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Nitrous oxide emissions and forage accumulation in the Brazilian Amazon forage-livestock systems submitted to N input strategies

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Abstract

In the Brazilian Amazon, nitrogen input strategies are required to maintain forage–livestock systems productivity. However, greenhouse gases (GHG) emissions mitigation from tropical soils is also a global demand. This research aims to assess productivity and nitrous oxide (N_2O) emissions from Oxisol cultivated with Marandu palisade grass (*Brachiaria brizantha* [Hochst. Ex A.Rich.] Stapf) submitted to nitrogen (N) input strategies (N fertilization and biological N fixation) in the Brazilian Amazon. The treatments were the following: control (unfertilized); U40 (fertilized with 40 kg N/ha as urea); U80 (fertilized with 80 kg N/ha as urea); AS40 (fertilized with 40 kg N/ha as ammonium sulfate); AS80 (fertilized with 80 kg N/ha as ammonium sulfate); and IAB (inoculated with *Azospirillum brasilense*). From January to March 2016, soil N_2O emission, forage accumulation (FA) and relative emission (RE) were assessed during two 28-day cycles. The FA was greater in the U80 and AS80 than in control and IAB. The highest peaks of soil N_2O flux occurred from 4 to 7 days after N fertilization, primarily in the highest N rates treatments. Overall, 40 kg N/ha resulted in higher N_2O flux than control and IAB, which were lower than 80 kg N/ha regardless of the N source. The lowest fluxes occurred in the control and IAB (below 20 μg N-N_2O m^{-2} hr^{-1}). All of the emission factors (EF) calculated for both fertilizers and rates were lower than 0.35%, which is below the 1% established by the IPCC. Our results indicate the need for discussion of the EF in the pasture intensification to contribute to avoid deforestation and mitigating emissions. The inputs of 40 kg N/ha per application with urea or ammonium sulfate, due to the low EF and RE, are recommended as a pasture N input strategy in the Brazilian Amazon.

KEYWORDS
climate change, fertilization, livestock, mitigation

1 | INTRODUCTION

The beef cattle production in Brazilian Amazon is a pasture-based system that uses forage plants of high productive potential; however, due to inadequate management and low fertilization input, forage plants lose productivity a few years after pasture establishment (Dias-Filho, 2011). In addition, the current lack of management in livestock systems conducted under Amazon's edaphoclimatic conditions may result in advanced pasture and soil degradation (Macedo 1999).

The production systems intensification has been highlighted as an alternative to avoid pasture degradation while focusing on sustain forage productivity and improving system efficiency (Pedreira & Pedreira, 2014). This strategy involves nutrient replenishment in soil via fertilization or biological agents responsible for extracting more nutrients from the soil and biological nitrogen (N) fixation that may became available for plants (Hungria, Nogueira, & Araujo, 2016). Due to the low natural fertility of Amazon soils, frequent nutrient replenishments are imperative for soil maintenance and improvement (Dias-Filho, 2015), and the fertilization strategies impact should be constantly evaluated, as well as their effects on the environment (Peters et al., 2012).

Fertilization using N sources, mainly urea and ammonium sulfate, is considered an important strategy to increases forage accumulation(FA) in pasture-based systems (Martha Júnior, Vilela, & Sousa, 2007). On the other hand, N fertilization is responsible for increasing soil nitrous oxide (N_2O) emission, which contributed, in 2011, for 14% of the total greenhouse gases (GHG) emissions from the agricultural sector worldwide (Tubiello et al. 2014). Nitrous oxide has a global warming potential 298 times greater than carbon dioxide (CO_2) and is involved in deleterious chemical processes to the ozone layer (IPCC 2013). Among the mitigation strategies for the soil N_2O emissions, the N fertilizer sources with low emission potential and with more efficiency, and/or the biological agents to N fixation, can replace or decrease N fertilizers application and reduce N_2O emission (Smith et al., 2008).

Biological N fixation using *Azospirillum brasilense* inoculation in grasses has become a promising practice for increasing forage productivity (Pedreira et al., 2017). Unlike biological N fixation in leguminous plants, in which there is a mutualistic relationship, N fixation in grass is intermediated by endophytic bacteria, which provide part of the N fixed to the associated plants (Hungria et al., 2016). Although less efficient, N fixation in grasses can mitigate N_2O emissions by reducing the need for mineral fertilizers (Hungria et al., 2016; Smith et al., 2008).

Overall, when evaluating only the N_2O emissions, fertilized agricultural systems have emitted more N_2O than unfertilized systems (Soares et al., 2016; Uchida & Clough, 2015), indicating that system intensification could generate a greater environmental impact. For this reason, the productivity should be considered when analyzing the influence of N input strategy on the GHG emissions (Burney, Davis, & Lobell, 2010).

Based on that, we hypothesize that N input strategies will enhance FA, although each strategy will present a different N_2O emission factor, and this knowledge contributes to developing sustainable forage-based systems. In order to test this hypothesis, we assessed FA and N_2O emissions from Oxisol cultivated with Marandu palisade grass (*Brachiaria brizantha* [Hochst. Ex A.Rich.] Stapf) pasture submitted to N input strategies (N fertilizer and biological N fixation) in the southern Brazilian Amazon.

2 | MATERIAL AND METHODS

2.1 | Field experiment

The experiment was carried out in the Amazon Biome at Embrapa Agrossilvipastoril, Sinop, Mato Grosso (latitude: 11°50'53" S - longitude: 55°38'57"W). The soil of the experimental area was classified as Oxisol (Hapludox) occurring in a flat relief (Soil Science Division Staff, 2017). The climate was classified according to the Koppen Climate Classification System as an Am monsoon climate, which alternates between a rainy and a dry season (Alvares, Stape, Sentelhas, Moraes Gonçalves, & Sparovek, 2013), with an average annual temperature of 25.5°C (20.2 °C minimum and 33.0°C maximum average temperatures). Average annual relative air humidity is 70%, with 2,250 mm of annual precipitation (Embrapa, 2017). Weather data were obtained from a record station located 500 m from the experiment site.

The experimental area was established with Marandu palisade grass intensely grazed during 2 years without fertilization to achieve a moderate degradation stage. Besides that, the area was divided into 18 plots (3 x 3 m), in a randomized complete block design with six N inputs strategies (treatments) and three replicates. The treatments were the following: control (unfertilized); U40 (fertilized with 40 kg N/ha as urea); U80 (fertilized with 80 kg N/ha as urea); AS40 (fertilized with 40 kg N/ha as ammonium sulfate); AS80 (fertilized with 80 kg N/ha as ammonium sulfate); and IAB (inoculated with *Azospirillum brasilense*).

To evaluate the N input strategy effect, two cycles of 28 days in the middle of the growth season were evaluated: cycle 1—from January 13 to February 10 and cycle 2—from February 11 to March 10, 2016. The urea (45% N) and ammonium sulfate (21% N) were applied manually on January 15 and February 12, on soil surface using the granular formula. The inoculation was sprayed on the post-harvest sward, at the same dates, using *Azospirillum brasilense* (2×10^8 colony forming unit/ml, strains AbV5 and AbV6) at a rate of 300 ml/ha diluted by a volume of 200 L/ha.

2.2 | Forage accumulation and relative forage accumulation

At the beginning of the experiment, all plots were harvested at 15 cm sward height. In each cycle, forage mass (FM) was quantified at preharvest by sampling the forage inside two quadrats (0.5 × 1 m) at 15 cm height. Forage mass harvested above 15 cm at the end of each cycle was used to calculate FA. Samples were dried at 55° C in a forced-air dryer until constant weight and weighed. The relative forage accumulation (RFA) was obtained by deducting control FA from the U40, U80, SA40 and SA80 values.

2.3 | Soil N_2O emissions

Gas samples were collected using rectangular vented static chambers (Parkin & Venterea, 2010). The metal chamber bases (5 cm height x 40 cm width x 60.5 cm length) were installed in the soil at a depth of 5 cm. The tops were constructed using polypropylene trays (9.2 cm height x 40 cm width x 60.5 cm length) coated with a double-sided thermo-reflective blanket to reduce the internal temperature of the chamber. Samples were collected over a 60-min period, with 4 samplings (0, 20, 40 and 60 min) between 8 and 10 a.m. (Parkin & Venterea, 2010). For sampling, 20 cm^3 polypropylene syringes were used with three-way couplings to avoid atmospheric air contamination. Samples in the syringes were transferred to 20 cm^3 glass bottles (vials), previously evacuated in the laboratory. Gas samples were collected daily during the first 15 days of each cycle, starting 2 days prior to fertilization. After 15 days, samples were taken every 5 days.

The sample gas concentrations were determined in a gas chromatograph (GC-2014, Shimadzu®) using an electron capture detector (ECD), for nitrous oxide quantification. The chromatograph system is fitted with Hayesep 80/100 mesh (1/8 "x 2.1 mm), T, D and *N* (two) series columns of 1, 2 and 1.5 m, respectively, and maintained at 75°C. Ultrapure nitrogen was used as the tracer gas at a flow rate of 25 ml/min, and injector pressure was maintained at 300 kPa. The injection volume was 1 ml, and the total analysis times were 5 min. In order to quantify the N_2O concentrations, three known standard concentrations of 382, 808 and 2,027 ppb were used in the chromatograph.

Based on the analytical results, it was possible to adjust the linear model by relating the variations in N_2O concentrations within the chamber as a time function (0, 20, 40 and 60 min). These data were then used to calculate N_2O flux from the soil to the atmosphere following the equation proposed by Hutchinson and Livingston (2001): Flux ($\mu g\ N\ m^2\ hr^{-1}$) = (dC/ dt) x V/ A x (m/ vm), where dC/ dt = change in gas concentration in the chamber as a function of time; V = chamber volume (L); A = area of the chamber (m^2); m = molecular weight (g); and Vm = molecular volume of the gas (L). Flux results were used to estimate the cumulative gas emissions over the evaluation period using the trapezoidal integration principle (Klein et al., 2015).

The EF, which considers the amount of N_2O emitted from the soil in relation to the amount of N applied, was calculated for urea and ammoniac sulfate treatments, as follows:

$$\mathrm{EF\ (\%)} = \frac{\text{kg of N} - N_2O \text{ in treatment} - \text{kg of N} - N_2O \text{ in the control}}{\text{kg of N applied} \times 100}$$

To determine the relative emission (RE), which is the ratio between total N_2O emissions and FA, RFA (previously described) was divided by accumulated emissions.

2.4 | Soil analysis

Disturbed soil samples from each treatment were collected from the 0–5 and 5–10 cm layers on the days 0, 2, 4, 6, 10, 14, 19, 24 and 28 of each cycle to determine the following attributes: gravimetric humidity, pH and inorganic forms of *N* (exchangeable ammonium and nitrate). Half of the sample volume collected in the field was stored in a freezer at −16°C to avoid transformations of mineral N in the soil until analysis (Li et al., 2012). Thereafter, 25% of the sample was used to determine gravimetric moisture and another 25% was air-forced dryer and sieved through 2-mm mesh, which was used for pH determination and for initial soil characterization.

Undeformed soil samples were collected in cylinders (98 cm^3) at the beginning of each cycle to determine soil bulk density, which, together with gravimetric moisture and particle density data, was used to calculate the water-filled pore space (WFPS) of the soil (Linn & Doran, 1984). Since soil particle density is a stable short-term attribute, it was determined at the onset of the experiment in triplicate at each studied depth (Embrapa, 2011), which revealed a result of 2.40 g/cm^3 and 2.69 g/cm^3 at depths of 0–5 and 5–10 cm, respectively. The pH was determined in water (deionized) using a soil:water ratio of 1:2.5. The 0–5 cm layer had 37%, 8% and 55%, while the layer of 5–10 cm had 37%, 7% and 56% of clay, silt and sand, respectively, resulting in a clayey texture for both layers (Santos et al. 2013). Cation exchange capacity, base saturation and aluminum saturation were determined according to Embrapa (2011) and presented 7.01 $cmol_c/kg$, 55% and 0% for the 0–5 cm layer, and 7.28 $cmol_c/kg$, 48% and 0% for the 5–10 cm layer, respectively. Total soil carbon (C) and N was determined using a dry combustion element analyzer (®Elementar Analysensysteme, GmbH) and revealed 3.24% C and 0.22% N for the 0–5 cm layer, and 2.10% C and 0.13% N for the 5–10 cm layer, respectively.

For the mineral N extraction (NH_4^+ and NO_3^-), 1 mol/L KCl solution was used at a soil:solution ratio of 1:5, stirred for 30 min and centrifuged at 3,800 *g* rpm for 5 min and then at 18,700 *g* rpm for 5 min, thus obtaining a limpid extract (Cantarella & Trivelin, 2001; Li et al., 2012). Centrifugation was used instead of filtration following recommendations of Cantarella and Trivelin (2001), which pointed out N contamination in extracts due to the use of filters. One day prior, samples were removed from the freezer to thaw in a refrigerator at 4°C. The determination of NH_4^+ and NO_3^- was performed using the colorimetric method (Sattolo, Otto, Mariano, & Kamogawa, 2016).

2.5 | Statistical analysis

Forage accumulation data were analyzed using a mixed models method with parametric structure in the covariance matrix, through the MIXED procedure of the statistical software SAS (SAS Studio, v. 9.4) (Littell, Milliken, Stroup, Wolfinger, & Schabenberger, 2006) with repeated measurements and using the maximum likelihood-restricted method (REML). Block and block × treatment interaction was considered as random effect, treatment and cycle as fixed effect. The covariance matrix used was the Akaike information criterion (AIC) (Wolfinger, 1993), and the correction of degrees of freedom was made using the method of Satterthwaite (1941) (DDFM = SATTERTHWAITE). The treatment means were estimated by least squares mean (LSMEANS), and comparison was performed using the probability of the difference (PDIFF) of Student's *t* test ($p < .05$).

The average and standard error was determined for daily N_2O flux, average N_2O flux of each cycle and for the entire experimental period, as well as for the RE, inorganic N and WFPS, since these data did not exhibit a normal distribution. Emission factor data were submitted to the variance analysis and, when significant, the Tukey test was applied at 5%.

3 | RESULTS AND DISCUSSION

Forage accumulation differed for strategies (p = .0114; Figure 1), but there was no cycle (p = .8248) or cycle × treatment interaction (p = .5025) effects. Once the essential nutrients do not limit the grasses growth potential, the N available will contribute to increasing FA. The greater N rate contributed to increased leaf area index, leaf and canopy photosynthesis rates and FA (Yasuoka et al., 2018). It occurs because N is a component of chlorophyll, an enzyme responsible for photosynthesis (Rubisco) and proteins (Taiz & Zeiger, 2013), which drives the process of energy capture and CO_2 fixation of by plants. Thus, biomass enhancement depends on leaf area development driven by cellular expansion and photosynthetic efficiency (Martins, Monteiro, & Pedreira, 2015).

In this scenario, FA was greater in the U80 and AS80 than in control and IAB. The U40 and SA40 presented FA intermediated to all N inputs strategies. Fertilization with higher rates may result in greater FA; however, the N source could also drive this process (Bourscheidt, Pedreira, Pereira, Zanette, & Devens, 2019). Although we would expect more FA using AS source, once it has sulfur (23%) in addition to *N* (Chien, Gearhart, & Villagarcía, 2011), in our study, there was no N source effects on FA at the same rates. Furthermore, the values obtained in this experiment are similar than those reported by Pedreira et al. (2017) in Marandu palisade grass pasture inoculated with *A. brasilense* or fertilized with 40 kg N/ha using urea in Oxisol dystrophic soils.

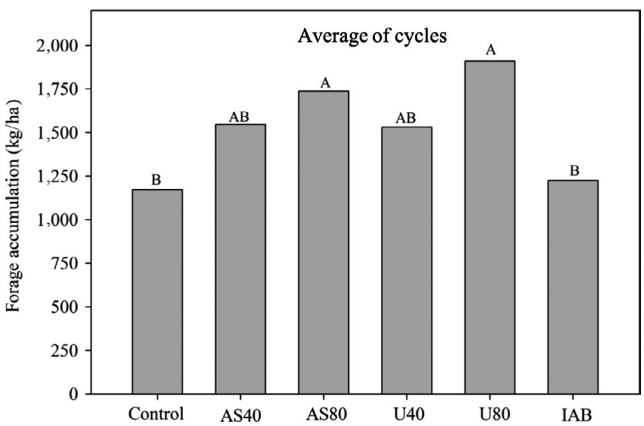

FIGURE 1 Forage accumulation (kg/ha, average of cycles) in pastures under N input strategies in the Brazilian Amazon. U40 (40 kg N/ha as urea); U80 (80 kg N/ha as urea); AS40 (40 kg N/ha as ammonium sulfate); AS80 (80 kg N/ha as ammonium sulfate); and IAB (Inoculated with Azospirillum brasilense). Means followed by a common uppercase letter in the bar are notdifferent by t test (p < .05)

The foliar application of *Azospirillum brasilense* did not contribute to increase FA, which presented values similar to the control. Lower nitrogen availability can affect root development, photoassimilate production and, consequently, reduce the growth rate (Gimenes et al., 2017). Probably, the bacteria would need longer period to colonize and offers nitrogen inputs to the plant.

Marandu palisade grass under pasture intensification strategies highlights the contribution to the N_2O emissions from Oxisol in the edaphoclimatic conditions of the Brazilian Amazon. Average soil N_2O flux (μg N m^2 hr^{-1}) in cycle 1, cycle 2 and in the average of the two cycles was higher in pastures fertilized with 80 kg N/ha as urea (Figure 2). Average soil N_2O flux in other treatments with N fertilization (U80, AS40 and AS80) did not differ in cycle 1. In cycle 2 and in the average of the two cycles, soil N_2O flux differed between AS40 and AS80. The flux in all cycles, including the average of both cycles, were similar in AS80 and U40, with values between 15 and 30 μg N m^{-2} hr^{-1}. The flux in U40 or AS40 did not differ in either cycle or in the average of the two cycles. The high N_2O fluxes from N fertilized soils are because the denitrification pathway, which would not be possible without soil moisture (precipitation) during this period. The difference in N_2O emissions between U80 and AS80, which was similar to AS40 and U40, is an important point that needs to be clarified. As the edaphoclimatic conditions were similar to both N rates, the different N_2O fluxes at the same N rate may be due to the fertilizer reactions in soils. Urea has an alkalizing hydrolysis which increases the nitrite accumulation, leading to higher N_2O emissions if compared to AS (Tierling & Kuhlmann, 2018).

The lowest average flux was measured in the control and inoculation treatments, with all values below 10 μg N m^{-2} hr^{-1}. Although, in cycle 1, the AS40 soil N_2O flux (14.4 μg N m^{-2} hr^{-1}) was similar to the control (8.4 μg N m^{-2} hr^{-1}). The low soil N_2O fluxes in control and IAB are due to the absence of the mineral N application. It highlights the low N mineralization in Oxisol, decreasing N availability to follow the nitrification/denitrification processes responsible for N_2O formation in soils (Butterbach-Bahl, Baggs, Dannenmann, Kiese, & Zechmeister-Boltenstern, 2013).

In the two cycles, the highest peaks of soil N_2O flux occurred a few days after N fertilization (Figure 3), and largely in the treatments with the highest N rates. The highest N_2O flux peaks were measured in U80, with values up to 140 and 90 μg N-N_2O m^{-2} hr^{-1} for cycles 1 and 2, respectively. In the AS80, the maximum peaks were up to 50 and 40 μg N-N_2O m^{-2} hr^{-1}, in cycles 1 and 2, respectively. At the rate of 40 kg N/ha, flux dynamics were similar for both fertilizers sources. The lowest fluxes were measured in the control and IAB, with values predominantly below 20 μg N-N_2O m^{-2} hr^{-1}.

The emissions were greatly until 10–12 days post-fertilization. In cycle 1, on the first day after fertilization, the flux increased for all N fertilization treatments; however, in cycle 2, this only occurred after the second day. The highest in N_2O flux increments started to occur at days 2 and 3 post-fertilization. Twelve days after fertilization, the fluxes were similar among treatments, which were equal to those on

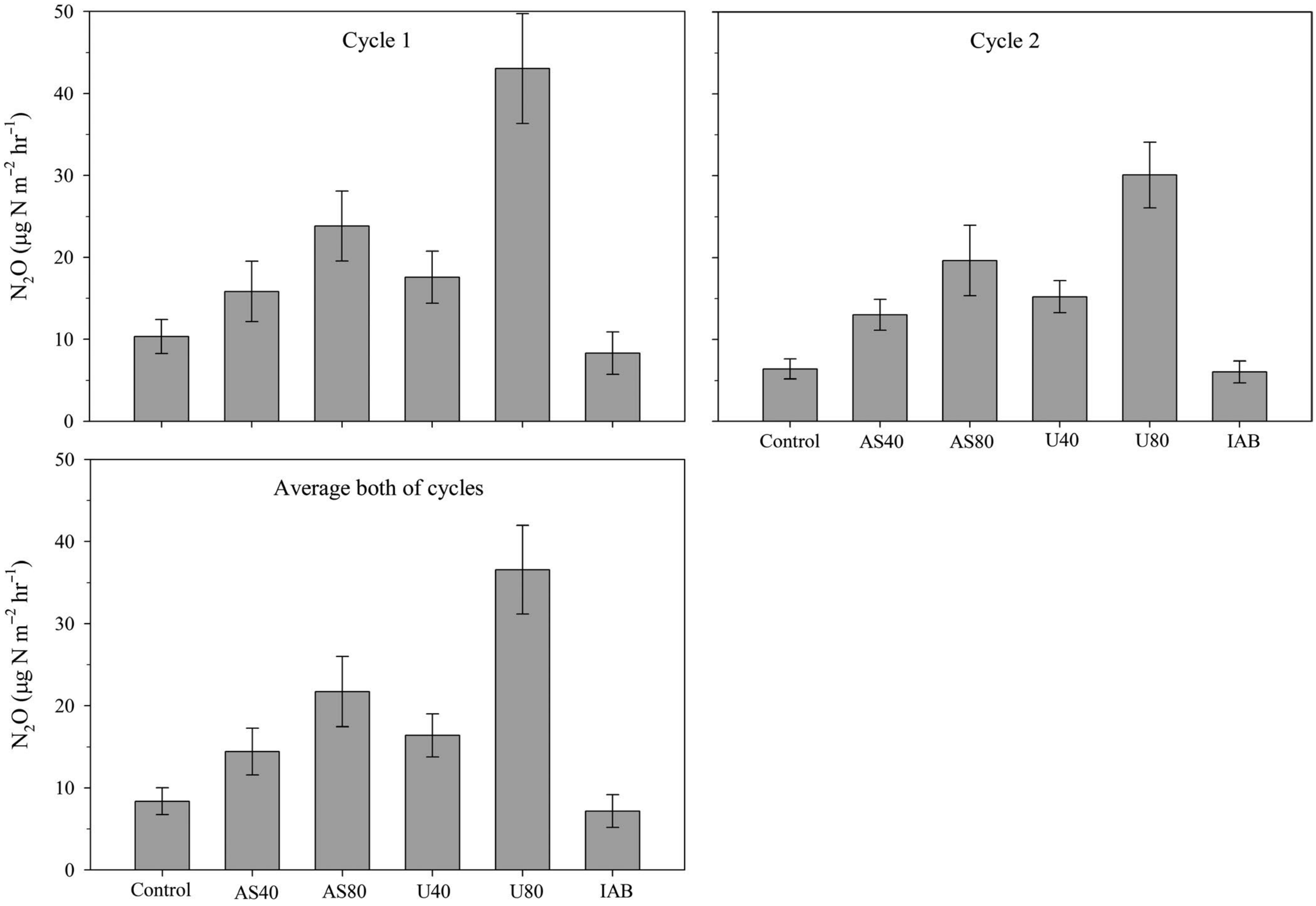

FIGURE 2 Average soil N_2O flux (µg N-N_2O m^{-2} hr^{-1}) under N input strategies in cycle 1, cycle 2 and the average of cycles in the Brazilian Amazon. Vertical bars correspond to the mean standard error. U40 (40 kg N/ha as urea); U80 (80 kg N/ha as urea); AS40 (40 kg N/ha as ammonium sulfate); AS80 (80 kg N/ha as ammonium sulfate); and IAB (Inoculated with Azospirillum brasilense)

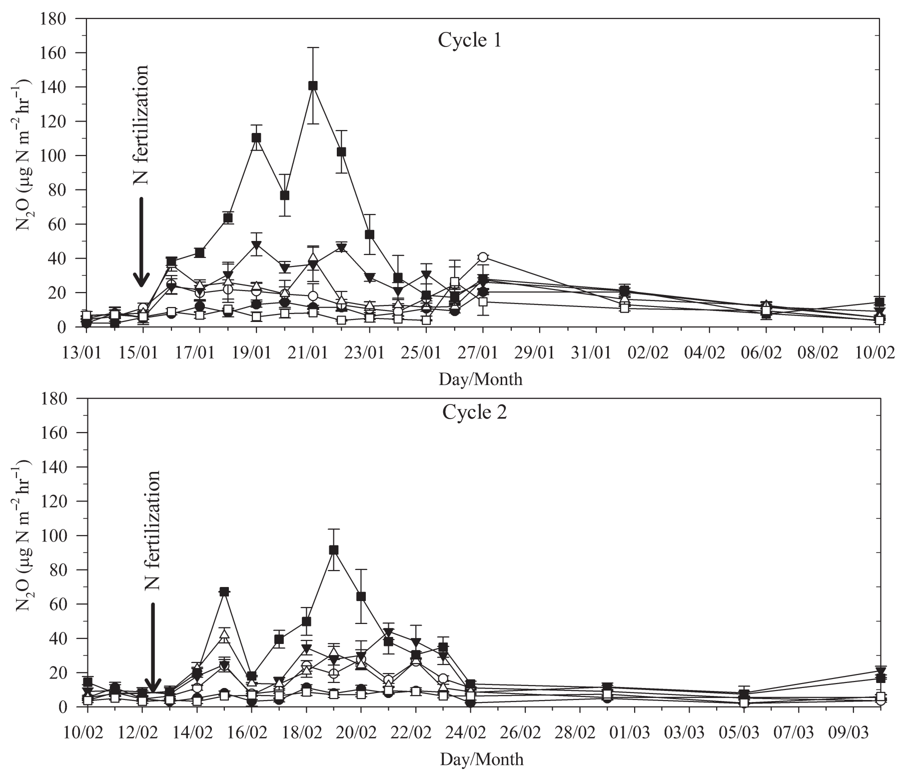

FIGURE 3 Soil N_2O flux (µg N-N_2O m^{-2} hr^{-1}) under N input strategies in the Brazilian Amazon

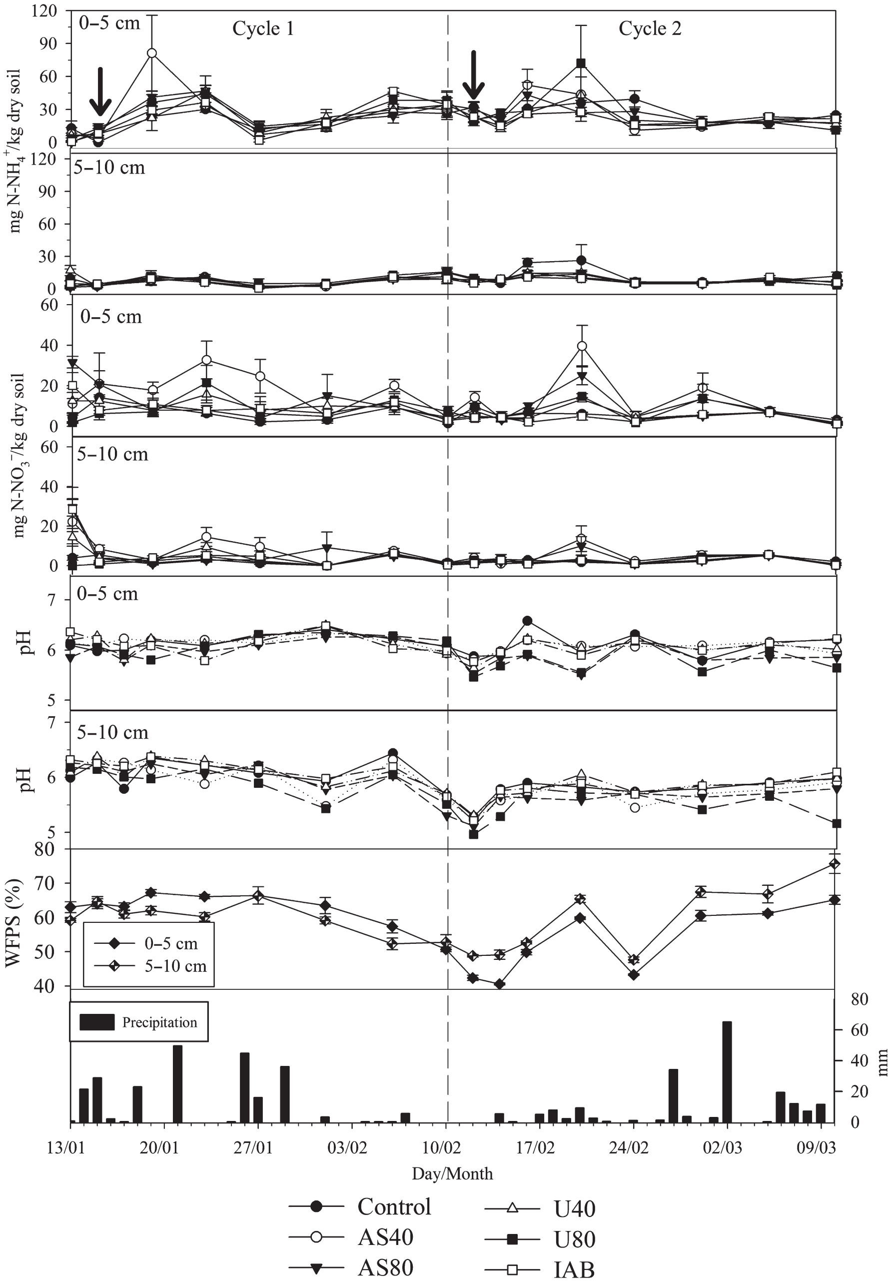

FIGURE 4 Mineral nitrogen availability (nitrate and ammonium), pH and water-filled pore space (WFPS) in the soil layers at 0–5 and 5–10 cm, and rainfall in pastures N input strategies in the Brazilian Amazon.U40 (40 kg N/ha as urea); U80 (80 kg N/ha as urea); AS40 (40 kg N/ha as ammonium sulfate); AS80 (80 kg N/ha as ammonium sulfate); and IAB (Inoculated with Azospirillum brasilense)

pre-fertilization period (values below 20 μg N-N_2O m^{-2} hr^{-1}). Similar to other studies, the duration of high soil N_2O flux and the flux level depend on the N rate which affects, with the environmental conditions, the inorganic N availability (Soares et al., 2016; Tierling & Kuhlmann, 2018). It demonstrates that under Brazilian Amazon edaphoclimatic conditions, the influence period of the N fertilization would be up to 2 weeks, depending of the precipitation.

In treatments receiving higher N rates (AS80 and U80), the highest flux peaks in cycle 1 may have been caused by higher rainfall (233 mm) than in cycle 2 (191 mm). The higher rainfall during cycle 1 provided, on average, a higher and more constant WFPS than during cycle 2 (Figure 4), suggesting a greater influence of denitrification on N_2O flux, which occurs when the soil has less oxygen (Van der Weerden, Kelliher, & Klein, 2012). However, this process only occurred following the fertilization input, which indicates that even in soil with more oxygen (as in the end of cycle 2), no soil N_2O emission occurs without mineral N being available.

The mineral N availability in the soil was similar among treatments (Figure 4). Although the availability of NH_4^+ and NO_3^- in the 0–5 cm layer was higher than in the 5–10 cm layer. In the AS40, the higher N availability in the 0–5 cm layer was observed 4 days after fertilization. In fact, the highest N rates treatments (80 kg N/ha) did not present a greater mineral N availability, a phenomenon that should be studied further. Since the present study fertilizers were manually distributed on the soil surface, partial surface runoff could have occurred, resulting in nutrient removal from the pasture (Burkitt, 2014). Furthermore, low N content may be related to its fast processing rate by microorganisms once the humidity and temperature conditions become adequate for nitrification and denitrification processes (Butterbach-Bahl et al., 2013). Future studies should

TABLE 1 Ammonium sulfate (AS) and urea (U) emission factors at rates of 40 and 80 kg N/ha in cycle 1, cycle 2 and the average of both cycles of Marandu palisade grass pastures in the Brazilian Amazon

Treatment	Cycle 1	Cycle 2	Average
	%		
AS40	0.100b	0.088b	0.108b
AS80	0.173b	0.205ab	0.189b
U40	0.086b	0.088b	0.126b
U80	0.318a	0.286a	0.321a

Note: Averages followed by the same letters in the column of each cycle do not differ by Tukey's test at 5% probability.

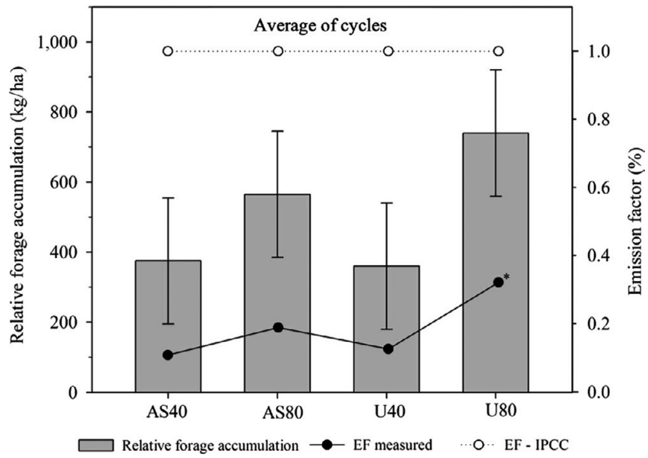

FIGURE 5 Relative forage accumulation (kg/ha) and emission factors (EF, %) in pastures under N input strategies in the Brazilian Amazon (* differs by Tukey's test at 5% probability). U40 (40 kg N/ha as urea); U80 (80 kg N/ha as urea); AS40 (40 kg N/ha as ammonium sulfate); AS80 (80 kg N/ha as ammonium sulfate); IAB (Inoculated with Azospirillum brasilense); and IPCC (Intergovernmental Panel on Climate Change)

elucidate N changes in the soil, enhancing the soil sampling frequency to improve the understanding of the N dynamics processes.

In cycle 1, the 0–5 cm soil layer in pastures fertilized with AS40 presented the highest NH_4^+availability of approximately 70 mg N/kg on January 19 and, after 4 days (January 23), exhibited a greater availability of NO_3^- (30 mg N/ha). This was not expected, since there was greater N rate; however, it indicates the occurrence of the nitrification process, which transforms N-ammoniac into N-nitric and must have been incorporated in N_2O emission via denitrification. This process is driven by the favorable conditions identified in cycle 1 (e.g., high WFPS), leading to microsites under anoxic soil conditions (Van der Weerden et al., 2012). The nitrification process was also observed in cycle 2, since the NO_3^- availability increased only after fertilization, and exhibited higher NH_4^+ availability, which was primarily due to enhanced rainfall. However, the nitrification process can be inhibited by grasses (Byrnes et al., 2017; Subbarao et al., 2012), which do not discard the low levels of N-nitric affected by Marandu palisade grass, especially with the ammoniacal fertilizers application that stimulates the production of biological nitrification inhibitors in grasses (Peters et al., 2012; Subbarao et al., 2012).

Notably, the WFPS values support an important role of moisture in N_2O emissions (Van der Weerden et al., 2012). In cycle 1, with higher WFPS in the 0–5 cm layer compared to the 5–10 cm layer, the emissions were higher than in cycle 2. In cycle 2, in which the layer underlying the surface exhibited higher WFPS, lower N_2O emissions were measured, indicating that the N_2O emissions occurred mainly in the superficial layer due to the greater mineral N availability.

The pH of the two soil layers was also similar among treatments. The 0–5 cm layer presented a slight acidification a few days after fertilization, with the highest rates in cycle 2, regardless of the nitrogen source (Tierling & Kuhlmann, 2018). The treatments effect on soil pH in the 5–10 cm layer was also small; however, a decrease in pH occurred a few days after the reduction in the overlying layer.

Soils with the pH range measured in our study allowed for higher N_2O emissions than those with lower pH, since they presented greater nitrite (NO_2^-) accumulation (Tierling & Kuhlmann, 2018). This is due to the higher ammonia (NH_3) availability during the nitrification process, which impairs the microorganisms development (Nitrobacter) responsible for nitrite oxidation to nitrate (Venterea et al., 2015). Under these conditions, the ammonium oxidation processes occur; however, due to the microorganisms sensitivity to the NH_3 presence in a higher proportion than in a lower pH soil, nitrite accumulates and leads to a N_2O emission via nitrifier denitrification (Tierling & Kuhlmann, 2018; Wrage, Velthof, Beusichem, & Oenema, 2001). This explains, in the cycle 2, a flux peak of up to 70 μg N m^{-2} hr^{-1} occurred 3 days after fertilization in pastures fertilized with U80 despite low rainfall and subsequently lower WFPS (Figure 4). With the return of the rainfall between 4 and 7 days after fertilization (Figure 3), a greatest N_2O flux peak occurred, which was probably due to the denitrification process since soil WFPS increased to over 60%, supporting the environmental conditions for this process (Butterbach-Bahl et al., 2013).

The highest EF was calculated to the U80, in the cycle 1, 2 and in the average of the two cycles (Table 1). In cycle 2, with lower rainfall, the rate of 80 kg N/ha for both sources (AS80 or U80) presented the similar EF, with values of 0.2% and 0.3%, respectively. In the others treatments (AS40, AS80 and U40), the EF were similar regardless of source, rate and cycle. The greatest EF was measured in our study to the U80 (0.321%) represents about 30% of the default EF suggested by the IPCC (2006), which established a factor of 1%. Thus, for the national inventories, we suggested that the contribution of nitrogen fertilization to the soil N_2O emissions under Brazilian Amazonia's edaphoclimatic conditions should be lower than 0.35%. The Brazilian inventory could achieve even lower reported emission values if was possible to also takes into account the N fertilizer source and rate.

Moreover, it is important to highlights the link between EF and RFA. Overall, as rate was increased from 40 to 80 kg N/ha, the RFA and EF also were enhanced (Figure 5). However, the ratio between

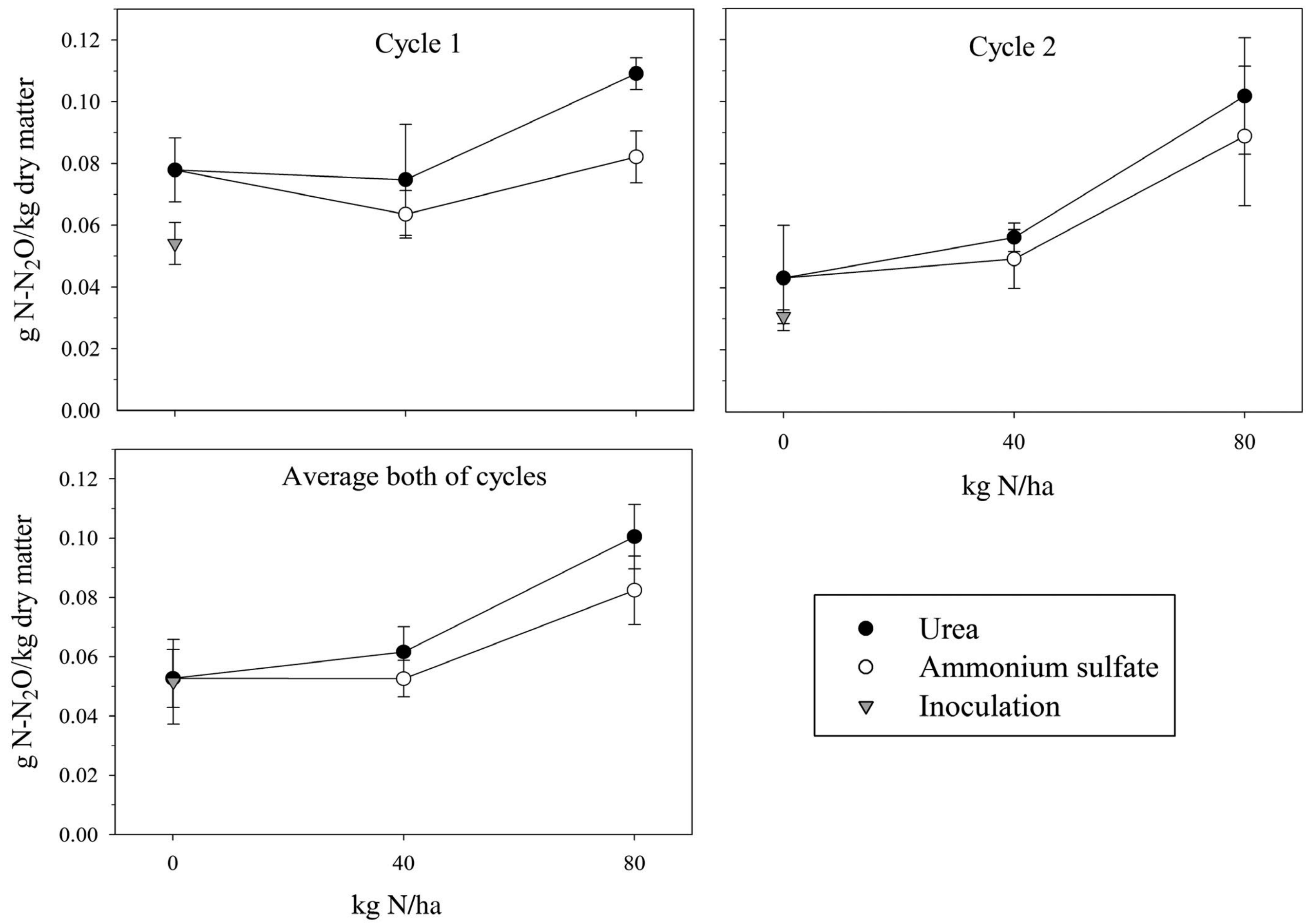

FIGURE 6 N_2o emission (g $N\text{-}N_2O$) per ton of dry matter produced in cycle 1, in cycle 2 and in two cycles (average) evaluated in an experiment of Marandu palisade grass submitted to N input strategies in the Brazilian Amazon

the amount of emitted $N\text{-}N_2O$ (g/ha) and FA (kg/ha) during the study period allows the evaluation of the optimal strategy to increase FA while resulting in lower N_2O emissions from soil. In all cycles, including the average of the two cycles, U80 resulted in higher RE when compared to the U40 and SA40 (Figure 6). This demonstrates the lowest efficiency of urea as nitrogen source at highest rates, because to accumulate a determinate amount of forage, the rate of 40 kg N/ha as urea or ammonium sulfate results in lower N_2O emission than 80 kg N/ha as urea. However, in the first cycle and on average of both cycles, AS80 resulted in the RE compared with the control. Thus, if high and well-distributed rainfall is expected, the application of AS80 could offer low N_2O emission and high productivity. This suggests that ammonium sulfate fertilization represents the best option, since it reflects greater FA in relation to control, specially at high rates. Profitability analyses should be performed to allow N input strategies decision-making in a production system.

The similar RE among the control and the 40 kg N/ha treatments in cycle 1, cycle 2 and the average of the two cycles suggests the application of both sources at this rate. This indicates that pasture fertilization strategy is highly recommended due to the greater potential to produce animal protein when compared with the control.

Excepted in the cycle 1, IAB resulted in the same RE as the control. In the cycle 2, IAB presented a lower RE than all N fertilizers; however, in cycle 1 and in the average of both cycles, RE IAB was similar to AS40 and U40. The IAB could be a N input strategy due to the similarity with the control; however, we should emphasize that foliar inoculation may not be the best application form for this technology (Pedreira et al., 2017). The seed inoculation during the pasture establishment should be tested for N_2O emissions and its relationship with grass productivity.

Based on the N_2O flux average and dynamics, EF, and RE, we affirmed that the optimal N input strategy for intensification of Marandu palisade grass pastures in the Brazilian Amazon would be at rate of 40 kg N/ha per application, using ammonium sulfate or urea. This would allow for increased FA with lowest N_2O emissions per unit of product when compared with highest fertilization rates. For the pastures management, a rate of 60 kg N/ha is the maximum recommended per application (Martha Júnior et al., 2007), which supports the results obtained by the our study focused on N_2O emissions. However, economic analyses were not included, it is recommended for each potential producer in each region, since prices can vary greatly according to the fertilizer industry distance (Pedreira, Pereira, & Paiva, 2013). For this reason, in some regions with even higher N_2O emissions, urea could be more economically advantageous than ammonium sulfate.

The adoption of 40 kg N/ha per application as a technologic tool could help mitigate GHG emissions, improving FA and, consequently, forage quality and animal production when compared to systems without fertilization (Tesk et al., 2018). Thus, sustainable pasture intensification will avoid new areas of natural vegetation being opened and incorporated as areas for agricultural production. Our data suggest that Brazilian Amazon has potential to support to forage–livestock systems with relatively high pasture productivity and low emissions that may minimize negative environmental impacts.

4 | CONCLUSION

The input of 80 kg N/ha using urea results in higher N_2O flux average and peak from soil, as well as a higher emission factor than 80 kg N/ha using ammonium sulfate and the 40 kg N/ha using urea and ammonium sulfate.

The application of 40 kg N/ha (urea or ammonium sulfate) is recommended as a pasture N input strategy in the Brazilian Amazon due to the lower emission factor and relative efficiency.

Further studies on inoculation should be performed, particularly with seed inoculation, to better examine this technique as a viable pasture N input strategy in the southern Amazon biome.

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