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Nitrification inhibitor addition to farm dairy effluent to reduce nitrous oxide emissions

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ABSTRACT: Increasing the use of nitrogen (N) fertilizers will be necessary to enhance grain and pasture yields to satisfy the growing world demand for food. Organic amendments, such as farm dairy effluents (FDE), are an alternative to traditional synthetic fertilizers. However, part of the applied N could be lost as ammonia (NH_3) volatilization or nitrous oxide (N_2O) emission, decreasing N availability to plants. Nitrification inhibitors, such as dicyandiamide (DCD), suppress the microbial process of nitrification, decreasing soil nitrate concentration and, therefore, N_2O emission. Reducing N_2O losses from agricultural soils is a key subject for sustainable production. This research aimed to quantify the effect of DCD addition to the FDE on the emissions of N_2O and the volatilization of NH_3 from the soil. A field trial was carried out in which NH_3 volatilization and N_2O emission were measured over 49 days after applying FDE, FDE with DCD (DCD), and control (C, without N added) treatments. The amount of N applied as FDE was 120 kg of N ha^{-1}. Accumulated N_2O emission during the 49 days after the application was 526, 237, and 174 g N_2O-N ha^{-1} from the soil in the FDE, DCD, and C treatments, respectively. No significant differences were observed in accumulated NH_3 volatilization. Pasture yield was higher in DCD treatment, followed by C and FDE. Under low temperatures and high soil moisture conditions, adding DCD to the FDE could be considered an effective alternative to increase pasture yields, decrease N_2O emissions, and maintain NH_3 volatilization, reducing total N losses to the atmosphere by about 14 %. Adding DCD to the FDE is a promising alternative for the more efficient N use of farm dairy effluents as fertilizer to mitigate N losses, tending to reduce N losses as N_2O emissions. More studies are necessary to verify the result of using FDE + DCD under different soils and climates.

Keywords: greenhouse gases, organic fertilizer, nitrogen, DCD.

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INTRODUCTION

For the next few years, the world population is expected to increase significantly, reaching 9.7 billion people in the year 2100, and that will be accompanied by an increase in food consumption (Koops and van Leeuwen, 2017). In the specific case of milk, it is expected that, by 2050, the demand will increase by 58 % compared to 2010 (Gerber et al., 2013). Increasing agricultural production is necessary to satisfy this growing demand. However, the intensification of livestock systems has severe consequences related to the increase in generation of effluents. Livestock effluents often do not have an alternative use, and dairy products do not escape this reality. Growing amount of farm dairy effluent (FDE) currently leads to the need to reuse them. A possible alternative is to use it as an organic amendment through modern technologies (Whalen et al., 2019).

In pastoral systems, each milking cow generates between 14 and 24 L of FDE per day (Taverna et al., 2007). Farm dairy effluent has a solid part comprising fecal matter, food remains, and mud; a liquid part comprises traces of milk, washing water, and urine (Charlón et al., 2007). Consequently, FDE has variable amounts of nutrients, which increases fertility when applied to the soil. Dairy systems are undergoing a process of rapid intensification (Lazzarini et al., 2019) accompanied by an increase in crop and pasture productivity, with a higher uptake of nutrients from soil.

When soil nitrogen (N) exceeds the uptake capacity of plants, N tends to be mobilized, causing negative environmental consequences (Fowler et al., 2013). In this sense, using slow-release organic fertilizers can minimize N losses to the environment, maintaining yields without increasing the application of synthetic fertilizers. In this context, the recovery of FDE as an N source for crops and pastures is undoubtedly a sustainable alternative. The recovery of FDE also provides a solution to the waste problem, contributing in turn to reducing synthetic fertilizer's use.

When N fertilizers are applied to the soil, part of the N is lost as ammonia (NH_3) volatilization by hydrolysis of ammonium (NH_4^+). In contrast, microbial processes quickly transform another part into nitrate (NO_3^-) (Huber et al., 1977). Most NO_3^- losses occur through leaching or production of nitrous oxide (N_2O) before plants can use it; this leads the system to have low efficiency in using N.

The N_2O has a global warming potential 273 times higher than CO_2 (Forster et al., 2021), and is one of the leading greenhouse gases emitted by the agricultural sector. The N_2O is mainly produced by the microbial processes of nitrification and denitrification. Nitrogen content of the soil, the temperature, and the soil moisture are the main regulatory factors (Steenwerth and Belina, 2008; Cosentino et al., 2013).

Nitrification inhibitors (NIs) are a group of chemical compounds that suppress the first step of nitrification by inhibiting enzymes from bacteria like *Nitrosomonas,* which oxidize NH_4^+ to nitrite (NO_2^-), thus delaying the nitrification process (Huber et al., 1977; Zerulla et al., 2001). Nitrification inhibition can thus promote N retention in the NH_4^+ form while reducing the content of NO_3^- in the soil. In addition, in the soil solution, the NH_4^+ is less mobile, and the lower NO_3^- concentration decreases the substrate for potential denitrification with N_2O production (Di et al., 2014; Gonzatto et al., 2016). Therefore, the IPCC recommends using NIs as a possible mitigation option to reduce agricultural N_2O emissions (IPCC, 2014).

Dicyandiamide (DCD) is an NI that inhibits the enzyme ammonia monooxygenase in bacteria like Nitrosomonas, limiting the nitrification process. Dicyandiamide is used in several commercial formulations because it is relatively inexpensive, nonvolatile, water-soluble, and efficient for use with N fertilizers (Di et al., 2011). In addition, numerous studies have shown that adding an NI to a fertilizer decreases the loss of N_2O from the soil (Di and Cameron, 2012; Gonzatto et al., 2016). However, there is little information

on applying NI with organic waste, especially with FDE. Knowing the effect of DCD on N_2O emissions and the volatilization of NH_3 from the soil with FDE will allow management strategies with greater productivity and less environmental impact. This research aimed to quantify the effect of the NI (DCD) addition to the FDE on the emissions of N_2O and the volatilization of NH_3 from the soil.

MATERIALS AND METHODS

The trial was carried out at the agricultural experimental station INTA AMBA (34° 37' 21.13" S - 58° 40' 12.44" W) from May 28 to July 15, 2019. Soil was classified as Typic Argiudoll, Las Cabañas series, with a silty-loam texture on the surface and clay-loam sub-surface (INTA, 2019). The experiment was carried out on a consociated pasture of fescue (Festuca sp.), alfalfa (*Medicago sativa*), white clover (*Trifolium repens*), and cebadilla (*Bromus sp.*) implanted in 2016. The experiment had a randomized complete block design with three blocks (replications) and three treatments in each block: 1) farm dairy effluent (FDE); 2) FDE applied in conjunction with NI (DCD); and 3) control: soil without application of any product (C). The amount of 8.5 L m^{-2} of FDE (equivalent to 120 kg of N ha^{-1}) was applied. An amount of FDE representative of this dose was applied in plots of 25 m^2 (5 × 5 m), inside each of the chambers for the determination of N_2O measurement and each tower for the determination of the volatilization of the NH_3. To treat FDE + DCD, 10 kg of DCD ha^{-1} was mixed with the FDE just before application. The FDE was collected fresh five days before the soil application. The FDE composition was: 1.44 g kg^{-1} of total N, 2.0 g kg^{-1} of total C, 0.4 g kg^{-1} of ammonium, non-detectable nitrate, a C/N ratio of 1.4, and dry matter content 14.69 g L^{-1}. During the test period, the average soil temperature was close to 12 °C, the average air temperature was close to 13 °C, and the accumulated precipitation was 93.6 mm.

N_2O emissions

Sampling of N_2O emissions was performed using closed-static chambers (surface 0.13 m^2, height 0.125 m) with an iron frame base and a PVC cover according to the criteria of Rochette and Eriksen-Hamel (2008). The chamber headspace was connected to the exterior by a two-way valve for gas sampling. Bases were inserted into the soil (0.05 m depth) 24 h before the beginning of the monitoring period, and they were not moved or rotated during the trial period (Alves et al., 2012). Gas samples were taken from the chamber headspace at 0, 15, and 30 min intervals after closing the chambers. A chamber was placed in each plot (replication), and N_2O emissions were measured in the first three days after the application (DAA) of the treatments, then three times a week until day 28, and once a week until the end of the trial. Nitrous oxide emissions over time were measured until no significant differences ($p<0.05$) were observed between the treatments and the control soil at 49 DDA of the treatments (Charteris et al., 2020). This sampling frequency enabled better capture of the changes in N_2O emissions and minimized the error.

The N_2O samples were collected with non-ventilated static chambers between 9 and 12 a.m. (Cosentino et al., 2012). Samples were taken from inside the chambers 0, 15, and 30 min after closing them. The N_2O was extracted with a vacuum pump and stored in 10 cm^3 vials. The N_2O was measured with a GC 6890 Agilent Technologies Network gas chromatograph.

The N_2O fluxes (f) were calculated according to equation 1:

$$f = \frac{\Delta C}{\Delta t} \times \frac{V}{A} \times \frac{n}{V_m} \quad \text{Eq. 1}$$

in which: ΔC is the change in the N_2O content throughout the incubation time (Δt), in a chamber of 16.7 dm^3 (V) and an area of 0.13 m^2 (A); m is the molecular mass of N_2O; and V_m is the molar volume of N_2O. Change in the N_2O content over the incubation time is the emission rate.

Volatilization of NH_3 and total N emitted

A NH_3 measurement absorption system was placed in each plot. The NH_3 volatilization was measured with a semi-open static absorption system proposed by Nommik in 1973 and modified by Videla (1994). Ammonia volatilization was sampled daily during the first nine DAA until the volatilization of NH_3 reached the same level as treatment C (Videla, 1994). In each sampling, the polyurethane plate of the lower grid was rinsed with distilled water, and the content of NH_4^+-N in the sponge washing solution was determined by the micro distillation method and subsequent titration (Bremner and Keeney, 1965). From the value of NH_4^+-N, the concentration of NH_3-N volatilized by the surface covered by the NH_3 capture chamber was obtained according to equations 2 and 3. Finally, the NH_3 volatilization per hectare was calculated with equation 4.

N captured in the cylinder = (Vm – Vb) × Factor Eq. 2

in which: V_m is the volume of sulfuric acid spent per sample (cm^3) and V_b is the volume of sulfuric acid spent per blank (cm^3).

Factor = Nac / (Vs × Vr × W) Eq. 3

in which: Nac is the normality of sulfuric acid (meq cm^{-3}), Vs is the volume of the sample (cm^3), Vr is the total sponge rinsing volume (500 cm^3) and W is the weight of meq of NH_4^+.

N volatilized = Nc × 10.000/Sc Eq. 4

in which: Nc is the N captured in the cylinder and Sc is the surface of cylinder.

Accumulated values were calculated for each repetition of each treatment, and an analysis of the daily rates accumulated during the nine days of monitoring was carried out. Videla (1994) reported that in the first six DAA, 80 % of the NH_3 volatilization is expected.

Linear interpolation method calculated the accumulated N_2O emissions during the first 49 DAA and the accumulated NH_3 volatilization during the first nine DDA of the treatments (Dorich et al., 2020). The fraction of N emitted (N_2O emission plus NH_3 volatilization) was obtained from the accumulated emission for each treatment. Nitrogen fraction was then calculated from the difference in the back-transformed total emitted from each treatment (MT) and the control treatment (MC), divided by the N applied, as described by equation 5.

$$NF = \frac{MT - MC}{\text{Treatment N applied}} \quad \text{Eq. 5}$$

in which: NF is the N fraction emitted (N emitted as % of N applied); and Treatment N applied is the N applied (kg of total N ha^{-1}).

Supporting variables

Within each plot and for each sampling date, a composite soil sample (five soil subsamples) from 0.00-0.20 m soil layer was carried out to determine soil moisture and nitrate

content (Cataldo et al., 1975). At the same time, soil temperature was measured at 0.10 m depth. Bulk density was calculated using the cylinder method (100 cm³ volume and 0.05 m diameter) (Blake, 1965).

The WFPS was calculated as described by equation 6.

$$\text{WFPS (\%)} = \frac{(\text{Soil moisture} \times \text{Bulk density} \times 100)}{\left[1 - \left(\frac{\text{Bulk density}}{2.65}\right)\right]} \quad \text{Eq. 6}$$

Pasture yield was determined 90 days after treatment application. An area of 0.50 m × 0.50 m was collected in each replication. Collected samples were dried in an oven at 40 °C and weighed to obtain dry weight. The data were subjected to analysis of variance (ANOVA) and linear regression with the InfoStat program (Di Rienzo et al., 2020).

RESULTS

Nitrous oxide emissions

Emission of N_2O showed a similar pattern for all treatments, presenting two peaks. The first peak occurred at the first DAA and the second at 21 DAA. The N_2O flux ranged from 0.44 to 1293 µg N_2O-N m^2 h^{-1} with high variability between repetitions. The N_2O emission values showed significant differences ($p<0.05$) between the treatments on four of the 16 sampling dates (Figure 1a). The first N_2O emission peak presented the maximum N_2O emissions values. These values decreased over time up to 16 DAA. During the first N_2O emission peak, the soil with FDE application presented the maximum N_2O emission value, followed by DCD and C. The second N_2O emission peak was observed at 21 DAA and did not present significant differences ($p<0.05$) between treatments (Figure 1a). The second N_2O emission peak was related to rainfalls that occurred 18-28 DAA and increased WFPS of the soil to values above 90 % (Figure 1b). Figure 1b shows the evolution in the average value of the WFPS. The average value is presented since no significant differences ($p<0.05$) in the WFPS value between treatments were observed on any of the sampling dates (data not shown). Second N_2O emission peak also coincided with the moment of the highest soil temperature (Figure 1c). Starting at 21 DAA, a decrease in N_2O emission values was again observed until the end of the trial.

From peak 1 DAA, the FDE treatment showed higher N_2O emission ($p<0.05$) than treatments DCD and C, and this pattern was observed until the end of the trial (Figure 2) without significant differences ($p<0.05$) between the DCD and C treatments. At 49 DAA, the accumulated N_2O emission values were, on average, 526 ± 162.2 g N_2O-N ha^{-1} from the soil with FDE application, 237.6 ± 89.5 g N_2O-N ha^{-1} from the soil with DCD application and 174 ± 91.1 g N_2O-N ha^{-1} from C. Therefore, N_2O emission from the soil with FDE application was approximately three times higher than the N_2O emission from C, while the N_2O emission from the soil with FDE plus DCD was 1.37 times greater than C (Figure 2). The fraction of N emitted as N_2O from the soil with FDE application was 0.35 (0.0013) %, a value significantly higher than that of 0.1 (0.0006) % obtained from the soil with DCD application.

Ammonia volatilization and total N emitted

Fluxes of volatilization of NH_3 were low and did not differ ($p<0.05$) among treatments neither the accumulated volatilization (Figure 3). Finally, the total N emitted (N-N_2O plus N-NH_3) from the DCD treatment showed a trend ($p<0.1$) of lower total N emission than the FDE treatment (Figure 4), decreasing total N losses to the atmosphere by almost 14 %.

Supporting variables

The NO_3^--N content in the soil presented significant differences ($p<0.05$) between the treatments on two of the eight sampling dates (Figure 5). The FDE treatment presented the highest value of NO_3^--N content at the beginning of the trial, which decreased over time. Treatments C and DCD did not show variations in the NO_3^--N content of the soil during the evaluated period (Figure 5). Pasture yield at 149 DAA was higher in the DCD treatment, followed by C and FDE (Figure 6).

DISCUSSION

Increase of N_2O fluxes immediately after applying the treatments was probably due to the incorporation of N readily available into the soil (Figures 1a and 4). Availability of NO_3^- for denitrifying bacteria is one of the main determinants in the denitrification process,

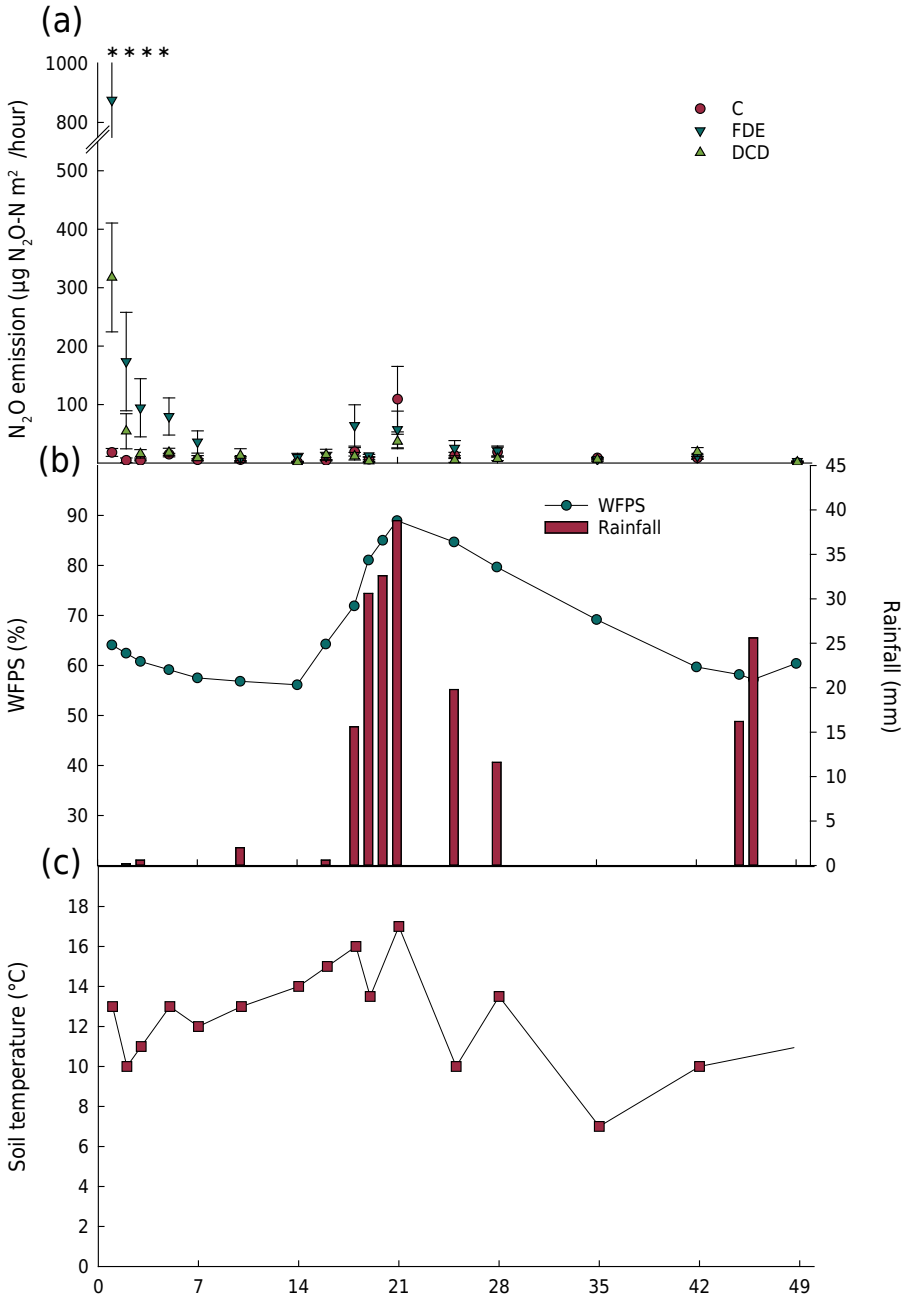

Figure 1. Evolution of (a) N_2O emission in the control soil (C), with an application of farm dairy effluent (FDE) and with farm dairy effluent plus the inhibitor (DCD); (b) water-filled pore space (WFPS) and rainfall, and (c) soil temperature. Bars indicate standard error. *: significant differences ($p<0.05$) between treatments for each measurement day.

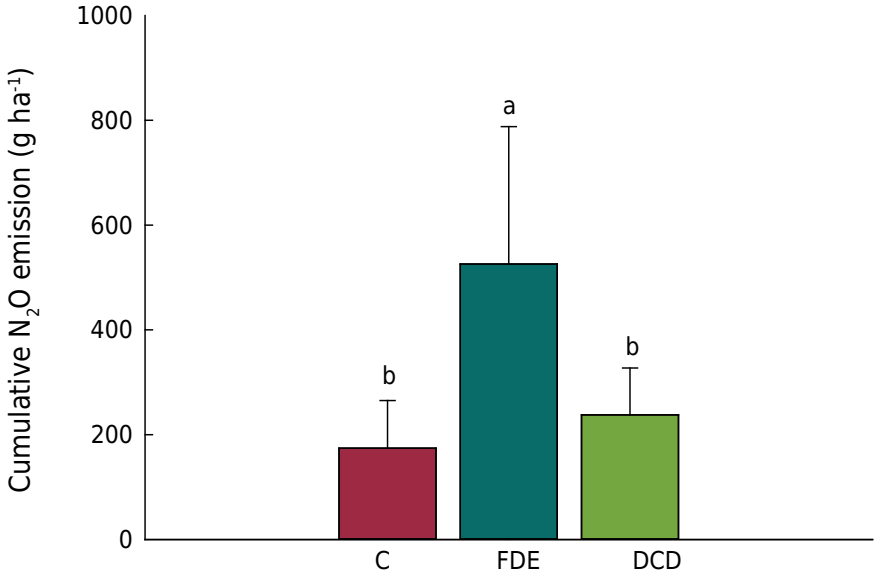

Figure 2. Cumulative N_2O emissions from the control soil (C) and after the application of the farm dairy effluent (FDE) and farm dairy effluent plus the inhibitor (DCD) at 49 DAA. Different letters correspond to significant differences ($p<0.05$) between treatments.

which leads to the production of N_2O (Wang et al., 2021). These results agree with those of Li et al. (2015), who carried out a trial of FDE application at different times of the year. These authors found that N_2O emissions increased immediately after effluent application in all three seasons of application, and also observed that N_2O emission peaks occurred within the first 24 h post-application in all treatments for the spring and summer seasons. Previously, Li et al. (2014) observed the highest N_2O losses within the first day after applying the effluent in pastures during the winter. Studies with other effluents showed similar N_2O emission responses within a few hours after application (Sharpe and Harper, 2002; Luo et al., 2008).

During the first N_2O emission peak, the soil with FDE application presented the highest emission value, followed by DCD and C (Figure 1a). This maximum value of N_2O emission from the soil with FDE application coincided with the soil's maximum value of NO_3^--N (Figure 5). These results agree with previous studies showing that NO_3^--N of the soil increased after adding organic amendments (Roig et al., 2012; Masaka et al., 2014). Low N_2O emission from C could be due to the lower NO_3^- content and soil moisture during the first DAA. On the other hand, because they are liquid, FDE and DCD treatments incorporate water at the time of application. The WFPS did not present significant differences ($p<0.05$) between the treatments for any sampling dates (data not shown). However, it is possible that the generation of anaerobic microsites with the incorporation of FDE (alone and applied in conjunction with DCD) led to an increase in the N_2O emission from the denitrification process (Bateman and Baggs, 2005). Lower N_2O emission from the DCD treatment compared to the FDE treatment was possibly due to the NO_3^- content. The NO_3^- content was lower in the treatment with DCD (Figure 5), possibly due to the inhibition of the nitrification process that slowed down the availability of NO_3^-, a substrate for the N_2O-producing denitrification process (Smith, 2017).

At 21 DAA, the second peak of N_2O emission was observed, probably related to the rainfalls that occurred between days 18-28 DAA, which raised the WFPS of the soil to values above 90 % (Figure 1b). The observed results do not align with the expected outcomes. According to Davidson (1991), when the WFPS values exceed 90 %, the denitrification process is complete, giving rise to N_2 emissions (Davidson, 1991). However, the WFPS value presented in our result corresponds to an average value. For this reason, it is possible that, due to the variability of the microrelief, some WFPS were at 100 % and, therefore, passed directly to N_2, while others were at 70-80 %, predisposing to the

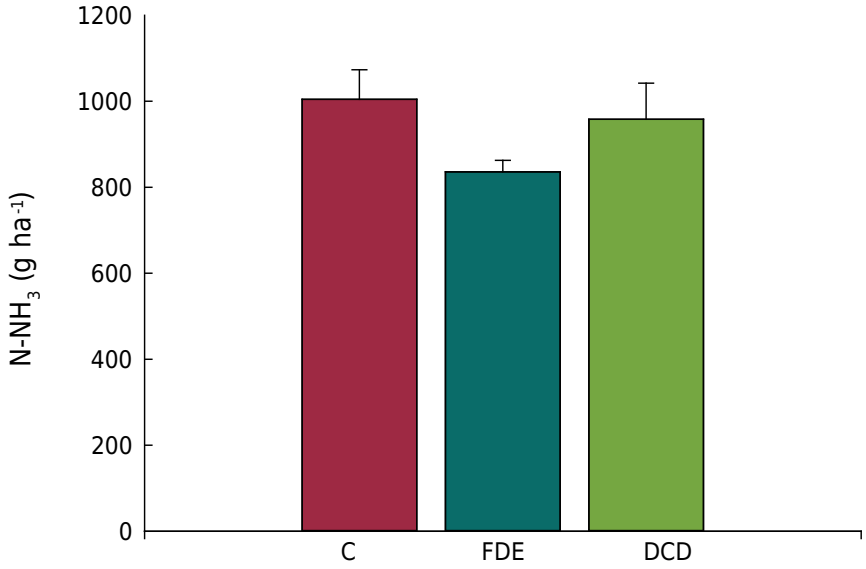

Figure 3. Accumulated volatilization of NH_3 from the control soil (C) and after the application of the farm dairy effluent (FDE) and farm dairy effluent plus the inhibitor (DCD).

maximum emission. Water content in the soil plays a fundamental role in the availability of oxygen and, therefore, in the activity of anaerobic microorganisms. When the WFPS is low, the primary process by which N_2O is emitted is nitrification, while when the WFPS increases, the denitrification process begins to gain relevance (Davidson, 1991). The second N_2O emission peak also coincided with the moment of the highest soil temperature (Figure 1c). Soil temperature affects N_2O emission by directly influencing microbial communities' reaction kinetics and growth (e.g., Pseudomonas) (Aguilera et al., 2013). Several authors observed a positive and significant correlation between temperature and N_2O flow (Meijide et al., 2007; Lee et al., 2009; Heller et al., 2010).

Accumulated N_2O emission at 49 DAA for the treatment with FDE application was approximately three times greater than that from C soil. In comparison, the emission from the soil with FDE plus DCD was only 1.37 times greater than C (Figure 2). These results coincide with what was observed by Li et al. (2015). After applying different residues to the soil, these authors observed DCD reduced the N_2O emission by 24-69 % in spring and 44-80 % in autumn. Also, Di et al. (2014) jointly applied 10 kg of DCD ha^{-1} with cow urine and observed a 65 % decrease in N_2O emission. Finally, Merino et al. (2002) found that applying cattle manure to the soil by adding 25 kg of DCD per ha inhibited nitrification and reduced up to 60 % N_2O emission.

Nitrogen fraction emitted as N_2O from the soil with the application of FDE presented a value of 0.35 %. This value was significantly higher than that obtained from the soil with DCD application, with a value of 0.1 %. Cumulative N_2O emission and the fraction of N emitted from the soils with FDE application were higher than that from the soil with the joint application of FDE plus DCD. This was possibly due to the form in which the N was found in the soil, with a lower NO_3^- content available for soil microorganisms in the treatment with DCD application. Dicyandiamide inhibits NH_4^+ oxidation by deactivating the activity of the ammonium monooxygenase enzyme (Di et al., 2009; Gonzatto et al., 2016). Low NO_3^- content in the soil can reduce adverse environmental effects such as deep N leaching or the emission of N_2O into the environment (Snyder, 2009).

Soil moisture content affects DCD effectiveness in reducing N_2O emissions (Di and Cameron, 2006). In this sense, Guo et al. (2022) investigated the efficacy of four NIs (including DCD) to reduce N_2O emissions from an N-fertilized and concluded that the efficiency in reducing N_2O emissions increased with increasing soil moisture. In the present trial, soil moisture was higher than 55 % WFPS (Figure 1b); therefore, a significant effect of the inhibitor is expected.

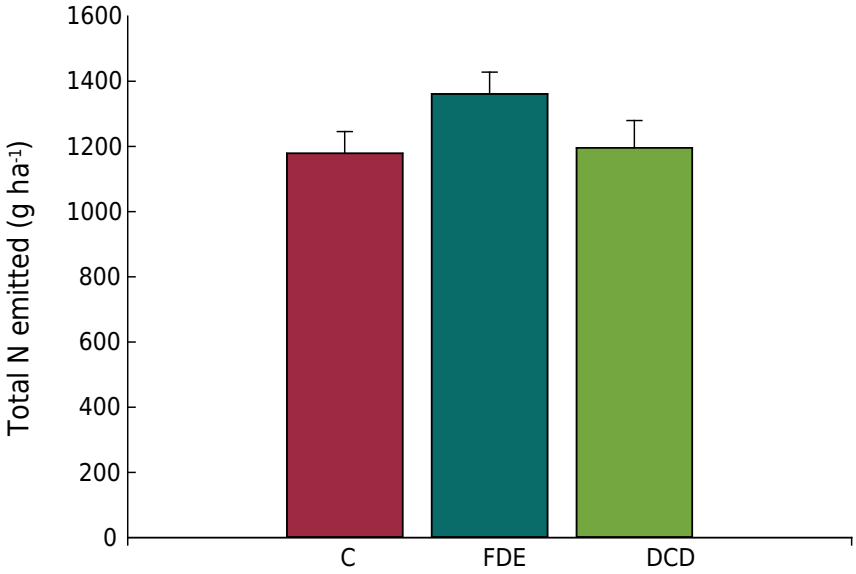

Figure 4. Total N emitted from the control soil (C) and after the application of the farm dairy effluent (FDE) and farm dairy effluent plus the inhibitor (DCD).

In the same sense, the effectiveness of DCD mitigating N_2O emissions depends strongly on the soil temperature. Bellow 15 °C, DCD has been reported to inhibit nitrification for up to 6 months, while above 15 °C, the efficacy decreases substantially (Guo et al., 2022). This is because the half-life of DCD decreases with increasing temperature (Kelliher et al., 2014). In this study the soil temperature never exceeded 17.3 °C (Figure 1c), this suggests a low DCD degradation rate and, therefore, a high DCD efficiency during this sampling period.

As of 35 DAA, the return of the N_2O emission to values similar to those of the control soil was observed. Similar values between treatments were maintained until the end of the trial at 49 DAA. These results show the importance of carrying out more frequent sampling in the first days after the application of the treatments to minimize the measurement error in the emission of N_2O from the soil.

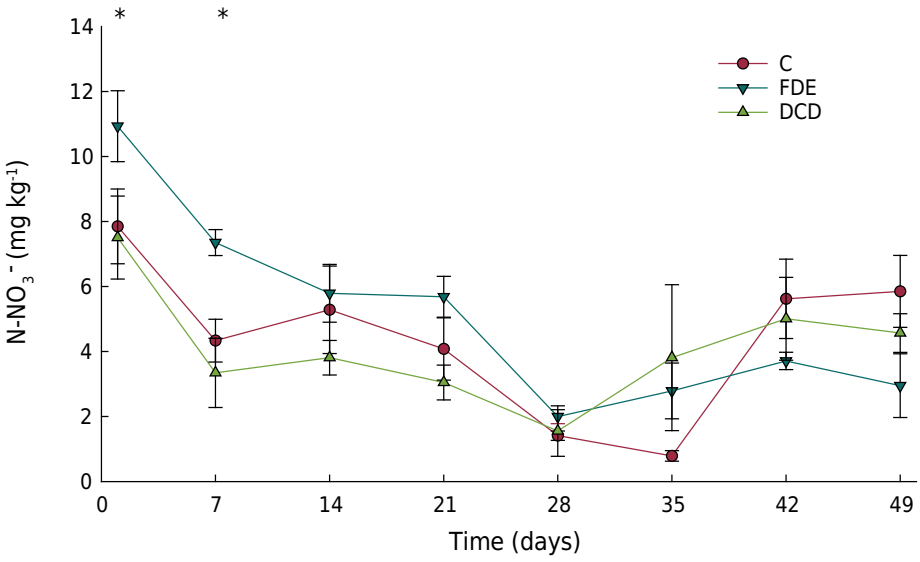

Figure 5. Evolution of the NO_3- content in the control soil (C) and after the application of the farm dairy effluent (FDE) and farm dairy effluent plus the inhibitor (DCD). Bars indicate the standard error of the mean SEM. *: significant differences ($p<0.05$) between treatments for each measurement day.

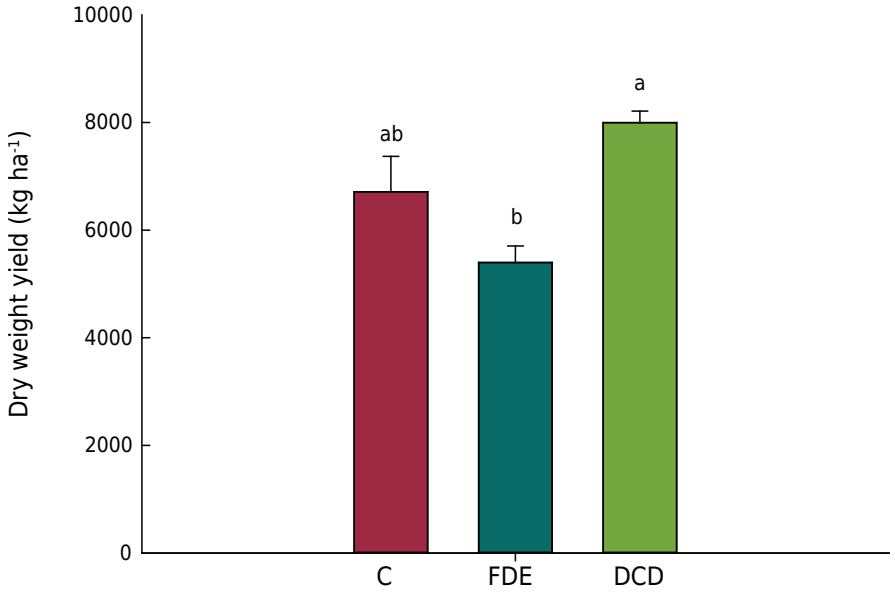

Figure 6. Pasture yield in the control soil (C) after the application of the farm dairy effluent (FDE) and farm dairy effluent plus the inhibitor (DCD) at 149 DAA. Different letters correspond to significant differences ($p<0.05$) between treatments.

How NIs affect NH_3 volatilization is widely discussed in the literature. Di et al. (2021) conducted a meta-analysis with 89 studies worldwide. It concluded that in most cases, the NI increased NH_3 volatilization by 35.7 % and increased indirect N_2O emission from NH_3 emission (and subsequent N deposition) by 2.9 %. However, these values strongly depend on the soil conditions under study. In the present study, the volatilization of NH_3 from the soil with the FDE and DCD treatments was similar (Figure 3). Thus, the application of DCD did not increase the loss of NH_3 by volatilization. The low volatilization of NH_3 from the soil with DCD application could be due to the high moisture of the studied soil. High moisture content in the soil makes it easier for NH_4^+ to be absorbed as a cation in the exchange sites, minimizing its volatilization (Zaman et al., 2008).

Pasture yield at 149 DAA was higher for the treatment with DCD, followed by C and FDE (Figure 6). The highest yield observed in the treatment with DCD application coincided with the lowest loss of N, while the minimum yield coincided with the maximum values of N loss (Figures 2, 3, 4 and 6). Therefore, it is possible that the N that was not lost as N_2O or NH_3 remained available for pasture growth.

CONCLUSION

Using effluent plus dicyandiamide (DCD) can decrease nitrogen losses to the atmosphere under low temperature conditions and high soil moisture. Applying farm dairy effluent (FDE) plus DCD proved to be an effective strategy for reducing N_2O emissions without increasing NH_3 volatilization. Reduction in N losses, specifically N_2O and NH_3, positively impacts pasture yield by retaining more nitrogen for plant growth. Consequently, managing soil NO_3^- levels and synchronizing its availability with plant demand by including DCD emerges as a promising approach for enhancing the efficient use of farm dairy effluents as fertilizers and mitigating N losses.

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AUTHOR CONTRIBUTIONS

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