excessive N use that has been observed in developed regions of the World (Herrero et al., 2016; Smerald et al., 2023; Velthof et al., 2009).

Various studies have described a strong positive response of tropical forage grasses to N addition in Latin America, particularly in Brazil (Delevatti et al., 2019; dos Santos et al., 2023; Euclides et al., 2022). However, except for only a few studies performed in Brazil (Dupas et al., 2016; Galindo et al., 2017), there is limited information about how to optimize the use of these N fertilizers to minimize the potential negative environmental impacts associated with their use. This situation contrasts with temperate regions where many studies have been conducted to evaluate different types and doses of N fertilizer to maximize the N use efficiency (NUE) and minimize the nitrous oxide (N_2O) emissions from soil (Cardenas et al., 2019; Dobbie and Smith, 2003; Harty et al., 2016; Velthof et al., 1996).

During the past three decades, the use of improved tropical forage grasses of the *Urochloa* and *Megathyrsus* genera has improved the productivity and quality of pasture systems (mainly in South America) due to their adaptability to different edaphoclimatic conditions and their pest resistance (Labarta et al., 2017). In Colombia, approximately 44% (10.4 million hectares) of the pasture and forage area is planted with the genera *Urochloa, Megathyrsus,* and *Cynodon* including different cultivars of each species (DANE, 2019). But how these common forage grasses respond to different types of N fertilizers is poorly understood despite the large area planted across Latin America (approximately 135 million ha in forage crops) (Fuglie et al., 2021). To our knowledge, no studies have compared the impact on NUE and N_2O emissions from the use of different N sources and rates by tropical forage grass cultivars. Previous studies in temperate regions demonstrated large differences in the NUE and N_2O emissions depending on the type and dose of N fertilizer used (Bell et al., 2016; Cardenas et al., 2019; Dobbie and Smith, 2003; Harty et al., 2016; Hinton et al., 2015). Thus, it is possible that such significant differences in NUE and N_2O emissions can be observed in tropical forage grasses, with consequences for animal productivity and the environment. We hypothesize that the specific interaction between tropical forage grass species and the form of N fertilizer applied will lead to distinct outcomes in NUE and N_2O emissions, underscoring the need for tailored N management strategies to optimize environmental and productivity outcomes from tropical pastures.

As an agronomic indicator of N utilization by crops, NUE has been proposed as one of the important indicators to assess progress in achieving the sustainable development goals (SDSN, 2014). Higher or lower NUE has implications on plant productivity, production-associated costs, and environmental pollution. In addition, other metrics such as N_2O emission intensity (i.e., the N_2O emission per unit of biomass produced), the emission factors (EF) (i.e., the quantity of N_2O-N emitted as a proportion of the N applied) or the crude protein (CP) concentration in plant biomass (reflecting the amount of N that could be consumed by animals) can help to design more sustainable N fertilization strategies (Cardenas et al., 2019; Hinton et al., 2015; Van Groenigen et al., 2010). Evaluating the response of these parameters to the addition of different N fertilizer types and doses to the improved tropical forage grasses can help to optimize the N fertilization strategies in Latin America. This strategy could help to improve the GHG inventories for the region while allowing to postulate more accurate mitigation goals.

This study aimed to optimize the use of N fertilizers in tropical forage grasses with a focus on global warming mitigation by evaluating the effect of different N fertilizer types and rates on the plant biomass production, NUE, and N_2O losses of six forage grasses that are being used by farmers in tropical areas of Latin America. More specifically, to compare the effect on plant biomass production, CP concentration, the calculated NUE and N uptake of three different sources of N (urea, calcium ammonium nitrate, and urea-ammonium sulfate) in six tropical forage grasses. In addition, the peak of N_2O emissions upon fertilization was evaluated for different N fertilization rates in three selected combinations of grass and fertilizer type. The optimization of the NUE for improving the agronomic efficiency of applied N fertilizers to six tropical forage grasses will contribute toward the sustainable intensification of tropical pastures in Latin America and for releasing land for other uses.

2. Material and methods

2.1. Experimental design

This study was conducted from 2018 to 2020 in Santander de Quilichao municipality (Department of Cauca, Colombia) at 1005 m above sea level (masl), at a location with a mean annual temperature of 28°C, under a bimodal rainfall distribution with tropical climate with an annual rainfall of 1992 mm, and a dry season between June and August. The soil is classified as an Inceptisol with a clay-loam texture with mean values of pH 4.93, 74.6 g kg^{-1} soil organic matter (SOM) content, 7.82 mg kg^{-1} of available phosphorus (P), 0.98 cmol kg^{-1} of exchangeable aluminum, 2.93 cmol kg^{-1} of exchangeable calcium, 1.4 cmol kg^{-1} of exchangeable magnesium, and 0.52 cmol kg^{-1} of exchangeable potassium in the 0–20 cm soil depth. Before the start of the experiment, an initial concentration of 25.03 mg N-nitrate (NO_3^-) kg soil^{-1} and 19.4 mg N-ammonium (NH_4^+) kg soil^{-1} was found, with a total available inorganic N content of 90.8 kg ha^{-1} (51.6 kg N-NO_3^- ha^{-1} and 39.2 kg N-NH_4^+ ha^{-1}). The soil was prepared with one pass of the plow and one pass of the subsoiler. In addition, 600 kg ha^{-1} of phosphate rock (calfos) was applied before sowing the forages and it was incorporated with two rake passes.

The study consisted of two phases, starting with the selection of the N fertilizer type (phase 1) for each forage grass and then, determining the appropriate doses of the selected N fertilizer type (phase 2). This strategy was adopted for more efficient resource management due to logistical considerations, as implementing all doses and fertilizer types simultaneously would imply a much larger experimental design for implementation with several operational challenges in the study area.

2.2. Experimental design of phase 1

The field trial was established under a split-plot design with three blocks (replications) and considering the type of N fertilizer as the main factor and the different forage grass cultivars as a second factor nested within the type of N fertilizer (Fig. 1A). Within each block, four larger plots (19 ×12 m) were established corresponding to the three types of N fertilizers evaluated (urea; calcium ammonium nitrate (CAN); and urea-ammonium sulfate (U-AS)) and a control treatment without application of fertilizer. The difference between CAN (N-P-K-Mg-Ca: 21–0–0–7.5–11) and U-AS (N-P-K-S: 40–0–0–6) is in their nutrient composition which we hypothesized could differentially influence NUE and N_2O emissions of tropical forage grasses. Within each type of N fertilizer plot, six smaller plots (5 × 5 m) were nested corresponding to the different forage grasses evaluated. The six forage grasses evaluated were *Urochloa humidicola* CIAT 679 (cv. Tully), *Urochloa* hybrid BR02/1794 (cv. Cobra), *Urochloa* hybrid BR02/1752 (cv. Cayman), *Urochloa decumbens* CIAT 606 (cv. Basilisk), *Megathyrsus maximus* CIAT 6962 (cv. Mombasa) and *Cynodon nlemfuensis* (cv. Stargrass). The main characteristics of the six different forage grasses evaluated are summarized in Table S1.

In total, 24 treatments were evaluated (4 N fertilization treatments x 6 forage grass cultivars), encompassing all feasible combinations between the levels of the aforementioned factors (Fig. 1A). The experiment comprised of 72 plots, each with the size of 25 m^2, with a 2-meter separation between plots within the same block, and 5 m between blocks to mitigate lateral movement of N applied to individual plots. When utilized, N fertilization rates used normally in livestock systems are high, exceeding 50 kg N ha^{-1} per grazing cycle (Fig. 1A). In phase 1 we used an application rate of 25 kg N ha^{-1} cut^{-1} which is on the lower side of the fertilizer N rates used in temperate regions (Cardenas et al., 2019; Gu et al., 2023) and also reported in other studies performed in the tropics (Dupas et al., 2016; Galindo et al., 2017). The purpose was to evaluate the different types of N fertilizers aiming to reduce the environmental impact of N fertilization and use a dose that potentially can be afforded economically by local farmers.

To simulate the effects of cattle grazing, the aerial plant biomass was cut and removed from the plot at a height of 15 cm for grasses Tully and Stargrass (short stoloniferous growing grasses), and at a height of 30 cm for grasses Mombasa, Cayman, Cobra, and Basilisk (tall grasses with tillering growth). Following the standardized mowing, plant material was removed from the plots to facilitate optimal forage growth and mimic the effect of biomass removal by cattle grazing. Three days following the standardization cut, N fertilizer was applied to a central 4 m x 4 m area within each 5 m x 5 m plot, situated at 0.5 m from the edges. The first forage dry matter (DM) production evaluation was done after the forage grasses had fully responded to the first N fertilization (approximately after 28 days). Three days later, the next N fertilization event and evaluation cycle started. This cutting frequency of 28–30 days was selected following the common grazing management practices by farmers in the region. Following this cutting frequency, the total application of N per year is estimated as 300 kg N ha^{-1} yr^{-1}. There was no

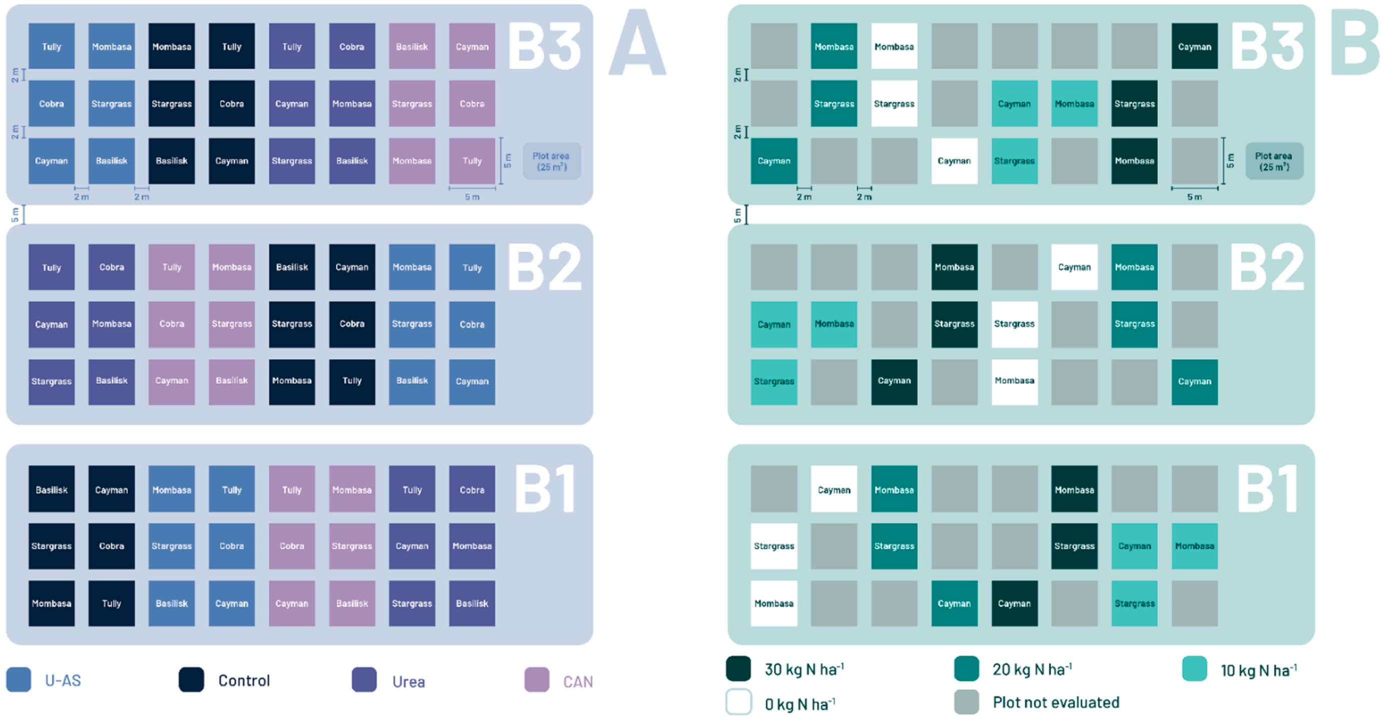


Fig. 1. Experimental design used for phase 1 of research (A): Response of six forage grasses (*Urochloa humidicola* CIAT 679 (cv. Tully), *Urochloa hybrid* BR02/1794 (cv. Cobra), *Urochloa* hybrid BR02/1752 (cv. Cayman), *Urochloa decumbens* CIAT 606 (cv. Basilisk), *Megathyrsus maximus* CIAT 6962 (cv. Mombasa) and *Cynodon nlemfuensis* (cv. Stargrass)) to different types of N fertilizer types (Urea; CAN: calcium ammonium nitrate; U-AS: urea-ammonium sulfate). Experimental design used for phase 2 of research (B): Response of forage grasses to different N rates (0, 10, 20, and 30 kg N ha^{-1}) of the selected N fertilizer type for each forage grass (Cayman and Stargrass CAN, Mombasa Urea). The same trial used in phase 1 was used again in phase 2 for evaluation. Before the establishment of this phase, four cycles of pasture growth and cutting regime without the addition of N fertilizers were performed (for a total of 120 days) aiming to avoid a potential residual effect of the fertilization from the preceding phase 1. Further details about the plot establishment and management are described in the text.

need to use pesticides to control insects during the experiment.

2.3. Experimental Design of Phase 2

After analyzing the results of the initial phase, the peak value of N_2O emissions upon fertilization was evaluated for different N fertilization rates in three selected combinations of forage grass and type of N fertilizers. We selected the forage grass and N fertilization type combinations that showed values of NUE higher than 85% (Cayman - CAN, Stargrass - CAN, and Mombasa - Urea) according to the results obtained in phase 1. This level of NUE is close to the NUE ≈ 90% described in the literature as the maximum for experimental non-grazed grassland plots (Balasubramanian et al., 2004), and it is indicative of an efficient utilization of the applied N with low N losses. Therefore, in phase 2 we evaluated Cayman – CAN, Stargrass – CAN and Mombasa - Urea at four N rates (0, 10, 20 and 30 kg N ha^{-1}) aiming to find the most adequate rate of N for each cultivar under the experimental setup used. The phase 2 activities were performed using the same field experiment (same soil and established forages) in which the four N sources were established, resulting in a total of 12 treatments and 36 plots (cultivar and fertilization rates were the two factors) (Fig. 1B). Before the establishment of this phase, four cycles of pasture growth and cutting regime without the addition of N fertilizers were performed (for a total of 120 days) aiming to avoid a potential residual effect of the fertilization that was conducted during the preceding phase 1. For the same reason, the control treatments were established over the control plots of the previous phase. N_2O measurements were also performed on the chosen treatments to evaluate the impact of these fertilization events on the atmosphere. The management of the plots in terms of biomass cutting and removal was the same as described above for phase 1.

2.4. Forage sampling and analysis

2.4.1. Forage dry matter production (DM)

In phase 1, two evaluations of forage dry matter (DM) production were carried out in the rainy season and two in the dry season following the protocol for agronomic evaluation of the International Network for Tropical Pastures Evaluation (RIEPT) (Toledo, 1982). The environmental conditions of the field site are shown in Fig. 2A. Briefly, after 120 days of sowing the forages, a standardization cut for all plots was made and the first forage DM production evaluation was done after the forage grasses had fully recovered since the standardization cut (approximately 28 days). To evaluate forage grass DM production, the plants were cut at the same height as previously described by gauging with a 0.25 m^2 frame, the total biomass of leaves and stems (with a diameter of less than 5 mm) were taken and weighed as available forage. Of the total green biomass, subsamples were weighed from each experimental plot (approximately 200 g) and oven-dried at 60°C under controlled ventilation for 72 hours. The final dry weight was used to estimate forage grass DM production. The average values of the four evaluations are presented in this study for phase 1.

In phase 2, the same protocol was used to evaluate the forage grass DM production although unfortunately due to the restrictions of the COVID-19 pandemic, a single evaluation was conducted to quantify forage grass DM productivity. The climatic conditions during this pasture recovery cycle are presented in Fig. 2B.

2.4.2. Crude protein concentration, nitrogen uptake, and nitrogen use efficiency

In phase 1, a subsample (200 g) of the forage grass biomass samples of each treatment was processed and analyzed by the Animal Nutrition Laboratory of the Tropical Forages Program of CIAT, according to ISO 12099:2017 (Mazabel et al., 2020). Crude protein and total N concentrations were measured using a FOSS Kjeltec ™ 8100 (Foss Company, Hillerøed, Denmark) according to the guidelines of the Association of Official Analytical Chemists AOAC, Method 2001.11 (AOAC International, 2002).

$$\text{(1)}\quad \textit{Crude protein}(\%) = \textit{N }(\%)\ *\ 6.25$$

N is the nitrogen concentration in plant tissue

Total N uptake by each of the forage grass cultivars under different N treatments was estimated as the product of forage DM production (kg ha^{-1}) and N concentration in plant tissues (Baptistella et al., 2020):

$$\text{(2)}\quad \textit{kg N uptake}(\textit{kg N ha}^{-1}) = \frac{DM(kg\ ha^{-1})*N(\%)}{100}$$

DM is the forage grass dry matter production (kg DM ha^{-1})

The NUE was estimated as the apparent N recovery fraction. This was calculated as the difference in N uptake in the fertilized treatment and the control of each plot, divided by the N application rate. It was expressed as the percentage of the N applied using the following the formula:

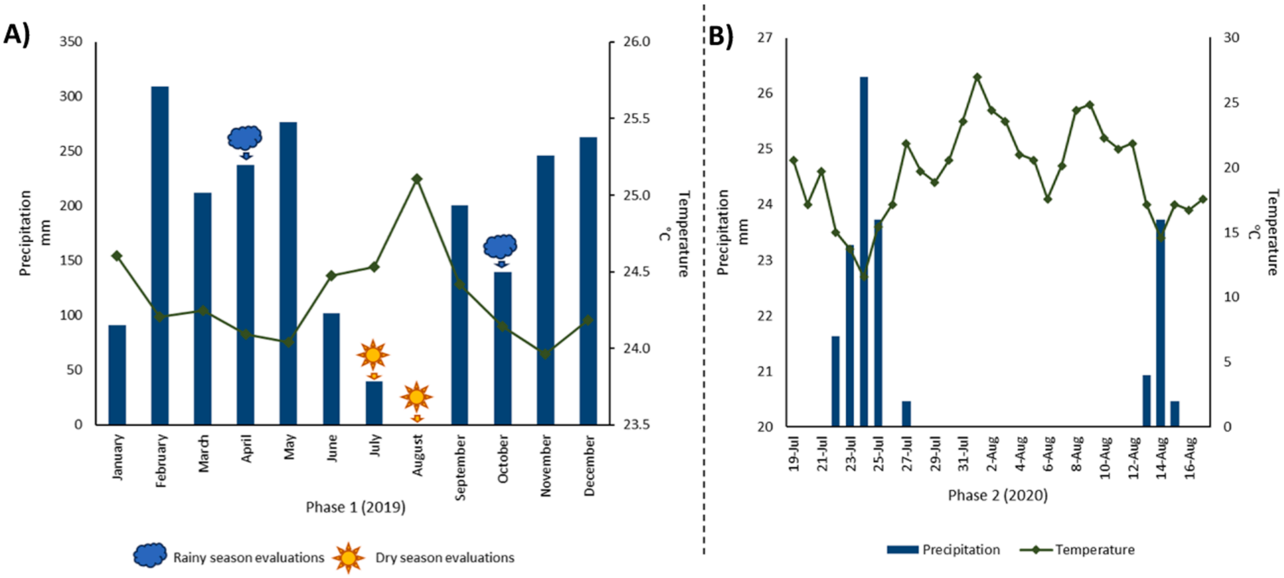


Fig. 2. Monthly precipitation and mean temperature during phase 1 of the experimental site of the study (A) including two evaluations during the rainy season and two during the dry season, and daily record of precipitation and ambient temperature during phase 2 of the study (B).

$$(3)\ NUE(\%) = \frac{kg\ NTi - kg\ NT0}{DN} \times 100$$

Where:

NT_i is the N uptake in the fertilized treatment (kg ha^{-1}).

NT_0 is the N uptake in unfertilized (control) treatment (kg ha^{-1}).

DN is the N rate of fertilized treatment (kg ha^{-1}).

Finally, the N surplus, a potential proxy for the potential N loss to the environment, was calculated as the amount of N added as a fertilizer that was not taken up by the crop following the formula:

$$(4)\ N\ surplus(kg\ N\ ha^{-1}cut^{-1}) = \frac{(100 - NUE(\%))x25(kg\ N\ ha^{-1}cut^{-1})}{100}$$

Where 25 kg N ha^{-1} cut^{-1} is the N fertilization rate

The NUE, N uptake and crude protein concentration were evaluated at each forage grass DM production evaluation performed and the average values from the four evaluations are presented from this study for phase 1.

2.5. Nitrous oxide emissions

During phase 2 of the study, N_2O emission peak upon N fertilization was also measured using the static chamber method for nine days. In each plot, a static chamber was placed (36 chambers in total). The chambers consisted of a PVC cylinder of 26 cm in diameter and 10 cm high, buried 5 cm in the ground, with a lid of the same dimensions that closed the gasket with a rubber band to prevent leakage of N. The lid had two plastic slack controls to prevent leakage to which the Gasmet DX4040 portable FTIR Gasmet DX4040 was connected for 10 min to determine the concentration of N_2O in the gas samples every 20 s (Costa et al., 2022; Teutscherova et al., 2019; Villegas et al., 2020). Measurements were conducted over nine days, starting from the day before N fertilizer application and then daily until day 8, when N_2O emissions had stabilized back to their starting point. The duration of N_2O measurements over a nine-day period allowed monitoring of peak emissions, and cumulative emissions over the monitoring period of 9 days which in this study was essential to discern differences between treatments. After this nine-day interval, N_2O emissions stabilized around the initial values prior to fertilization.

Soil N_2O fluxes were calculated using the following formula:

$$(5)\ N_2O\ flux(\mu g\ N - N_2O) = \frac{dN_2O}{dt} \times \frac{V \times M}{A \times Vm}$$

Where:

dN_2O/dt is the accumulation rate of N_2O in the air inside the chamber

V is the volume of chamber headspace + analyzer tubing and sample cell

M is the mass of N per mol of N_2O

A is the area of the soil surface covered by the chamber

Vm is the molecular volume for N_2O

The daily emissions of N_2O were calculated by assuming a constant efflux over 24 hours. Linear interpolation of gas concentration was used to calculate the accumulated emissions of N_2O between two time points (t) using formula (5). Finally, the cumulative emissions for the entire 8-day sampling period were calculated by adding up the emissions from each time interval. This approach has been previously used in studies by Shen et al. (2018), (2019).

$$(6)\ Cummulative\ flux(\mu g\ N - N_2O) = \frac{(flux1 + flux2)}{2} \times (t2 - t1)$$

The equation used to calculate the N_2O-N emission factor (EF) for treatments that underwent N_2O measurements is as follows:

$$(7)\ EF(\%) = \frac{(N_2O - N\ \text{emitted from fertilized treatment}) - (N_2O - N\ \text{control treatment})}{N\ \text{applied}} \times 100$$

2.6. Statistical analysis

Since the main aim of the present study was to optimize the N fertilizer type and N fertilization rate for each of the six evaluated forage grasses instead of identifying general trends across all the forage grasses, we evaluated separately the results obtained for each forage grass. Therefore, statistical analyses were performed separately for each forage grass comparing either N fertilizer type (phase 1) or N fertilization rate (phase 2). ANOVA were performed using N fertilizer type (phase 1) or N fertilization rates (phase 2) as fixed factors. When a significant treatment effect was found ($p < 0.05$), the Tukey post hoc test ($p < 0.05$) was used to compare mean values of different N fertilizer treatments or N fertilization rates means. Statistical analyses were performed using R v3.4.4, and the figures were constructed using "ggplot2" v2.2.1.

3. Results

3.1. Phase 1: Effects of N source on forage dry matter production, crude protein concentration, nitrogen uptake and NUE

After four evaluation cycles, differential behavior for different N sources was observed among the six forage grasses evaluated as revealed by improved forage DM production, crude protein concentration, N uptake and NUE (Table 1). Among the six grasses, four responded positively to N fertilization, one responded negatively, and one showed no significant effect. Apparently, the N fertilization had a more pronounced effect on the crude protein concentration than on the overall DM production. Therefore, the results thereafter are described independently for each forage grass.

No significant effect in forage DM production between N fertilizer application treatments was observed in Cayman after four evaluation cycles, although the DM production was increased by 19% in CAN compared to control reaching a biomass production of 2.47 t DM ha^{-1} cut^{-1} (Table 1). However, a strong effect of N fertilizer type was observed for crude protein concentration and total N uptake in Cayman, with the three types of N fertilizers increasing significantly in both parameters compared to control ($p<0.05$) and CAN showing the highest values (Table 1). The NUE of CAN (87.8%) treatment was significantly higher ($p<0.05$) than with U-AS (34.6%) and Urea (40.4%) for Cayman (Table 1). Finally, the N surplus ranged between 3.1 and 16.4 kg N ha^{-1} cut^{-1} in CAN and urea, respectively (Fig. 3).

Forage DM production of Cobra grass showed a significant negative effect upon Urea and U-AS application compared to control (2.61 t DM ha^{-1} cut^{-1}) while no significant effect of CAN application was observed after the four evaluation cycles (Table 1). In contrast, the crude protein concentration in Cobra was significantly increased ($p<0.05$) by the application of the three N fertilizers while the urea treatment resulted in the highest value (11.8%) (Table 1). No significant differences between control and the three types of N fertilizers were observed in the total N uptake (Table 1). Cobra showed the lowest NUE value among the forage grasses evaluated and no significant differences among N fertilizer types were observed, although urea (21.3%) and CAN (20.1) promoted NUE value by four times higher than U-AS (4.8%) (Table 1). In consequence, the N surplus ranged between 19.7 and 23.8 kg N ha^{-1} cut^{-1} with urea and UAS, respectively (Fig. 3).

The application of urea to Basilisk grass increased its DM productivity by 74% ($p < 0.05$) compared to control reaching a value of 2.33 t DM ha^{-1} cut^{-1} (Table 1). Both urea and U-AS increased significantly ($p<0.05$) the crude protein concentration and the total N uptake compared to control and CAN treatments (Table 1). The highest NUE values for Basilisk were achieved when fertilized with urea (76.4%) compared to the other two N fertilizer types (8.0% and 42.5% for CAN and U-AS, respectively). Therefore, the N surplus was 5.9, 14.4 and 23.0 kg N ha^{-1} cut^{-1} with urea, U-AS, and CAN, respectively (Fig. 3).

In the case of the Tully grass, the addition of U-AS significantly increased the forage DM production by 51% compared to the control,

Table 1
Forage dry matter production, crude protein, total N uptake, and NUE of six tropical forage grasses in response to the application of different types of N fertilizer at the experimental field located in Santander de Quilichao County (Department of Cauca, Colombia). The data presented are the average of four evaluation cycles. Control: non-fertilized forages; CAN: calcium ammonium nitrate; Urea; U-AS: urea-ammonium sulfate. Values in parentheses represent the standard deviation of the mean, $n = 3$. Different letters indicate statistical differences according to the Tukey HSD test ($\alpha = 0.05$).

Forage cultivar		N fertilizer	Forage dry matter production (ton DM ha^{-1} cut^{-1})	Crude Protein concentration (%)	Total N uptake (kg N ha^{-1} cut^{-1})	NUE (%)
Cayman		Control	2.08 (0.17)^{a}	8.3 (0.2)^{d}	27.8 (2.8)^{c}	-
		CAN	2.47 (0.06)^{a}	12.1 (0.32)^{a}	47.9 (0.2)^{a}	87.8 (7.1)^{a}
		Urea	2.14 (0.08)^{a}	11.1 (0.2)^{b}	37.9 (1.6)^{b}	40.4 (8.2)^{b}
		U-AS	2.28 (0.28)^{a}	10.0 (0.1)^{c}	36.4 (4.8)^{b}	34.6 (22.2)^{b}
	P value		0.106	0.000	0.000	0.011
Cobra		Control	2.61 (0.23)^{a}	8.4 (0.3)^{c}	35.1 (3.2)^{a}	-
		CAN	2.24 (0.25)^{ab}	10.6 (0.4)^{b}	38.1 (3.2)^{a}	20.1 (11.9)^{a}
		Urea	2.15 (0.11)^{b}	11.8 (0.4)^{a}	40.5 (3.3)^{a}	21.3 (14.7)^{a}
		U-AS	2.12 (0.32)^{b}	10.7 (0.0)^{b}	36.3 (5.4)^{a}	4.8 (10.4)^{a}
	P value		0.018	0.000	0.118	0.285
Basilisk		Control	1.34 (0.04)^{c}	8.7 (0.0)^{b}	18.6 (0.5)^{c}	-
		CAN	1.47 (0.01)^{bc}	8.6 (0.5)^{b}	20.4 (1.4)^{c}	8.0 (7.7)^{b}
		Urea	2.33 (0.25)^{a}	10.1 (0.2)^{a}	37.7 (5.0)^{a}	76.4 (18.3)^{a}
		U-AS	1.75 (0.06)^{b}	10.4 (0.2)^{a}	29.2 (1.7)^{b}	42.5 (5.5)^{ab}
	P value		0.000	0.001	0.000	0.001
Tully		Control	1.44 (0.10)^{b}	9.9 (0.6)^{c}	22.7 (0.4)^{c}	-
		CAN	1.57 (0.21)^{b}	12.7 (0.7)^{a}	31.9 (4.5)^{b}	37.8 (18.3)^{b}
		Urea	1.67 (0.03)^{b}	11.2 (0.3)^{bc}	29.8 (0.3)^{b}	28.4 (1.8)^{b}
		U-AS	2.18 (0.13)^{a}	11.4 (0.3)^{ab}	39.6 (1.6)^{a}	67.6 (5.0)^{a}
	P value		0.001	0.001	0.000	0.016
Mombasa		Control	2.11 (0.12)^{c}	9.6 (0.1)^{c}	32.3 (2.1)^{c}	-
		CAN	2.81 (0.11)^{b}	11.7 (0.7)^{ab}	52.5 (3.0)^{a}	81.2 (16.5)^{a}
		Urea	3.04 (0.10)^{a}	11.3 (0.6)^{b}	54.8 (3.8)^{a}	89.9 (7.2)^{a}
		U-AS	2.17 (0.01)^{c}	12.9 (0.3)^{a}	44.7 (0.72)^{b}	49.7 (7.2)^{b}
	P value		0.000	0.000	0.000	0.019
Stargrass		Control	1.45 (0.06)^{c}	12.8 (0.2)^{c}	29.7 (1.6)^{b}	-
		CAN	2.28 (0.30)^{a}	14.4 (0.2)^{b}	52.7 (7.4)^{a}	91.7 (22.9)^{a}
		Urea	2.09 (0.30)^{ab}	11.9 (0.3)^{d}	40.1 (5.6)^{ab}	41.5 (26.5)^{a}
		U-AS	1.58 (0.06)^{bc}	15.3 (0.3)^{a}	38.7 (2.3)^{ab}	36.2 (5.6)^{a}
	P value		0.011	0.000	0.001	0.082

reaching a production of 2.18 t DM ha^{-1} cut^{-1} while no effect of CAN or Urea compared to control was observed (Table 1). For crude protein concentration, CAN showed the highest values (12.7%), being 28% higher than the crude protein concentration in the control (Table 1). In addition, total N uptake was 74%, 41%, and 31% higher with U-AS, CAN, and urea, respectively than that of the control treatment (Table 1). In terms of NUE, we observed that U-AS (67.6%) had significantly higher efficiency compared to CAN (37.8%) and urea (28.4%) with the cultivar Tully. The lowest N surplus in Tully was obtained with U-AS fertilizer (8.1 kg N ha^{-1} cut^{-1}) (Fig. 3).

The addition of the three types of N fertilizers increased the forage DM production compared to control of Mombasa (Table 1). After the four cycles of evaluation, the forage DM production with urea reached the highest value of 3.04 t DM ha^{-1} cut^{-1}, representing an increase of 44.8% compared to the control (Table 1). Similarly, U-AS, CAN, and urea increased the crude protein concentration compared to the control by 34%, 22% and 18%, respectively (Table 1). The total N uptake was increased by 38%, 63% and 70% compared to control by the addition of urea, CAN, and U-AS, respectively (Table 1). In terms of NUE, the urea and CAN treatments had the highest values with 89.9% and 81.2%, respectively, while the U-AS treatment was 49.7% (Table 1). Therefore, the N surplus when Mombasa was fertilized with CAN and urea was very low (4.7 and 2.5 kg N ha^{-1} cut^{-1}) (Fig. 3).

In Stargrass, the forage DM production was 57.9% higher with the application of CAN than with the control treatment ($p < 0.05$), showing production of 2.28 t DM ha^{-1} cut^{-1} after the four cycles of evaluation (Table 1). The crude protein concentration was increased with CAN and U-AS and decreased with urea compared to the control treatment ($p<0.05$). The highest total N uptake occurred with the CAN treatment with the value being 77.3% higher than the control, with N uptake rates of 52.7 kg N ha^{-1} cut^{-1} and 29.7 kg N ha^{-1} cut^{-1}, respectively ($p<0.05$). The NUE in Stargrass was 91.7% with CAN, 41.5% with urea, and 36.2% with U-AS, although the differences among treatments were not statistically significant (Table 1). The lowest N surplus value was observed with CAN (2.1 kg N ha^{-1} cut^{-1}) (Fig. 3).

Fig. 3 shows an inverse relationship between N surplus and N uptake of the treatments evaluated (Forage cultivar and N fertilizer combinations). Treatments located close to or inside the shaded area of the graph (Mombasa-Urea, Cayman-CAN, and Stargrass-CAN) present a higher N uptake, a higher NUE and a reduced N surplus. In contrast, treatments located above the shaded area show higher N surplus, are generally associated with lower N uptake, lower NUE, and therefore a higher probability of N loss to the environment. The dispersion of the points in the figure indicates that there is significant variability in pasture response to different fertilizer sources.

3.2. Phase 2: Effects of N fertilization rate on forage dry matter production

After analyzing the results of phase 1, the forage grass and type of N fertilizer combinations that showed NUE values higher than 85% (Cayman - CAN, Stargrass - CAN, and Mombasa - Urea) were selected for evaluation in phase 2.

In Cayman grass, we observed an increase in forage DM production with N addition, although only the rate of 20 kg N ha^{-1} increased the forage DM production significantly compared to the control ($p < 0.05$), reaching a maximum value of 3.86 ton DM ha^{-1} cut^{-1} (Table 2).

Forage DM production of Stargrass did not show significant differences with the different doses of N applied ($p > 0.05$), although an absolute maximum value of 2.39 ton DM ha^{-1} cut^{-1} was recorded with the

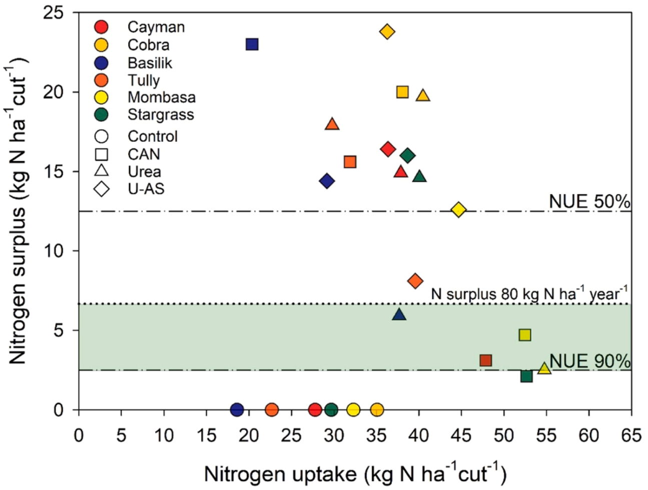


Fig. 3. Relationship between nitrogen uptake and nitrogen surplus for the six tropical forage grasses evaluated in response to the application of different types of nitrogen fertilizer at the experimental field located in Santander de Quilichao County (Department of Cauca, Colombia). The data presented are the average values from four cycles of evaluation. The dash-dotted lines indicate possible target values for NUE (50 and 90%), and the dotted line indicates the maximum surplus per cut equivalent to an annual surplus of 80 kg N ha^{-1} year^{-1} (6.67 kg N ha^{-1} cut^{-1} considering 12 cutting events per year). The shaded area represents the desirable range for NUE. Control: non-fertilized forage grasses; CAN: calcium ammonium nitrate; Urea; U-AS: urea-ammonium sulfate.

application of 20 kg N ha^{-1} rate (Table 2).

In Mombasa grass, significant differences were observed in forage DM production, with 30 kg N ha^{-1} dose compared to the control ($p < 0.05$) (Table 2). This rate of 30 kg N ha^{-1} showed a forage DM production of 3.68 ton DM ha^{-1} cut^{-1} which was two times the value obtained with the control treatment.

3.3. Phase 2: Effects of N fertilization rate on N_2O emissions

Fluxes of N_2O emission from Cayman, Stargrass, and Mombasa grass with the 0 kg N ha^{-1} dose were close to zero, during the evaluation period, while the N_2O emissions were gradually increasing for the different treatments with the application of increasing N fertilizer rates (Fig. 4, Table 2). We observed with Cayman and Stargrass grasses that the highest peaks in N_2O emissions were on the second day after N fertilizer application, and then gradually decreasing until reaching stable values after the seventh day of measurement (Fig. 4a, b). However, with Mombasa grass the N_2O emission peak was slightly delayed reaching the peak value at the third day after N fertilization and the shape of the peak was less prominent compared to Cayman and Stargrass (Fig. 4c).

Table 2
Forage dry matter production, N_2O cumulative emissions, and N_2O emission factor of six tropical forage grasses in response to the application of different nitrogen fertilizer rates at the experimental field located in Santander de Quilichao County (Department of Cauca, Colombia). Values in parentheses represent the standard deviation of the mean, n = 3. Different letters indicate statistical differences according to the Tukey HSD test (α = 0.05).

Forage cultivar	N rates (kg N ha^{-1})	Forage dry matter production (ton DM ha^{-1} cut^{-1})	Cumulative emissions (μg N-N_2O m^{-2})	Emission factors (%)
Cayman-CAN	0	1.36 (0.54)^{b}	1.31 (1.64)^{b}	-
	10	2.28 (0.22)^{ab}	7.31 (2.69)^{a}	0.60 (0.11)^{a}
	20	3.86 (1.42)^{a}	6.35 (2.09)^{ab}	0.25 (0.18)^{a}
	30	3.01 (0.62)^{a}	10.54 (1.42)^{a}	0.31 (0.03)^{a}
P value		0.042	0.008	0.772
Stargrass-CAN	0	1.91 (0.29)^{a}	0.85 (0.70)^{a}	-
	10	1.93 (0.32)^{a}	7.21 (3.01)^{a}	0.64 (0.29)^{a}
	20	2.39 (0.45)^{a}	11.00 (7.89)^{a}	0.51 (0.41)^{a}
	30	2.19 (0.14)^{a}	14.92 (3.73)^{a}	0.47 (0.10)^{a}
P value		0.063	0.068	0.843
Mombasa-Urea	0	1.91 (0.30)^{b}	0.38 (0.34)^{b}	-
	10	2.09 (0.17)^{b}	4.89 (2.19)^{a}	0.45 (0.23)^{a}
	20	2.01 (0.33)^{b}	4.24 (1.22)^{ab}	0.19 (0.08)^{a}
	30	3.68 (0.54)^{a}	5.25 (2.59)^{a}	0.16 (0.09)^{a}
P value		0.001	0.019	0.053

The Stargrass, Cayman, and Mombasa grasses fertilized with 30 kg N ha^{-1} showed higher values of N_2O emissions (14.92, 10.54, and 5.25 mg N-N_2O m^{-2}, respectively) although the differences compared to the control were only statistically significant for Cayman and Mombasa (Table 2). In addition, Stargrass and Cayman grasses, fertilized with CAN, showed higher N_2O emissions compared to Mombasa grass, which was fertilized with urea.

In Table 2, EF values for different N fertilization rates in Cayman, Stargrass, and Mombasa grasses are presented. These calculated values of EF represent the percentage value of N emitted as N_2O from the total amount of N fertilizer applied. It is highlighted that EF values were higher with the lowest dose (10 kg N ha^{-1}) of the three grasses although the differences were not statistically different. In addition, it is observed that the EFs are higher in the grasses fertilized with CAN compared to those that received urea in the case of Mombasa. An interesting fact is that in Mombasa, the EF value with 10 kg N ha^{-1} was twice as high as in the other doses. These results highlight the influence of the dose and type of N fertilizer on the EFs of N_2O for these forage grasses.

For Cayman grass, it is observed that the EF value of N_2O goes from 0.60% with 10 kg N ha^{-1} to a lower value of EF (0.25%) with 20 kg N ha^{-1}. However, in Cayman with a dose of 30 kg N ha^{-1}, the EF value increased again to 0.31% compared to the 10 kg N ha^{-1} treatment. Similarly, in Stargrass with 10 kg N ha^{-1}, the EF value is 0.64%, which decreased to 0.51% with 20 kg N ha^{-1} and it further decreased to 0.47% with 30 kg N ha^{-1}. For Mombasa, the EF value decreased from 0.45% at 10 kg N ha^{-1} to 0.19% at 20 kg N ha^{-1}, and then to an even lower value of 0.16% at 30 kg N ha^{-1}.

4. Discussion

We evaluated the effect of different types of N fertilizer and N fertilization rates on the performance of six tropical forage grasses grown for improving livestock productivity while possibly avoiding pasture degradation in Latin America. To our knowledge, this is the first study performed in the Andean region in which different types of N fertilizers and N fertilization rates are studied using different tropical forage grasses by evaluating agronomic and environmental performance based on the DM production, crude protein concentration, NUE, and N_2O emissions. Our results showed that each of the studied forage grass preferred a different type of N fertilizer which can affect strongly the NUE, N losses, and the agronomic performance. Several studies have found similar results using temperate grasses (Cardenas et al., 2019; Dobbie and Smith, 2003; Harty et al., 2016; Velthof et al., 1996). Results from this study could help to design N fertilization strategies aiming toward sustainable intensification of grass-based systems, maintaining productivity, avoiding soil degradation and grassland expansion in Latin America.

4.1. Type of N fertilizer strongly affects the NUE of tropical forages

Based on the NUE data obtained after four cycles of evaluation, Cayman and Stargrass grasses respond better with CAN; Basilisk and Mombasa grasses with urea; and Tully grass with U-AS (Fig. 3, Table 1). This indicates that the NUE response to different types of N fertilizer largely depends on the forage grass cultivar. The N fertilizer type (NH_4^+,

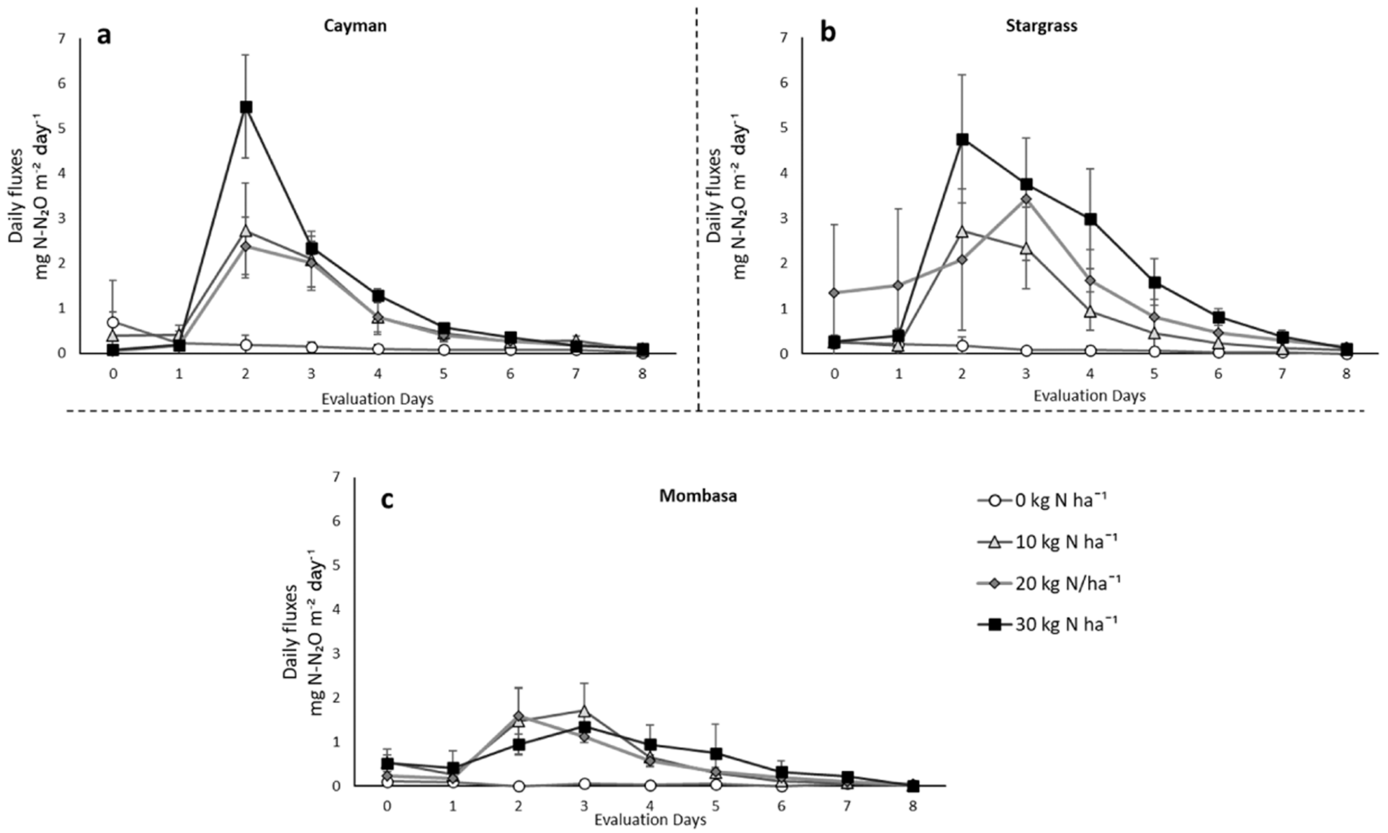


Fig. 4. Daily N_2O fluxes for Cayman (a) Stargrass (b) and Mombasa (c) tropical forage grasses in response to the application of different N fertilizer rates (0, 10, 20, and 30 kg N ha^{-1}) at the experimental field located in Santander de Quilichao county (Department of Cauca, Colombia). The type of N fertilizer used for Cayman and Stargrass was CAN while urea was used for Mombasa based on the results obtained from phase 1. Error bars indicate the standard deviation of the mean (n=3). (*) represent significant differences according to the Tukey HSD test at a 5% level. NS, non-significant.

NO_3^- or organic N) impacts NUE due to the differential response of each grass cultivar to different forms of N (Xu et al., 2012) and it is well known that the type of N fertilizer affects the growth and NUE of major crops like rice, cotton, wheat or maize (Bhatia et al., 2023; Fageria et al., 2008; Iqbal et al., 2020; Xu et al., 2012). Previous studies have also shown that the response to the same N fertilizer differs among different tropical forage grasses (Miranda et al., 1994); however, different types of fertilizers are not evaluated simultaneously.

The type of N fertilizer strongly affected the NUE of the six tropical forage grasses as observed after four cycles of evaluation performed during phase 1. Indeed, four of the six evaluated forage grasses showed significant differences in the NUE depending on the type of N fertilizer used (Fig. 3, Table 1). For example, Cayman grass showed a NUE of 87.8% with CAN and 40.4% with urea, while the NUE of Basilisk grass with CAN was 8.0% and 76.4% with urea (Fig. 3, Table 1). Thus, the type of N fertilizer used can shift the NUE values of these forage grasses from desirable to undesirable values according to the recommendations of the EU Nitrogen Expert Panel, (2015) which recommended NUE values ranging between 50% and 90%. For all the grasses except Cobra, at least one of the N fertilizer types reached NUE values within the desirable NUE range of 50–90%. In addition, combinations such as Stargrass and Cayman grass fertilized with CAN or Mombasa grass with urea showed NUE values of 87.8%, 91.7% and 89.9%, respectively, (Fig. 3, Table 1) which are remarkably high compared to the values observed with the other crops (Balasubramanian et al., 2004; Bhatia et al., 2023; Guardia et al., 2018). These results highlight the capacity for reaching a high NUE of these tropical forage grasses if the type of N fertilizer is properly selected, and this capacity confirms potential for mitigating N losses by redistributing N fertilizer across global croplands and regions (Smerald et al., 2023). Our high NUE values contrasted with the low NUE values obtained with Mombasa and *Urochloa brizantha* cv. Marandu (another widely used tropical forage grass that was not evaluated in our study) in studies performed in Brazil (Dupas et al., 2016; Galindo et al., 2017). We explain our higher NUE values by the lower N dose used in phase 1 of our study (25 kg N ha^{-1} cut^{-1}) compared to high N doses used in the above-mentioned studies (100 kg N ha^{-1} cut^{-1} and 50–200 kg N ha^{-1} cut^{-1}, respectively) suggesting that high N application rates decrease the NUE values as expected. This supports our decision to use a lower N dose in phase 1 as a strategy to minimize the N losses and reach high NUE values. However, we assume that our high NUE values have been obtained under a cutting regime and these values could become markedly lower under grazing conditions at a farm level (Balasubramanian et al., 2004; Cardenas et al., 2019).

Based on the NUE values, Basilisk and Mombasa grasses responded better with urea while Tully grass with U-AS fertilizer, i.e., both are urea-based fertilizers (Table 1). Similar results were obtained with temperate grasses where higher NUE and lower N losses were observed when urea was used instead of CAN (Cardenas et al., 2019; Harty et al., 2016). However, this result is surprising under tropical conditions, where the high temperature and soil moisture could lead to high NH_3 volatilization and N losses (de Morais et al., 2013; Martins et al., 2017). The good performance of some tropical forage grasses with urea-based fertilizers could be explained by the biological nitrification inhibition (BNI) capacity, as well as the high and quick NH_4+ microbial immobilization that were observed in the soil grown with some tropical forage grasses that can reduce the N losses and increase the NUE (Byrnes et al., 2017; Subbarao et al., 2009; Teutscherovaá et al., 2022; Vázquez et al., 2020; Villegas et al., 2020). These forage grasses with their BNI capacity may prefer NH_4^+ sources rather than NO_3^- due to the low levels of NO_3^- under non-fertilized conditions (Bradley et al., 2006; Teutscherova et al., 2019). This BNI capacity and high NH_4^+ immobilization has been largely observed in Tully (CIAT 679) (Vázquez et al., 2020) and Mombasa (CIAT 6962) which may explain their better response to urea-based fertilizers (Villegas et al., 2022).

4.2. N uptake and surplus as affected by the type of N fertilizer

Except for Cobra forage grass, the addition of N fertilizers increased the total N uptake compared to the control treatment (Table 1) due to higher N availability in soil upon N fertilization. Considering the combinations of forage grass and type of N fertilizer showing higher NUE values, an extra N uptake of approximately 20 kg N ha^{-1} cut^{-1} was observed compared to the control. Under the evaluated cutting frequency (28–30 days), the annual N uptake can be increased up to 250 kg N ha^{-1} yr^{-1} compared to control if the proper type of N fertilizer is selected for application (from 203 kg N ha^{-1} yr^{-1} in Tully with U-AS to 276 kg N ha^{-1} yr^{-1} in Stargrass with CAN). Furthermore, the annual N output from these grasses considering the best combination of forage grass and type of N fertilizer ranges between 452 and 658 kg N ha^{-1} yr^{-1} for Basilisk and Mombasa grasses, respectively. These values are by far higher than the minimum desirable N output values set for EU agriculture (i.e., 80 kg N ha^{-1} yr^{-1}) (EU Nitrogen Expert Panel, 2015) and these values are approximately two times higher than those of temperate grasses cultivated under similar fertilization regimes (Cardenas et al., 2019). This indicates the higher productivity and N uptake of the studied tropical grasses compared to temperate grasses and suggests that the minimum desirable N output values for tropical forage grasses could need revision.

When the annual N surplus (a proxy for the potential N loss to the environment) is calculated for the combinations of forage grass and type of N fertilizer showing higher NUE values, the potential N losses to the environment can range between 24.9 kg N ha^{-1} yr^{-1} in Stargrass with CAN and 97.2 kg N ha^{-1} yr^{-1} in Tully with U-AS. In contrast, the N surplus in Stargrass fertilized with UAS can reach up to 191 kg N ha^{-1} yr^{-1} indicating that the use of a inappropriate N fertilizer can increase N losses to an order of magnitude and/or increase the N pool in soil that might not be always readily available for the plant. The combinations of Cayman, Stargrass, and Mombasa grasses with CAN, and Basilisk and Mombasa with urea were under the limit of 80 kg N ha^{-1} yr^{-1} of maximum desirable N output set by EU Nitrogen Expert Panel (2015) (Fig. 3). This indicates the importance of selecting the appropriate type of N fertilizer for each forage grass to minimize the negative environmental impact of N addition while enhancing forage productivity.

4.3. Agronomic performance of each tropical forage grass as affected by the type of N fertilizer

Except for Cobra grass, we observed a positive effect for at least one type of N fertilizer on the forage DM production after four cycles of evaluation performed during phase 1 (two in the rainy and two in the dry season). This confirms the commonly observed N limitation for the growth of tropical forage grasses (da Cruz Corrêa et al., 2021; Delevatti et al., 2019; Dupas et al., 2016; Euclides et al., 2022; Galindo et al., 2017; Hernández Garay et al., 2004) as their biomass production responds positively to N addition, although the response magnitude varies largely among studies due to variation in soil and climatic conditions. We highlight that we used a lower N application rate (25 kg N ha^{-1} cut^{-1}) than the above-mentioned studies despite the total annual N load being in the same range. The higher level of increases in forage DM production was observed with Basilisk grass fertilized with urea (+73%), Stargrass with CAN (+57%), Tully with U-AS (+51%), or Mombasa with urea (+44%). In contrast, other combinations of forage grass and types of N fertilizer barely showed any response to N fertilization. It is also important to note that our field study was conducted in a soil with a higher value of SOM (74.6 g kg^{-1}) that could meet the partial N requirement of the grass as indicated by the high DM production of control treatment. However, in the medium-term the lack of N fertilization can lead to soil N mining and decreasing DM production. The higher SOM and the fraction of particulate organic matter in the soil could also contribute to immobilization of applied N.

We did not observe any positive response to N fertilization to Cobra despite it showing the highest forage DM production compared with the control treatment. Likely, Cobra biomass production was limited by another factor such as another nutrient, soil pH, water, or light which hampered its further growth when N was added. In consequence, no effect of any type of N fertilizer was observed in the total N uptake leading to very low NUE value and high N losses including N_2O emission and NO_3^- leaching. When a low portion of the added N is taken up by grasses due to plant growth limitation by another nutrient, light or water, the added N tends to accumulate in soil in mineral form leading to N losses (Craine and Jackson, 2010; Vázquez et al., 2023). Indeed, global grasslands are in general co-limited by N and phosphorus (P) and they show a synergistic response to the combined addition of both which boosts biomass production while reducing the N losses (Craine and Jackson, 2010; Fay et al., 2015; Vázquez et al., 2023).

The addition of N increased the crude protein concentration of all six tested forage grasses because of the higher N availability in soil (Table 1). This increase in crude protein concentration in grasses with the addition of N is well documented by previous research (da Cruz Corrêa et al., 2021; Delevatti et al., 2019; Dupas et al., 2016; Euclides et al., 2022; Hernández Garay et al., 2004; Leite et al., 2021). According to Detmann et al. (2014), a minimum crude protein concentration of 124 g kg^{-1} DM is required to ensure a good ruminal N availability for an optimum animal metabolism under tropical conditions. This crude protein level was reached in the forage grass and type of N fertilizer combinations that also showed a high NUE such as Stargrass and Cayman grass fertilized with CAN. In contrast, Mombasa grass fertilized both with CAN and urea displayed high NUE, but the crude protein concentration was slightly under this recommended level which may limit the digestibility of the forage. The use of a higher N fertilization dose for Mombasa grass could increase the crude protein concentration although its impact on NUE should be evaluated.

4.4. Optimization of N fertilization rate to minimize the peak value of N_2O emission

In phase 2, we aimed to evaluate the effect of different N fertilization rates on the N_2O peak value upon fertilizer application for the best combinations of forage grass and type of N fertilizer based on their NUE values. We selected the combinations of Stargrass and Cayman grass fertilized with CAN or Mombasa grass fertilized with urea because their NUE values are close to the maximum NUE registered in the literature for non-grazed experimental plots (Balasubramanian et al., 2004), and their values were at the upper limit of desirable NUE values recommended by the EU Nitrogen Expert Panel (2015).

We observed an N_2O emission peak value upon N fertilizer application for the three evaluated forage grasses and type and fertilizer combination, although with Cayman and Stargrass greater N_2O emission rates were observed after two days of N fertilizer application while with Mombasa grass greater values were observed after four days of application (Fig. 4). Similar delays in peak values of N_2O emission with urea compared to CAN were observed by Harty et al. (2016) and these may be due to differences in formulations of the fertilizers evaluated. CAN addition immediately increased the N_2O emissions from the nitrification and denitrification processes of NH_4^+ and NO_3^-, respectively, while urea addition requires a previous step of hydrolysis which can reduce the N_2O emissions (Cardenas et al., 2019; Harty et al., 2016). Indeed, the accumulated N_2O emissions from urea application were lower with Mombasa grass than with Cayman and Stargrass which were fertilized with CAN. However, the use of urea can be linked to high NH_3 volatilization under tropical conditions which can limit the NUE values (de Morais et al., 2013; Martins et al., 2017).

In general, higher N fertilization rates increased cumulative N_2O emissions, although the differences were not significant for Stargrass because of the large standard deviation of the mean value (Table 2). Similar results were found with both temperate (Cardenas et al., 2010, 2019; Hinton et al., 2015) and tropical grasses (da Cruz Corrêa et al., 2021; do Nascimento et al., 2021; Raposo et al., 2020). Elevated N fertilization rates resulted in a rise in overall N_2O emissions. Nevertheless, we noted that fertilizing Stargrass and Cayman grass at a rate of 20 kg N^{-1} cut^{-1} and Mombasa grass at a rate of 30 kg N^{-1} cut^{-1} proved more advantageous compared to alternative N fertilization rates, considering both the quantity of emitted N_2O and biomass production simultaneously. This indicates that those doses could be preferred to increase the forage productivity with a lower environmental cost in terms of N_2O emissions. In contrast, the N_2O emission was higher with increasing the N fertilization doses for Stargrass, thus the increase of forage production seemed to be linked to higher N loss through N_2O emissions within the range of the rate of N fertilization evaluated.

Worth noting that the range of EF calculated here for all treatments was between 0.16% and 0.64%, which is lower than the IPCC default value of 1.17% for tropical grasses (IPCC, 2019). This discrepancy in values with respect to the IPCC standard has been consistently documented in previous research, underscoring the importance of field experiments that measure N_2O emissions where region-specific EFs can be derived (Byrnes et al., 2017; Durango Morales et al., 2021; Lombardi et al., 2021, 2022). However, we acknowledge the short duration of our campaign of N_2O measurements, thus the EF values may have been underestimated in our study.

We are also aware of the complexity of soil N processes including immobilization, mineralization, and potential N leaching, which together with gaseous emissions, constitute the overall N dynamics in the system. While N surplus is used as a proxy for potential N loss, it encompasses a range of outcomes including N_2O emissions, ammonia volatilization, and N leaching and immobilization in soil that we did not measure to the full extent in this study.

Further research is needed to elucidate the mechanisms driving the observed responses to N fertilization, including plant-soil-microbe interactions and the role of soil physical and chemical properties. Our findings lay the groundwork for such investigations by identifying promising fertilizer types and doses that warrant closer examination.

4.5. Implications for sustainable intensification of tropical grass pastures

Our results showed that each of the studied forage grasses preferred a different type of N fertilizer which can affect strongly the NUE values, N losses, and agronomic performance of the six tropical forage grasses evaluated. However, we acknowledge the limitations of our study and encourage further studies to be performed under different edaphoclimatic conditions. We recommend simultaneous evaluations of N fertilizer types, N rates, and the inclusion of grazing animals. We also suggest longer monitoring periods to obtain more robust and representative results. For example, monitoring N_2O emissions over a complete pasture recovery cycle or several grazing cycles.

According to the stocking rate estimations performed in phase 1 (Figure S1), the use of CAN with a rate of 25 kg N^{-1} cut^{-1} can increase by 17% and 58% the stocking rate for Cayman and Stargrass grasses, respectively; and the addition of urea to Mombasa grass can increase the stocking rate by 44%. Those increased stocking rates could be achieved with a low environmental impact in terms of N losses due to higher values of NUE of the N fertilizers and rates used. This confirms the potential for a better redistribution of N fertilizer use across global regions and crops to mitigate the global N challenge (Smerald et al., 2023). Our results have implications for the sustainable use of N fertilizer that could contribute toward meeting the increasing demand for livestock products as well as to improve the profitability of small and medium size farms in the region. In addition, this sustainable intensification of pastures in Latin America can enhance the land use efficiency of the livestock sector and may help to protect tropical forests and natural ecosystems by reducing the need to increase the area of pasturelands and thereby sparing land for nature or other uses (Matson and Vitousek, 2006; Strassburg et al., 2014; White et al., 2001). However, to realize this land-sparing potential of a sustainable N fertilization to tropical grass pastures, there is a need to develop policies such as territorial planning and improved land use monitoring, land tenure security and law enforcement (Strassburg et al., 2014).

The benefits and challenges associated with the use of organic fertilizers such as livestock manure should also be considered in future studies. Including this aspect will provide a more holistic view of sustainable N management in tropical pastures. In general, applying organic fertilizers like livestock manure can enhance soil health and nutrient availability, leading to improved N cycling and increased productivity of tropical pastures, but it may also elevate N_2O emissions due to increased N availability and microbial activity (Bhunia et al., 2021; Köninger et al., 2021).

5. Conclusion

We evaluated the effect of different types and rates of N fertilizer applied to six tropical forage grasses grown in Latin America. Our results highlight the importance of considering the variability in the responses of different forage grasses to different types of N fertilizers, for increasing the forage productivity while limiting the negative environmental impact and potentially reducing the possible degradation of grass pastures. We observed that depending on the forage grass, the use of a different type of N fertilizer changes the NUE values from near optimum range reaching up to 90% (Cayman, Stargrass and Mombasa fertilized with CAN or Mombasa with urea) compared to very inefficient NUE values of $< 50\%$ (e.g., Cayman and Stargrass fertilized with urea or U-AS). Further, when the plant N uptake and the N surplus (a proxy for the potential N loss to the environment) are calculated for the different types of N fertilizers evaluated, we observed that the use of an inappropriate N fertilizer can increase the gaseous N losses to an order of magnitude which indicates the importance of using proper N fertilizer type and rate to reduce the N losses.

Optimizing NUE values for reducing N losses is a key challenge for sustainable intensification of livestock sector in Latin America. We show that use of a proper N fertilization strategy aiming at higher NUE values can increase the forage production with a limited environmental impact. In addition, our study indicates that the N fertilization recommendations cannot be universal for all the tropical forage grasses and a tailor-made design should be developed for each forage grass under local soil and climatic conditions. However, further research and validation of our results are required through long-term studies under diverse soil and climatic conditions, including grazing and cattle production aspects. These additional efforts will consolidate knowledge and guide the development of sustainable N fertilization strategies for tropical livestock production, promoting more sustainable land use in the region and a greater resilience of the agricultural sector to current economic, environmental, and climate challenges. Collaboration among scientists, producers, and policy makers is essential to promote sustainable management practices that contribute to more productive, efficient, and environmentally friendly livestock production in tropical Latin America.

CRediT authorship contribution statement

Jacobo Arango: Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Miguel Amado:** Validation, Resources, Funding acquisition. **Carlos Berdugo:** Visualization, Resources, Funding acquisition. **Eduardo Vázquez:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Daniel M. Villegas:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation. **Mike Bastidas:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Idupulapati M. Rao:** Writing – review & editing. **Jhon F. Gutierrez:** Methodology, Conceptualization. **Nelson J. Vivas-Quila:** Writing – review & editing, Methodology, Investigation, Data curation.

Declaration of Competing Interest

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

Data Availability

Data will be made available on request.

Acknowledgments

We acknowledge the financial support from YARA, Colombia. This work was conducted as part of the CGIAR Initiative on Livestock and Climate. We thank all donors who globally support our work through their contributions to the CGIAR System. We also thank Alejandro Ruden for his contribution to this work, and GARCES EDER SAS, owners of the La Campiña farm where the field experiments were carried out.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2024.108970.

References

AOAC International, 2002. AOAC Official Method 2001.11 Protein (crude) in animal feed, forage (plant tissue), grain, and oilseeds. J. AOAC Int. http://www.eoma.aoac.org/methods/info.asp?ID=32924.

Arango, J., Ruden, A., Martinez-Baron, D., Loboguerrero, A.M., Berndt, A., Chacón, M., Torres, C.F., Oyhantcabal, W., Gomez, C.A., Ricci, P., Ku-Vera, J., Burkart, S., Moorby, J.M., Chirinda, N., 2020. Ambition meets reality: achieving GHG emission reduction targets in the livestock sector of Latin America. Front. Sustain. Food Syst. *4* (April) https://doi.org/10.3389/fsufs.2020.00065.

Balasubramanian, V., Alves, B., Aulakh, M., Bekunda, M., Cai, Z., Drinkwater, L., Mugendi, D., Kessel, C. van, Oenema, O., 2004. Crop, Environmental, and Management Factors Affecting Nitrogen use Efficiency, 65. SCOPE, pp. 19–33..

Baptistella, J.L.C., de Andrade, S.A.L., Favarin, J.L., Mazzafera, P., 2020. *Urochloa* in tropical agroecosystems. Front. Sustain. Food Syst. *4* (August), 1–17. https://doi.org/10.3389/fsufs.2020.00119.

Barbieri, P., Pellerin, S., Seufert, V., Smith, L., Ramankutty, N., Nesme, T., 2021. Global option space for organic agriculture is delimited by nitrogen availability. Nat. Food *2* (5), 363–372. https://doi.org/10.1038/s43016-021-00276-y.

Bell, M.J., Cloy, J.M., Topp, C.F.E., Ball, B.C., Bagnall, A., Rees, R.M., Chadwick, D.R., 2016. Quantifying N_2O emissions from intensive grassland production: the role of synthetic fertilizer type, application rate, timing and nitrification inhibitors. J. Agric. Sci. *154* (5), 812–827. https://doi.org/10.1017/S0021859615000945.

Bhatia, A., Cowan, N.J., Drewer, J., Tomer, R., Kumar, V., Sharma, S., Paul, A., Jain, N., Kumar, S., Jha, G., Singh, R., Prasanna, R., Ramakrishnan, B., Bandyopadhyay, S.K., Kumar, D., Sutton, M.A., Pathak, H., 2023. The impact of different fertiliser management options and cultivars on nitrogen use efficiency and yield for rice cropping in the Indo-Gangetic Plain: Two seasons of methane, nitrous oxide and ammonia emissions. Agric., Ecosyst. Environ. *355* (March), 108593 https://doi.org/10.1016/j.agee.2023.108593.

Bhunia, S., Bhowmik, A., Mallick, R., Mukherjee, J., 2021. Agronomic efficiency of animal-derived organic fertilizers and their effects on biology and fertility of soil: A review. Agronomy *11* (5), 1–25. https://doi.org/10.3390/agronomy11050823.

Bradley, R., Mosquera, D., Mendez, J., 2006. Inhibition of nitrate accumulation in tropical grassland soils: Effect of nitrogen fertilization and soil disturbance. J. Soil Sci. *39*, 407–416. https://doi.org/10.1111/j.1365-2389.1988.tb01226.x.

Byrnes, R.C., Nùñez, J., Arenas, L., Rao, I., Trujillo, C., Alvarez, C., Arango, J., Rasche, F., Chirinda, N., 2017. Biological nitrification inhibition by *Brachiaria* grasses mitigates soil nitrous oxide emissions from bovine urine patches. Soil Biol. Biochem. *107*, 156–163. https://doi.org/10.1016/j.soilbio.2016.12.029.

Cardenas, L.M., Thorman, R., Ashlee, N., Butler, M., Chadwick, D., Chambers, B., Cuttle, S., Donovan, N., Kingston, H., Lane, S., Dhanoa, M.S., Scholefield, D., 2010. Quantifying annual N_2O emission fluxes from grazed grassland under a range of inorganic fertiliser nitrogen inputs. Agric. Ecosyst. Environ. *136* (3), 218–226. https://doi.org/10.1016/j.agee.2009.12.006.

Cardenas, L.M., Bhogal, A., Chadwick, D.R., McGeough, K., Misselbrook, T., Rees, R.M., Thorman, R.E., Watson, C.J., Williams, J.R., Smith, K.A., Calvet, S., 2019. Nitrogen use efficiency and nitrous oxide emissions from five UK fertilised grasslands. Sci. Total Environ. *661*, 696–710. https://doi.org/10.1016/j.scitotenv.2019.01.082.

Corrêa, da Cruz, Cardoso, D.C., Ferreira, A. da S., Siniscalchi, M.R., Gonçalves, D., Lumasini, P.H. de A., Reis, R.N., A, R., Ruggieri, A.C., 2021. Ammonia volatilization, forage accumulation, and nutritive value of marandu palisade grass pastures in different N sources and doses. Atmosphere *12* (9). https://doi.org/10.3390/atmos12091179.

Costa, C., Villegas, D., Bastidas, M., Rubio, N., Rao, I., Arango, J., 2022. Soil carbon stocks and nitrous oxide emissions of pasture systems in Orinoquía Region of Colombia: Potential for developing land-based greenhouse gas removal projects. Front. Clim. 4, 916068 https://doi.org/10.3389/fclim.2022.916068.

Craine, J.M., Jackson, R.D., 2010. Plant nitrogen and phosphorus limitation in 98 North American grassland soils. Plant Soil *334* (1), 73–84. https://doi.org/10.1007/s11104-009-0237-1.

DANE. (2019). *Encuesta nacional agropecuaria (ENA)*. Departamento Administrativo Nacional de Estadística. https://www.dane.gov.co/index.php/estadisticas-por-tema/agropecuario/encuesta-nacional-agropecuaria-ena.

Delevatti, L.M., Cardoso, A.S., Barbero, R.P., Leite, R.G., Romanzini, E.P., Ruggieri, A.C., Reis, R.A., 2019. Effect of nitrogen application rate on yield, forage quality, and animal performance in a tropical pasture. Sci. Rep. *9* (1), 1–9. https://doi.org/10.1038/s41598-019-44138-x.

Detmann, E., Valente, É.E.L., Batista, E.D., Huhtanen, P., 2014. An evaluation of the performance and efficiency of nitrogen utilization in cattle fed tropical grass pastures with supplementation. Livest. Sci. *162*, 141–153. https://doi.org/10.1016/j.livsci.2014.01.029.

Dobbie, K.E., Smith, K.A., 2003. Impact of different forms of N fertilizer on N_2O emissions from intensive grassland. Nutr. Cycl. Agroecosyst. *67* (1), 37–46. https://doi.org/10.1023/A:1025119512447.

Dupas, E., Buzetti, S., Silveira Rabêlo, F.H., Sarto, A.L., Cheng, N.C., Minhoto Teixeira Filho, M.C., Galindo, F.S., Dinalli, R.P., de Niro Gazola, R., 2016. Nitrogen recovery, use efficiency, dry matter yield, and chemical composition of palisade grass fertilized with nitrogen sources in the cerrado biome. Aust. J. Crop Sci. *10* (9), 1330–1338. https://doi.org/10.21475/ajcs.2016.10.09.p7854.

Durango Morales, S.G., Barahona, R., Bolívar, D.M., Arango, J., Verchot, L., Chirinda, N., 2021. Apparent nitrogen recovery in milk and early dry season nitrous oxide emission factors for urine deposited by dual-purpose cattle on different soil types. Front. Sustain. Food Syst. *4* (January) https://doi.org/10.3389/fsufs.2020.602657.

ENA, 2019. Boletín Técnico - Encuesta Nacional. Agropecu. (ENA) *2019*. ⟨https://geoportal.dane.gov.co/descargas/ena_result/boletin_ena_2019.pdf⟩.

Ermgassen, zu, de Alcântara, E.K.H.J., Balmford, M.P., Barioni, A., Neto, L., Bettarello, F. B., de Brito, M.M.F., Carrero, G., Florence, G.C., de, E., Garcia, A.S., Gonçalves, E., da Luz, E.T., Mallman, C.T., Strassburg, G.M., Valentim, B.B.N., F, J., Latawiec, A., 2018. Results from on-the-ground efforts to promote sustainable cattle ranching in the Brazilian Amazon. Sustain. (Switz.) *10* (4). https://doi.org/10.3390/su10041301.

EU Nitrogen Expert Panel, 2015. Nitrogen Use Effic. (NUE). Indic. Util. Nitrogen Agric. Food Syst. (http://www.inms.international/inms-meeting-lisbon/NExpertPanelNUESession1.pdf. December)..

Euclides, V.P.B., Montagner, D.B., de Araújo, A.R., de Aragão Pereira, M., dos Santos Difante, G., de Araújo, I.M.M., Barbosa, L.F., Barbosa, R.A., Gurgel, A.L.C., 2022. Biological and economic responses to increasing nitrogen rates in Mombaça guinea grass pastures. Sci. Rep. *12* (1) https://doi.org/10.1038/s41598-022-05796-6.

Fageria, N.K., Baligar, V.C., Li, Y.C., 2008. The role of nutrient efficient plants in improving crop yields in the twenty first century. J. Plant Nutr. *31* (6), 1121–1157. https://doi.org/10.1080/01904160802116068.

Fay, P.A., Prober, S.M., Harpole, W.S., Knops, J.M.H., Bakker, J.D., Borer, E.T., , … Lind, E.M., MacDougall, A.S., Seabloom, E.W., Wragg, P.D., Adler, P.B., Blumenthal, D.M., Buckley, Y.M., Chu, C., Cleland, E.E., Collins, S.L., Davies, K.F., Du, G., Feng, X., Yang, L.H., 2015. Grassland productivity limited by multiple nutrients. Nat. Plants *1* (7), 15080. https://doi.org/10.1038/nplants.2015.80.

Figueroa, D., Galicia, L., Suárez Lastra, M., 2022. Latin American cattle ranching sustainability debate: an approach to social-ecological systems and spatial-temporal scales. Sustain. (Switz.) *14* (14), 1–17. https://doi.org/10.3390/su14148924.

Fuglie, K., Peters, M., Burkart, S., 2021. The Extent and Economic Significance of Cultivated Forage Crops in Developing Countries. Front. Sustain. Food Syst. *5* (October), 1–8. https://doi.org/10.3389/fsufs.2021.712136.

Galindo, F.S., Buzetti, S., Filho, M.C.M.T., Dupas, E., Ludkiewicz, M.G.Z., 2017. Application of different nitrogen doses to increase nitrogen efficiency in Mombasa guinegrass (*Panicum maximum* cv. mombasa) at dry and rainy seasons. Aust. J. Crop Sci. *11* (12), 1657–1664. https://doi.org/10.21475/ajcs.17.11.12.pne907.

Garay, Hernández, Sollenberger, A., McDonald, L.E., Ruegsegger, D.C., Kalmbacher, G.J., S, R., Mislevy, P., 2004. Nitrogen fertilization and stocking rate affect stargrass pasture and cattle performance. Crop Sci. *44* (4), 1348–1354. https://doi.org/10.2135/cropsci2004.1348.

Gerber, P.J., Uwizeye, A., Schulte, R.P.O., Opio, C.I., de Boer, I.J.M., 2014. Nutrient use efficiency: A valuable approach to benchmark the sustainability of nutrient use in global livestock production? Curr. Opin. Environ. Sustain. *9*, 122–130. https://doi.org/10.1016/j.cosust.2014.09.007.

Gu, B., Zhang, X., Lam, S.K., Yu, Y., van Grinsven, H.J.M., Zhang, S., Wang, X., Bodirsky, B.L., Wang, S., Duan, J., Ren, C., Bouwman, L., de Vries, W., Xu, J., Sutton, M.A., Chen, D., 2023. Cost-effective mitigation of nitrogen pollution from global croplands. Nature *613* (7942), 77–84. https://doi.org/10.1038/s41586-022-05481-8.

Guardia, G., Sanz-Cobena, A., Sanchez-Martín, L., Fuertes-Mendizábal, T., González-Murua, C., Álvarez, J.M., Chadwick, D., Vallejo, A., 2018. Urea-based fertilization strategies to reduce yield-scaled N oxides and enhance bread-making quality in a rainfed Mediterranean wheat crop. Agric., Ecosyst. Environ. *265* (May), 421–431. https://doi.org/10.1016/j.agee.2018.06.033.

Harty, M.A., Forrestal, P.J., Watson, C.J., McGeough, K.L., Carolan, R., Elliot, C., Krol, D., Laughlin, R.J., Richards, K.G., Lanigan, G.J., 2016. Reducing nitrous oxide emissions by changing N fertiliser use from calcium ammonium nitrate (CAN) to urea based formulations. Sci. Total Environ. *563–564*, 576–586. https://doi.org/10.1016/j.scitotenv.2016.04.120.

Herrero, M., Henderson, B., Havlík, P., Thornton, P.K., Conant, R.T., Smith, P., Wirsenius, S., Hristov, A.N., Gerber, P., Gill, M., Butterbach-Bahl, K., Valin, H., Garnett, T., Stehfest, E., 2016. Greenhouse gas mitigation potentials in the livestock sector. Nat. Clim. Change *6* (5), 452–461. https://doi.org/10.1038/nclimate2925.

Hinton, N.J., Cloy, J.M., Bell, M.J., Chadwick, D.R., Topp, C.F.E., Rees, R.M., 2015. Managing fertiliser nitrogen to reduce nitrous oxide emissions and emission intensities from a cultivated Cambisol in Scotland. Geoderma Reg. *4*, 55–65. https://doi.org/10.1016/j.geodrs.2014.12.002.

ILRI, 2019. Meat: the Future series Options for the Livestock Sector in Developing and Emerging Economies to 2030 and Beyond. *World Economic. Forum, Jan.* https://www.weforum.org/whitepapers/meat-the-future-series-options-for-the-livestock-sector-in-developing-and-emerging-economies-to-2030-and-beyond.

Instituto Colombiano Agropecuario - ICA. (2020). *Censo Pecuario año 2020*. https://www.ica.gov.co/areas/pecuaria/servicios/epidemiologia-veterinaria/censos-2016/censo-2018.aspx.

IPCC (Intergovernmental Panel on Climate Change), 2019. 2019 the refinement to the 2006 IPCC guidelines for national greenhouse gas inventories. Fundam. Appl. Climatol. *2*, 5–13. https://doi.org/10.21513/0207-2564-2019-2-05-13.

Iqbal, A., Dong, Q., Wang, X., Gui, H.-P., Zhang, H., Pang, N., Zhang, X., Song, M., 2020. Nitrogen preference and genetic variation of cotton genotypes for nitrogen use efficiency. J. Sci. Food Agric. *100* (6), 2761–2773. https://doi.org/10.1002/jsfa.10308.

Köninger, J., Lugato, E., Panagos, P., Kochupillai, M., Orgiazzi, A., Briones, M.J.I., 2021. Manure management and soil biodiversity: Towards more sustainable food systems in the EU. Agric. *Syst. 194* (April) https://doi.org/10.1016/j.agsy.2021.103251.

Labarta, R., Martinez, J.M., Yaccelga, A., Reyes, B., Gomez, L., Maredia, M., DeYoung, D., Carriazo, F., Toro, M., 2017. Assessing the adoption and economic & environmental impacts of *Brachiaria* grass forage cultivars in Latin America focusing in the experience of Colombia. Present. Overv. 45. https://hdl.handle.net/10568/118414.

Leite, R.G., Cardoso, A. da S., Fonseca, N.V.B., Silva, M.L.C., Tedeschi, L.O., Delevatti, L. M., Ruggieri, A.C., Reis, R.A., 2021. Effects of nitrogen fertilization on protein and carbohydrate fractions of Marandu palisadegrass. Sci. Rep. *11* (1), 1–8. https://doi.org/10.1038/s41598-021-94098-4.

Lerner, A.M., Zuluaga, A.F., Chará, J., Etter, A., Searchinger, T., 2017. Sustainable cattle ranching in practice: Moving from theory to planning in Colombia's livestock sector. Environ. Manag. *60* (2), 176–184. https://doi.org/10.1007/s00267-017-0902-8.

Lombardi, B., Alvarado, P.I., Ricci, P., Guzmán, S.A., Gonda, H.L., Juliarena, M.P., 2021. Methane and nitrous oxide emissions from dung patches deposited by grazing cattle supplemented with maize grain. Anim. Feed Sci. Technol. *279*, 115029 https://doi.org/10.1016/j.anifeedsci.2021.115029.

Lombardi, B., Loaiza, S., Trujillo, C., Arevalo, A., Vázquez, E., Arango, J., Chirinda, N., 2022. Greenhouse gas emissions from cattle dung depositions in two *Urochloa* forage fields with contrasting biological nitrification inhibition (BNI) capacity. Geoderma *406*. https://doi.org/10.1016/j.geoderma.2021.115516.

Martins, M.R., Sant'Anna, S.A.C., Zaman, M., Santos, R.C., Monteiro, R.C., Alves, B.J.R., Jantalia, C.P., Boddey, R.M., Urquiaga, S., 2017. Strategies for the use of urease and nitrification inhibitors with urea: Impact on N_2O and NH_3 emissions, fertilizer-15N recovery and maize yield in a tropical soil. Agric., Ecosyst. Environ. *247*, 54–62. https://doi.org/10.1016/j.agee.2017.06.021.

Matson, P., Vitousek, P., 2006. Agricultural intensification: Will land spared from farming be land spared for nature? Conserv. Biol. *20*, 709–710. https://doi.org/10.1111/j.1523-1739.2006.00442.x.

Mazabel, J., Worthington, M., Castiblanco, V., Peters, M., Arango, J., 2020. Using near infrared reflectance spectroscopy for estimating nutritional quality of *Brachiaria humidicola* in breeding selections. Agrosystems, Geosci. Environ. *3* (1), 1–9. https://doi.org/10.1002/agg2.20070.

Miranda, C.H.B., Cadisch, G., Urquiaga, S., Miranda, C.H.B., Boddey, R.M., Giller, K.E., 1994. Mineral nitrogen in an oxisol from the Brazilian cerrados in the presence of *Brachiaria* spp. Eur. J. Agron. *3* (4), 333–337. https://doi.org/10.1016/S1161-0301(14)80163-9.

de Morais, R.F., Boddey, R.M., Urquiaga, S., Jantalia, C.P., Alves, B.J.R., 2013. Ammonia volatilization and nitrous oxide emissions during soil preparation and N fertilization of elephant grass (*Pennisetum purpureum* Schum.). Soil Biol. Biochem. *64*, 80–88. https://doi.org/10.1016/j.soilbio.2013.04.007.

do Nascimento, A.F., de Oliveira, C.M., Pedreira, B.C., Pereira, D.H., Rodrigues, R.R., de, A., 2021. Nitrous oxide emissions and forage accumulation in the Brazilian Amazon forage-livestock systems submitted to N input strategies. Grassl. Sci. *67* (1), 63–72. https://doi.org/10.1111/grs.12287.

Notenbaert, A.M.O., Douxchamps, S., Villegas, D.M., Arango, J., Paul, B.K., Burkart, S., Rao, I., Kettle, C.J., Rudel, T., Vázquez, E., Teutscherova, N., Chirinda, N., Groot, J. C.J., Wironen, M., Pulleman, M., Louhaichi, M., Hassan, S., Oberson, A., Nyawira, S. S., , … Peters, M., 2021. Tapping into the environmental co-benefits of improved tropical forages for an agroecological transformation of livestock production systems. Front. Sustain. Food Syst. *5* (November) https://doi.org/10.3389/fsufs.2021.742842.

Rao, I., Peters, M., Castro, A., Schultze-Kraft, R., White, D., Fisher, M., Miles, J., Lascano, C., Blümmel, M., Bungenstab, D., Tapasco, J., Hyman, G., Bolliger, A., Paul, B., Van Der Hoek, R., Maass, B., Tiemann, T., Cuchillo, M., Douxchamps, S., , … Rudel, T., 2015. LivestockPlus - The sustainable intensification of forage-based agricultural systems to improve livelihoods and ecosystem services in the tropics. Trop. Grassl. -Forrajes Trop. *3* (2), 59–82. https://doi.org/10.17138/TGFT(3)59-82.

Raposo, E., Brito, L., Janusckiewicz, E., Freitas de Oliveira Melo, L., Versuti, J., Assumpção, F., Cardoso, A., Siniscalchi, D., Delevatti, L., Malheiros, E., Reis, R., Ruggieri, A., 2020. Greenhouse gases emissions from tropical grasslands affected by nitrogen fertilizer management. Agron. J. *112* https://doi.org/10.1002/agj2.20385.

Rincón, Á., Flórez, H., Ballesteros, H., León, L.M., 2018. Effects of fertilization of *Brachiaria humidicola* cv. Llanero on pasture productivity in the foothills region of the Llanos Orientales, Colombia. Trop. Grassl. -Forrajes Trop. *6* (3), 158–168. https://doi.org/10.17138/TGFT(6)158-168.

dos Santos, C.A., Monteiro, R.C., Homem, B.G.C., Salgado, L.S., Casagrande, D.R., Pereira, J.M., de Paula Rezende, C., Alves, B.J.R., Boddey, R.M., 2023. Productivity of beef cattle grazing *Brachiaria brizantha* cv. Marandu with and without nitrogen fertilizer application or mixed pastures with the legume *Desmodium ovalifolium*. Grass Forage Sci. *78* (1), 147–160. https://doi.org/10.1111/gfs.12581.

SDSN (Sustainable Development Solutions Network), 2014. Indic. a Monit. Framew. Sustain. Dev. Goals 233.

Shen, J., Treu, R., Wang, J., Nicholson, F., Bhogal, A., Thorman, R., 2018. Modeling nitrous oxide emissions from digestate and slurry applied to three agricultural soils in the United Kingdom: Fluxes and emission factors. Environ. Pollut. *243*, 1952–1965. https://doi.org/10.1016/j.envpol.2018.08.102.

Shen, J., Treu, R., Wang, J., Hao, X., Thomas, B.W., 2019. Modeling growing season and annual cumulative nitrous oxide emissions and emission factors from organically fertilized soils planted with barley in Lethbridge, Alberta, Canada. Agric. Syst. *176*, 102654 https://doi.org/10.1016/j.agsy.2019.102654.

Smerald, A., Kraus, D., Rahimi, J., Fuchs, K., Kiese, R., Butterbach-Bahl, K., Scheer, C., 2023. A redistribution of nitrogen fertiliser across global croplands can help achieve food security within environmental boundaries. Commun. EArth Environ. *2023* https://doi.org/10.1038/s43247-023-00970-8.

Strassburg, B.B.N., Latawiec, A.E., Barioni, L.G., Nobre, C.A., da Silva, V.P., Valentim, J. F., Vianna, M., Assad, E.D., 2014. When enough should be enough: Improving the use of current agricultural lands could meet production demands and spare natural habitats in Brazil. Glob. Environ. Change *28* (1), 84–97. https://doi.org/10.1016/j.gloenvcha.2014.06.001.

Subbarao, G.V., Nakahara, K., Hurtado, M.P., Ono, H., Moreta, D.E., Salcedo, A.F., Yoshihashi, A.T., Ishikawa, T., Ishitani, M., Ohnishi-Kameyama, M., Yoshida, M., Rondon, M., Rao, I.M., Lascano, C.E., Berry, W.L., Ito, O., 2009. Evidence for biological nitrification inhibition in *Brachiaria* pastures. Proc. Natl. Acad. Sci. USA *106* (41), 17302–17307. https://doi.org/10.1073/pnas.0903694106.

Teutscherova, N., Vazquez, E., Arevalo, A., Pulleman, M., Rao, I., Arango, J., 2019. Differences in arbuscular mycorrhizal colonization and P acquisition between genotypes of the tropical *Brachiaria* grasses: is there a relation with BNI activity? Biol. Fertil. Soils 325–337. https://doi.org/10.1007/s00374-019-01353-y.

Teutscherová, N., Vázquez, E., Lehndorff, E., Pulleman, M., Arango, J., 2022. Nitrogen acquisition by two *U. humidicola* genotypes differing in biological nitrification inhibition (BNI) capacity and associated microorganisms. Biol. Fertil. Soils *58* (3), 355–364. https://doi.org/10.1007/s00374-022-01635-y.

Toledo, J.M., 1982. Man. Para. la Eval. Agron. Riept. Cent. Int. De. Agric. Trop. (CIAT) https://hdl.handle.net/10568/54148.

Van Groenigen, J.W., Velthof, G., van Groenigen, K.J., Kessel, C., 2010. Towards an agronomic assessment of N_2O emissions: A case study for arable crops. Eur. J. Soil Sci. *61*, 903–913. https://doi.org/10.1111/j.1365-2389.2009.01217.x.

Vázquez, E., Teutscherova, N., Dannenmann, M., Töchterle, P., Butterbach-Bahl, K., Pulleman, M., Arango, J., 2020. Gross nitrogen transformations in tropical pasture soils as affected by *Urochloa* genotypes differing in biological nitrification inhibition (BNI) capacity. Soil Biol. Biochem. *151* (August) https://doi.org/10.1016/j.soilbio.2020.108058.

Vázquez, E., Borer, E.T., Bugalho, M.N., Caldeira, M.C., McCulley, R.L., Risch, A.C., Seabloom, E.W., Wheeler, G.R., Spohn, M., 2023. The synergistic response of primary production in grasslands to combined nitrogen and phosphorus addition is caused by increased nutrient uptake and retention. Plant Soil *0123456789*. https://doi.org/10.1007/s11104-023-06083-7.

Velthof, G.L., Oenema, O., Postma, R., Van Beusichem, M.L., 1996. Effects of type and amount of applied nitrogen fertilizer on nitrous oxide fluxes from intensively managed grassland. Nutr. Cycl. Agroecosystems *46* (3), 257–267. https://doi.org/10.1007/BF00420561.

Velthof, G.L., Oudendag, D., Witzke, H.P., Asman, W.A.H., Klimont, Z., Oenema, O., 2009. Integrated assessment of nitrogen losses from agriculture in EU-27 using MITERRA-EUROPE. J. Environ. Qual. *38* (2), 402–417. https://doi.org/10.2134/jeq2008.0108.

Villegas, D., Arevalo, A., Nuñez, J., Mazabel, J., Subbarao, G., Rao, I., De Vega, J., Arango, J., 2020. Biological nitrification inhibition (BNI): Phenotyping of a core germplasm collection of the tropical forage grass *Megathyrsus maximus* under greenhouse conditions. Front. Plant Sci. *11* (June), 1–14. https://doi.org/10.3389/fpls.2020.00820.

Villegas, D., Arévalo, A., Sotelo, M., Nuñez, J., Moreta, D., Rao, I., Ishitani, M., Subbarao, G.V., Arango, J., 2022. Phenotyping of *Urochloa humidicola* grass hybrids for agronomic and environmental performance in the Piedmont region of the

Orinoquian savannas of Colombia. Grass Forage Sci. 10 (1) https://doi.org/10.1111/gfs.12582.

White, D., Holmann, F., Fujisaka, S., Reategui, K., Lascano, C., 2001. Will intensifying pasture management in Latin America protect forests - or is it the other way round? Agric. Technol. Trop. Deforestation 91–111. https://doi.org/10.1079/9780851994512.0091.

Xu, G., Fan, X., Miller, A.J., 2012. Plant nitrogen assimilation and use efficiency. Annu. Rev. Plant Biol. *63*, 153–182. https://doi.org/10.1146/annurev-arplant-042811-105532.