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3,4-Dimethylpyrazole Phosphate (DMPP) Reduces N_2O Emissions from a Tilled Grassland in the Bogotá Savanna

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Abstract: Grasslands are subject to a wide range of land management practices that influence the exchange of the three main agricultural greenhouse gases (GHGs) that are related to agriculture: carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4). Improving nitrogen fertilization management practices through the use of nitrification inhibitors (NIs) can reduce GHGs emissions. We conducted a field experiment at the Colombian Agricultural Research Corporation with four fertilization treatments: urea (typical fertilizer used in this region), ammonium sulfate nitrate (ASN), ASN plus the NI 3,4-dimethylpyrazole phosphate (ASN+DMPP), and an unfertilized control. The highest grassland yields (1956 and 2057 kg DM ha^{-1}, respectively) and apparent fertilizer nitrogen recoveries (34% and 33%, respectively) were generated by the conventional urea fertilizer and ASN+DMPP. Furthermore, the use of ASN+DMPP reduced the N_2O emissions that were related to N fertilization to the level of the unfertilized treatment (ca. 1.5 g N_2O-N ha^{-1}), with a significant reduction of N-yield-scaled N_2O emissions (ca. 20 g N_2O-N kg N $uptake^{-1}$). These results support the application of DMPP as an alternative strategy to increase grassland yield while simultaneously reducing the environmental impact of N fertilization.

Keywords: methane; nitrous oxide; pasture; tillage; yield-scaled N_2O emissions

1. Introduction

Agriculture is responsible for just under a quarter (10–12 Gt CO_2-eq $year^{-1}$) of global anthropogenic greenhouse gas (GHG) emissions [1]. Among the GHGs that were emitted through human activity, carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) have the greatest links with intensive agriculture, mainly because of deforestation, livestock emissions, and soil and nutrient management [1,2].

The atmospheric concentration of N_2O has increased by 20% over the last two and a half centuries, from 271 ppb in 1750 to 324.2 ppb in 2011 [3]. Very few studies have been conducted in Colombia dealing with GHGs emissions from grasslands; however, GHGs emissions from agriculture are estimated to be 158.6 Gt CO_2-eq $year^{-1}$, of which, 10% corresponds to N_2O [4]. The main source of N_2O in agricultural soils comes from microbial activity in processes such as nitrification, denitrification, and nitrifier denitrification [5,6]. Therefore, the use of nitrification inhibitors (NIs) has become an increasingly important strategy in recent decades to help reduce N_2O emissions [1]. 3,4-dimethylpyrazole phosphate (DMPP) is one of the most widely used NIs. It is incorporated pre-blended in N fertilizers and delays the bacterial oxidation of ammonium (NH_4^+) by inhibiting the activity of the ammonia monooxygenase enzyme [7].

CO_2 is a major GHG. Its atmospheric concentration has increased by 40% since 1750 and from 278 ppm to 390.5 ppm in 2011 [3]. It is generated by natural processes, such as respiration and decomposition of organic matter and by anthropogenic processes. Agricultural CO_2 emission is considered to be almost neutral because it is also associated with annual cycles of carbon fixation through photosynthesis.

The atmospheric concentration of CH_4 has increased by 150% since 1750 and from 722 ppb to 1803 ppb in 2011 [3]. The main sources of agricultural emissions of CH_4 are the intestinal metabolism of domesticated ruminants, biomass burning, and production systems in flooded soils [8], because methanogenic bacteria generate CH_4 in anoxic environments. Contrastingly, grasslands and arable lands act as sinks because methanotrophic bacteria oxidize and therefore consume CH_4 in aerobic environments. When considering that methane monooxygenase can also oxidize NH_4^+ [8], increasing soil NH_4^+ concentration through nitrogen fertilization may induce an increase in the net soil CH_4 emission into the atmosphere.

Managed grasslands are one of the most extensive agricultural systems in the world, occupying more than 25% of the global land surface [9]. Depending on edaphoclimatic conditions fertilization, and the type of management, grasslands can be significant sources of CO_2, CH_4, and N_2O emissions [1]. Thus, the intensification of these managed productive systems with large inputs of nitrogen (N) fertilizers can lead to serious environmental risks, mainly N_2O emissions. Soil tillage is another management practice that is used to increase grassland yield by introducing improved grass species. It can affect, amongst other parameters, the size and distribution of soil organic matter aggregates, as well as soil porosity, and therefore its aeration and drainage. Accordingly, as a result, it can influence the N_2O and CO_2 fluxes [10].

Regarding grassland species, perennial ryegrass is one of the main forage grasses sown in the world due to its rapid recovery after grazing, its forage quality, its adaptability to different soil conditions and its productivity over long growing seasons [11,12]. Forage quality is based on parameters such as nutritional composition, crude protein (CP) content and dry matter digestibility [11]. Dry matter digestibility is related to the proportion of insoluble fraction of the fiber that can be assimilated by livestock. Both CP content and dry matter digestibility are primarily determined by the genetic characteristics of the grass and its maturity, while the application of N fertilizers is the most widely used strategy to increase CP content.

According to the National Administrative Department of Statistics, in 2014, around 80% of the agricultural surface in Colombia was assigned to grasslands and forage production [13], and Colombia constitutes one of the major consumers of fertilizers in the Latin America and Caribbean region. While the most commonly used N fertilizer in the world is urea, which in 2013 and 2014 represented more than half (57%) of the global N fertilizer demand [14], in Colombia, in 2016, ca. 70% of the imports of N fertilizers were urea [15]. Thus, the objective of this study was to evaluate the use of ammonium sulfate nitrate (ASN) in combination with DMPP as an alternative to the conventional application of urea in a tilled grassland in the Bogotá savanna. The effect of the different fertilization management strategies was measured in terms of their environmental impact (GHGs emissions) and forage yield and quality.

2. Materials and Methods

2.1. Study Area

The field experiment was carried out in 2013 at the Colombian Agricultural Research Corporation (CORPOICA) in a long-term permanent grassland (eight years old) that was located in the Bogotá savanna (4°42′ N, 74°12′ W), with an average annual temperature of 15 °C and an average annual rainfall of 660 mm. Figure 1F shows the rainfall during the study period. The soil had a loamy texture and it was classified as a Typic Haplustands (Table 1).

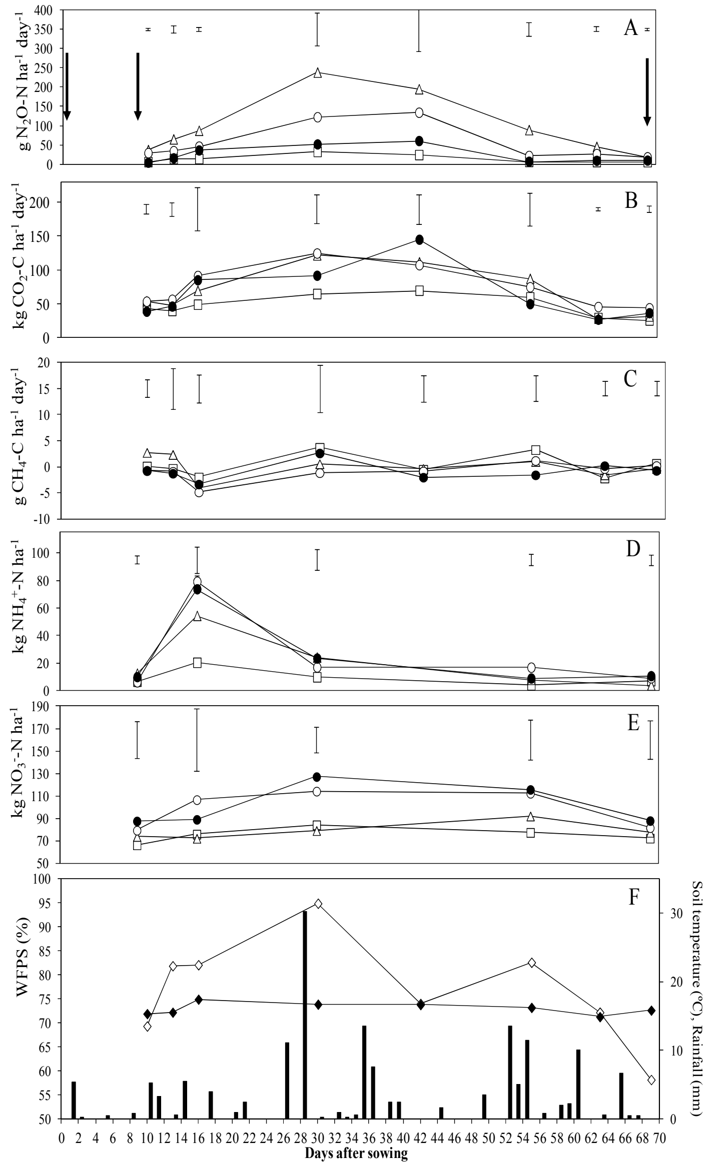

Figure 1. Daily greenhouse gas (GHGs) emissions (**A**–**C**), soil NH_4^+-N (**D**) and NO_3^--N (**E**) contents, soil water-filled pore space (WFPS) (◊), soil temperature (⧫) and rainfall (black bars) (**F**). Unfertilized (□), urea (△), ammonium sulfate nitrate (ASN) (○) and ASN plus the NI 3,4-dimethylpyrazole phosphate (ASN+DMPP) (•). Arrows show in order from left to right: sowing, fertilizer application and harvest. Vertical lines indicate least significant difference (LSD) ($p < 0.05$; $n = 4$) for each sampling day.

Table 1. Physical and chemical properties of soil (0–30 cm depth).

Soil Texture			**Soil Chemical Properties**							
Sand (%)	**Silt (%)**	**Clay (%)**	**pH**	**OC * (%)**	**N (%)**	**ECEC ** cmol (+) kg^{-1}**	**P (ppm)**	**Ca (ppm)**	**Mg (ppm)**	**K (ppm)**
36	47	17	5.7	9	0.74	21.6	85.3	1880	629	2254

* Organic carbon, ** Effective cation exchange capacity (Sum of exchangeable basic and acidic cations).

The experiment was initiated with mechanical tillage (ploughing and harrowing) (0–10 cm depth) on the 18th September. Perennial ryegrass (*Lolium perenne* L. var. Samson) was sown at a density of

50 kg ha^{-1}. On the 3rd October, a randomized block design was established with an individual plot size of 10 m^2. In all treatments, N-fertilizer was applied at a rate of 80 kg N ha^{-1} on 11th October. Three different fertilizers were used and four treatments were then evaluated: urea, ammonium sulfate nitrate 26% (ASN), a combination of ASN with DMPP (ASN+DMPP) that was marketed under the name of Entec® 26 (trademark registered by EuroChem Agro GmbH, Mannheim, Germany), and an untreated control. The N content in ASN was 7.5% as nitrate and 18.5% as ammonium, and the DMPP content in Entec® 26 was 0.8% of the total ammonium content.

2.2. GHGs Emissions

GHGs emissions were measured using dark closed chambers (headspace of 0.01 m^3) and the technique that was described by Chadwick et al. [16]. The samples were taken between 9.00 and 12.00 am to avoid the effects of daytime variation when determining daily emission rates. By previously measuring gas accumulation over a period of 30 min, the linearity of GHGs emissions was validated. Four chambers were placed randomly in each plot and the fluxes from two chambers were alternatively measured every sampling day. The initial gas concentration was determined by sampling 30% of the chambers. The concentration of each of the emitted gases (N_2O, CO_2, and CH_4) was determined using a gas chromatograph (Agilent, 7890A; Agilent Tech., Wilmington, DE, USA) that was equipped with an electron capture detector (ECD) for N_2O detection, a flame ionization detector (FID) for CH_4, and a methanizer to convert CO_2 to CH_4. A capillary column (IA KRCIAES 6017: 240 °C, 30 m × 320 µm; Agilent Tech., Wilmington, DE, USA) was used and the samples were injected by means of a headspace autosampler (Teledyne Tekmar HT3; Teledyne Tech., Manson, OH, USA). The standards for the three gases were stored and then analyzed at the same time as the samples. Soil temperature and water content (0–10 cm depth) were also measured each time that gas samples were collected.

Cumulative GHGs emissions were estimated throughout the crop cycle using the trapezoidal integration method (linear interpolation and numerical integration between sampling times) [17]. In the fertilized treatments, the N_2O emission factor (EF) was calculated as a percentage of the N fertilizer that was applied by subtracting the cumulative N_2O-N emission of the unfertilized treatment from that of the fertilized treatment and dividing the result by the total amount of N applied. Cumulative N_2O and CH_4 emissions were converted to CO_2 equivalents following the recommendations of the Intergovernmental Panel on Climate Change (IPCC) [18], using global warming potential (GWP) factors of 298 for N_2O and 25 for CH_4.

2.3. Soil Mineral N and Soil Water Content

Soil mineral N content was calculated from a sample that was taken from a mixture of three subsamples of soil per plot (0–10 cm depth and 2.5 cm diameter) before and after the application of the fertilizer, and at a frequency of one or two weeks after the application. 100 g of soil were extracted with 200 mL of KCl (1 M). The extracts were filtered through Sep-Pak C18 filters (Waters; Waters Corp., Milford, MA, USA) and then frozen until the day of measurement. The concentrations of nitrate (NO_3^-) and ammonium (NH_4^+) in the extracts were determined by colorimetry, using the method that was described by Cawse [19] and the Berthelot reaction (indophenol method), respectively.

Soil water content was gravimetrically determined and then expressed as the percentage of water-filled pore space (WFPS), which was calculated as Linn and Doran [20]:

$$\text{WFPS (\%)} = (\theta v/\text{TP}) \times 100, \quad (1)$$

where:

θv = volumetric percent of water content = (% gravimetric water content) × soil bulk density

TP = percent of total soil porosity = [1 − (soil particle density/soil bulk density)] × 100

The standard value of 2.65 Mg/m^3 was assumed as soil particle density. The used value of soil bulk density was 0.96 Mg/m^3, which was determined using an intact core soil sampler (0–10 cm depth and determining its weight after drying.

2.4. Ryegrass Yield and Quality, and Nitrogen Use Efficiency

Ryegrass was harvested on 10th December. Two subsamples of 2 m^2 per plot were randomly selected and cut to a height of 2 cm above the soil. The samples were dried at 70 °C for 48 h before measuring the dry matter (DM) yield. A segment of the dried samples was used to determine the plant N content by the Kjeldahl method. CP content was calculated, multiplying the empirical coefficient of 6.25 to the $N_{Kjeldahl}$ content [21]. Forage digestibility was estimated by determining the acid detergent fiber (ADF) content using the standardized method [21].

The apparent nitrogen recovery (ANR) rate is a means of determining N fertilization efficiency. It was calculated from the yield (DM) and plant N content based on the following formula:

$$\text{ANR (\%)} = [(\text{kg } N_F - \text{kg } N_U)/\text{kg } N_{Ap}] \times 100, \quad (2)$$

where:

N_F = N uptake in plants from fertilized treatments
N_U = N uptake in plants from the unfertilized control treatment
N_{Ap} = amount of N applied.
Nitrogen use efficiency (NUE) was also calculated, as follows:

$$\text{NUE} = (\text{kg } DM_F - \text{kg } DM_U)/\text{kg } N_{Ap}, \quad (3)$$

where:

DM_F = dry matter obtained from fertilized treatments
DM_U = dry matter obtained from the unfertilized control treatment

2.5. Statistical Analysis

The means and standard errors are presented for each parameter. One-way ANOVA was performed using IBM SPSS 21.0 (IBM Corp., Armonk, NY, USA). Duncan's multiple-range test and the least significant difference (LSD) were used to determine significantly different means. Pearson's correlation coefficient was used to determine the relationship between the CO_2 fluxes and soil parameters.

3. Results

3.1. N_2O Emissions

Under the conditions of the study, daily N_2O fluxes did not exceed 32 g N_2O-N ha^{-1} day^{-1} from the unfertilized treatment, whereas the maximum emission rate was up to seven times higher from the fertilized treatments, reaching a value of 237 g N_2O-N ha^{-1} day^{-1} in the treatment with urea (Figure 1A). For the fertilized treatments, the highest emission rates were obtained 30 and 42 days after sowing, coinciding with WFPS values of 95% and 74%, respectively (Figure 1F).

Cumulative N_2O emissions in the whole experiment (60 days) (Table 2) for the ASN+DMPP treatment were 4.0 and 2.2 times lower than for the urea and ASN treatments, respectively. Thus, DMPP efficiency mitigating N_2O emissions could be verified. Additionally, the emission factor (EF), which represents the percentage of the applied N-fertilizer that has been lost in the form of N_2O, was 86% and 71% lower with the use of ASN+DMPP with respect to the use of urea and ASN (Table 2).

Table 2. CO_2, CH_4, and N_2O cumulative emissions, global warming potential (GWP), and N_2O emission factor (EF). In brackets: percentage of reduction of EF by ASN+DMPP. Values in cells with different letters are significantly different ($p < 0.05$; $n = 4$).

Treatment	CO_2-C (kg ha^{-1})	CH_4-C (g ha^{-1})	N_2O-N (g ha^{-1})	GWP (Mg CO_2 eq ha^{-1})	EF (%)
Unfertilized	2506 ± 635 a	46 ± 29 a	1019 ± 170 c	9.7 ± 2.3 a	
Urea	3954 ± 1065 a	−20 ± 47 a	7839 ± 732 a	18.3 ± 4.0 a	8.5 (86%)
ASN	4189 ± 1214 a	−77 ± 41 a	4287 ± 541 b	17.4 ± 4.4 a	4.1 (71%)
ASN+DMPP	3530 ± 1079 a	−53 ± 65 a	1958 ± 351 c	13.9 ± 4.0 a	1.2
ANOVA Components	$F_{(3,12)}$ = 0.537; p = 0.666	$F_{(3,12)}$ = 1.277; p = 0.327	$F_{(3,12)}$ = 37.628; p = 0.000	$F_{(3,12)}$ = 1.079; p = 0.395	

3.2. CO_2 and CH_4 Emissions

Daily CO_2 fluxes ranged from 25 to 145 kg CO_2-C ha^{-1} day^{-1} (Figure 1B). Under the conditions of this study, there was a significant positive correlation ($r = 0.526$; $p < 0.01$) between CO_2 flux and WFPS (Figure 2A). We observed that even small temperature changes could enhance CO_2 emissions, because the small oscillations in soil temperature between 14.9 and 17.4 °C showed a positive correlation with CO_2 emission rates ($r = 0.638$; $p < 0.01$) (Figure 2B). Despite a slight increase in the daily CO_2 emission rates for the fertilized treatments (Figure 1B), the cumulative CO_2 emissions did not present significant differences between the treatments (Table 2), with the average value for the four treatments being 3555 kg CO_2-C ha^{-1}.

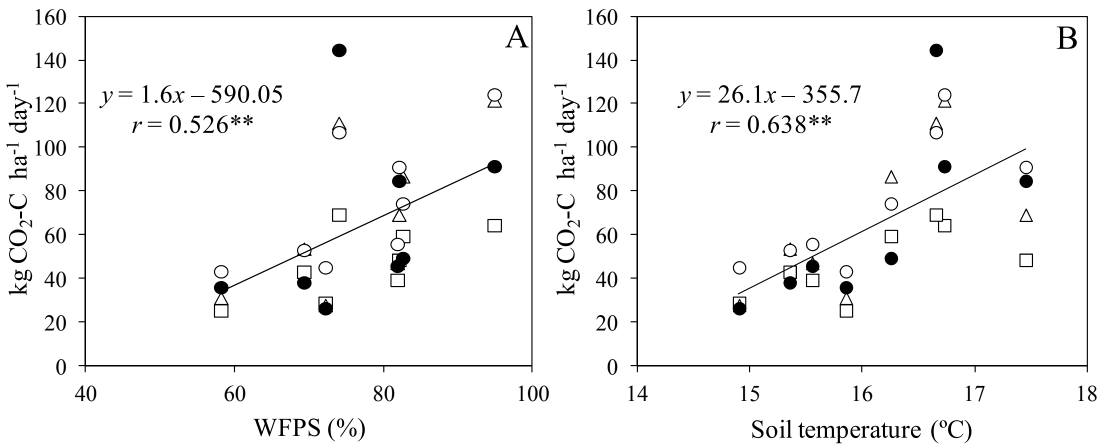

Figure 2. Daily CO_2 emissions for the different treatments related to WFPS (**A**) ($p = 0.002$) and soil temperature (**B**) ($p = 0.000$). Unfertilized (□), urea (△), ASN (○), and ASN+DMPP (●).

Regarding CH_4 fluxes, maximum uptake rates of around −5 g CH_4-C ha^{-1} day^{-1} and a maximum emission rate to the atmosphere of 4 g CH_4-C ha^{-1} day^{-1} (Figure 1C) were observed.

3.3. Ryegrass Yield and Quality

The ryegrass yields were close to 2000 kg DM ha^{-1} in the fertilized treatments sixty days after fertilization (Table 3).

With respect to forage quality, the CP content can widely vary in ryegrass species, from 8% to 27% [22]. In our study, this content was already as high as 26% in the unfertilized treatment (Table 3) and a significant increment of this content was obtained with the use of urea. Another key factor in forage quality is digestibility, which is evaluated through ADF content. Neither fertilization nor DMPP application affected the concentration of ADF (Table 3).

Regarding the efficiency of N uptake (ANR), as DM yield was higher in the urea and ASN+DMPP treatments, and the CP content was higher in the urea treatment; the ANR was higher in both urea and ASN+DMPP treatments, with values of ca. 34% (Table 3). When ASN was applied without an inhibitor, the ANR was as low as 17%.

Table 3. Yield (dry matter (DM) production), crude protein (CP) content, acid detergent fiber (ADF) content, nitrogen use efficiency (NUE), and apparent nitrogen recovery (ANR). Values in cells with different letters are significantly different ($p < 0.05$; $n = 4$).

Treatment	Yield (kg DM ha^{-1})	CP (% DM)	ADF (% DM)	NUE (kg DM/kg N Applied)	ANR (%)
Unfertilized	1456 ± 76 c	26 ± 0.23 b	44 ± 0.65 a		
Urea	1956 ± 69 ab	28 ± 0.24 a	43 ± 1.93 a	6.2 ± 0.98 a	34 ± 3.45 a
ASN	1836 ± 83 b	25 ± 0.54 b	43 ± 2.05 a	4.7 ± 1.36 a	17 ± 2.90 b
ASN+DMPP	2057 ± 51 a	26 ± 0.51 b	47 ± 0.91 a	7.5 ± 1.01 a	33 ± 6.09 a
ANOVA components	$F_{(3,12)} = 13.667$; $p = 0.000$	$F_{(3,12)} = 6.538$; $p = 0.007$	$F_{(3,12)} = 1.225$; $p = 0.343$	$F_{(2,9)} = 1.275$; $p = 0.326$	$F_{(2,9)} = 4.064$; $p = 0.049$

4. Discussion

4.1. N_2O Emissions

We observed that N_2O emissions from the urea treatment were 1.8 times higher than from ASN (Table 2), as reported by Clayton et al. [23], who also observed 2.0 times higher cumulative N_2O emissions in urea-fertilized plots when compared to ASN-fertilized plots. Davidson [24] reported that denitrification is a predominant process when soil moisture remains at a WFPS of above 70% because of the lower availability of O_2. Moreover, under high soil NO_3^- content conditions (Figure 1E), the competitive behavior of NO_3^- versus N_2O as a terminal electron acceptor during denitrification can inhibit the reduction of N_2O to N_2 [25–27], generating the high daily N_2O emission rates that were observed in our study for the fertilized treatments. Another factor that could have also induced the observed N_2O emissions is the high soil organic carbon content of 9%. For example, in our unfertilized treatment, N_2O cumulative emissions were up to 38 times higher than those that were reported by Misselbrook [28] in different United Kingdom (UK) soil types with soil organic carbon contents <5%. In this regard, Bouwman [29] measured a significant increase in N_2O emissions in soils with high organic carbon contents (>3%), indicating that this organic carbon effect is favored by a fine soil texture and a neutral to slightly acidic pH, as was the case of the present study. In fact, under the same climatic conditions and a similar N fertilizer dose (70 kg ha^{-1}), but with an organic carbon content of 4.2%, Huérfano et al. [30] recorded N_2O emissions rates for fertilized treatments that were four times lower than those that were found in the present study. Even under high soil water content conditions (90% WFPS), Ruser et al. [27] observed peak N_2O emission rates. They also attributed this to the influence of the availability of soil organic carbon (1.63%), since this availability enhances the consumption of O_2, thus favoring the formation of anoxic zones that promote the emission of N_2O, especially when NO_3^- is readily available [31], as was the case in our experiment.

With respect to the effect of temperature and WFPS on the efficiency of DMPP, in Atlantic grasslands, Merino et al. [32] reported that DMPP was more efficient in reducing N_2O emissions at temperatures ranging between 6 to 11 °C than at temperatures above 16 °C, and Menéndez et al. [33] reported that, at 80% WFPS, DMPP's capacity to reduce N_2O emissions decreased when soil temperature increased from 10 to 20 °C. In the present study, we have confirmed that DMPP efficiently reduces N_2O emissions at soil temperatures close to 16 °C and prevalent conditions of WFPS >70%.

It is also worth highlighting that the efficiency that was observed after DMPP application was the highest which could possibly be obtained, based on the assumption that DMPP could reduce N_2O emissions down to the level of the unfertilized control.

When calculating N_2O emission due to fertilization in terms of emission factor (EF), we must bear in mind that the N_2O emission factors that were obtained in this study should not be directly compared to the default value that was proposed by the IPCC [34], because they were calculated for a short period of 60 days, corresponding to one ryegrass cut. Regardless, when considering the non-seasonality and therefore the low climatic variation of the study area (the Bogotá savanna can produce up to six cuts per year), we can assume that the emission factor that was obtained for a single cut is very similar to what would be obtained for one year when applying fertilizer after each cut. Our results show that the N_2O emission factors for the urea and ASN treatments were 8.5 and 4.1 times higher, respectively, than the 1% default value that was proposed by the IPCC [34]. However, the ASN+DMPP treatment, whose EF was 86% and 71% lower than in the urea and ASN treatments, respectively, gave an EF of 1.2% (Table 2). This value is within the range that was proposed by the IPCC, thereby highlighting DMPP's capacity to reduce high N_2O emissions that are induced by fertilization.

4.2. CO_2 and CH_4 Emissions

Kiese and Butterbach-Bahl [35] and Davidson et al. [36] reported a negative correlation between CO_2 emissions and soil moisture content that is above 60% WFPS, which they attributed to the anoxic conditions prevailing at high moisture content. By contrast, other authors, such as Ruser et al. [27] and Frank et al. [37], did not observe a clear influence of soil moisture content on CO_2 emissions. In the evaluated edaphoclimatic conditions, the positive correlation between CO_2 flux and WFPS (Figure 2A) indicates that the soil respiration rate was stimulated by the increase of soil water content in the range of 58% < WFPS < 94%. In this sense, Doran et al. [38] proposed a model to describe the relationship between WFPS and respiration in soils with different textures, defining that the highest respiration rates occur in the range of 40% < WFPS < 70%; therefore, the positive correlation that we observed could be explained, because most of the measurements were determined in that interval. Temperature is also a determining factor of CO_2 fluxes, because it favors the microbial activity of the soil [39]. In this experiment, we have been able to determine that even small changes in this variable can favor the increase of CO_2 emissions, since the small oscillations of temperature of the soil (Figure 1F) presented a significant positive correlation with the emissions of this gas ($r = 0.638$) (Figure 2B).

Agricultural soils have demonstrated the capacity to oxidize CH_4 at non-flooded aerobic conditions [40,41]. In our study, WFPS was in the range of 70–95% during most of the study time, therefore the O_2 availability was low but it did not generate anaerobic conditions. Hence, it was not either enough to favor CH_4 consumption [42]. Thus, the daily emissions were close to zero, fluctuating between -5 to 4 g CH_4 ha^{-1} day^{-1} (Figure 1C). The cumulative CH_4 emissions were similar for all treatment (Table 2). Accordingly, under the edaphoclimatic conditions of this study, and according to Huérfano et al. [40], we did not observe any effect of N fertilization or DMPP application on CH_4 emissions.

4.3. Effect on Global GHGs Emissions

Regarding environmental implications, the GWP has been established as a relative measure that reflects, on average, how long a GHG remains in the atmosphere and how strongly it absorbs energy. For conducting national inventories of GHGs emissions, the GWP is calculated in CO_2 equivalents and, when establishing a horizon of time of 100 years, the GWP of CO_2 is 1, of CH_4 is 25, and of N_2O is 298 [18]. In the present study, the GWP was determined based on the total emission of the three GHGs (Table 2). Despite that the GWP (expresed in CO_2 equivalents) of N_2O is considerably larger than the GWP of CO_2, the small amount of grams of N_2O emitted with respect to CO_2, makes the emission of N_2O only contribute 4% and 6–18% of the total GWP in the unfertilized and the fertilized treatments, respectively. Therefore, CO_2 emissions were the determining factor in the magnitude of the GWP. When considering that the evaluated treatments did not exert any effect on CO_2 emissions, neither did they exert any efect on the GWP.

4.4. Effect on Yield and Quality

The efficiency of fertilization was measured in terms of NUE, where 1 kg of N fertilizer induced an increase of ca. 6 kg DM ha^{-1} in every treatment. Thus, no significant differences in NUE were observed, which was probably due to the high soil nitrate content (Figure 1E) prior to fertilization. On the other hand, the ANR values were low (Table 3) when compared to a two-year study with eight varieties of ryegrass and fertilizer additions above 100 kg N ha^{-1}, where ANR values of ca. 70% were obtained [43]. As was mentioned before, ANR was higher in the urea and ASN+DMPP treatments. When comparing these two treatments, the use of DMPP was shown to compensate for the decrease in CP content in ASN and ASN+DMPP treatments through an increase in yield (kg DM ha^{-1}).

On the other hand, given that, in our trial, NH_4^+ remains available for the first 15 days after fertilization (Figure 1D), our results confirm the tolerance of ryegrass to ammonium nutrition that was reported by Belastegui-Macadam et al. [44]. As a conclusion, N fertilization that was based on urea or ASN+DMPP could be good alternatives in terms of yield and nitrogen uptake (ANR) under our experimental conditions.

4.5. Efficiency of the N Fertilization Managements

The high pre-fertilization soil NO_3^- content that was observed in the unfertilized treatment (Figure 1E) could be the result of tillage that oxygenated the soil and would have subsequently induced the mineralization of organic matter, as observed by Pinto et al. [45] after tilling a permanent pasture. Despite the high soil NO_3^- content that was measured before fertilization, we still observed yield response to fertilization, and the urea and ASN+DMPP treatments presented significantly higher yields, even in comparison to the ASN treatment.

Van Groenigen et al. [46] established a parameter called "N-yield-scaled N_2O emission" based on the expression of N_2O emissions in relation to crop productivity (above-ground N uptake). When considering that the fertilizer treatments in our experiment had different effects on yield and CP content, we also calculated the relation between N_2O emissions and the total DM that was harvested as the "yield-scaled N_2O emission". Each treatment that was assessed in our study was observed to have a similar effect on both N-yield-scaled N_2O emission and yield-scaled N_2O emission (Table 4). While for the urea treatment the N-yield-scaled N_2O and yield-scaled N_2O emissions presented values of 89 g N_2O-N kg N $harvested^{-1}$ and 4 g N_2O-N kg DM $harvested^{-1}$, respectively, these values were reduced by 36% and 42% when ASN was applied. Moreover, in the ASN+DMPP treatment, they were further reduced to the level of the unfertilized treatment (17–23 g N_2O-N kg N $harvested^{-1}$ and 0.7–1 g N_2O-N kg DM $harvested^{-1}$). Provided that the N-yield-scaled N_2O emission is a relevant factor when assessing the environmental cost (in terms of N_2O) of food production [47], the use of DMPP in the Bogotá savanna grasslands constitutes an effective management practice to increase the efficiency of N fertilization, while simultaneously reducing its negative environmental impact.

Table 4. N-yield-scaled N_2O emissions and yield-scaled N_2O emissions. Values in cells with different letters are significantly different ($p < 0.05$; $n = 4$).

Treatment	N-Yield-Scaled N_2O Emission (g N_2O-N kg N $Uptake^{-1}$)	Yield-Scaled N_2O Emission (g N_2O-N kg DM $Harvested^{-1}$)
Unfertilized	17 ± 4 c	0.7 ± 0.2 c
Urea	89 ± 7 a	4.0 ± 0.3 a
ASN	57 ± 6 b	2.3 ± 0.3 b
ASN+DMPP	23 ± 4 c	1.0 ± 0.1 c
ANOVA components	$F_{(3,12)} = 35.477$; $p = 0.000$	$F_{(3,12)} = 40.289$; $p = 0.000$

Conventional nitrogen fertilization management with urea and the use of ASN+DMPP generated the highest ryegrass yields and apparent nitrogen recoveries. However, in the case of the application of ASN+DMPP, the significant reduction of N-yield-scaled N_2O emissions down to the unfertilized control level is evidence that the use of DMPP can effectively increase grassland yield while also reducing the environmental impact of N fertilization.

5. Conclusions

This study provides the first data on GHGs emissions from grasslands that were grown under the climatic conditions of the Bogotá savanna. This is an important starting point for the measurement and assessment of GHGs mitigation practices to be implemented in the most important productive system in this region. Both the conventional nitrogen fertilization management with urea and the use of ASN+DMPP generated the highest ryegrass yield and apparent nitrogen recovery. However, the significant reduction of N-yield-scaled N_2O emissions by ASN+DMPP down to the unfertilized control level support the effectiveness regarding the use of DMPP to increase grassland yield while reducing the environmental impact of N fertilization. Regarding forage quality, neither the application of nitrogen nor the use of DMPP affected the digestibility of the forage in ADF content terms.

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Abbreviations

ADF, acid detergent fiber; ASN, ammonium sulfate nitrate; ANR, apparent nitrogen recovery; CP, crude protein; DM, dry matter; DMPP, 3,4 dimethylpyrazole phosphate; EF, emission factor; GWP, global warming potential; GHG, greenhouse gas; LSD, least significant difference; Mg, Megagrams; NI, nitrification inhibitor; NUE, nitrogen use efficiency; WFPS, soil water filled pore space.

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