



Mitigation of nitrous oxide emissions in grazing systems through nitrification inhibitors: a meta-analysis

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Abstract Grasslands are the largest contributor of nitrous oxide (N₂O) emissions in the agriculture sector due to livestock excreta and nitrogen fertilizers applied to the soil. Nitrification inhibitors (NIs) added to N input have reduced N₂O emissions, but can show a range of efficiencies depending on climate, soil, and management conditions. A meta-analysis study was conducted to investigate the factors that influence the efficiency of NIs added to fertilizer and excreta in reducing N₂O emissions, focused on grazing systems. Data from peer-reviewed studies comprising

2164 N₂O emission factors (EFs) of N inputs with and without NIs addition were compared. The N₂O EFs varied according to N source (0.0001–8.25%). Overall, NIs reduced the N₂O EF from N addition by 56.6% (51.1–61.5%), with no difference between NI types (Dicyandiamide—DCD; 3,4-Dimethylpyrazole phosphate—DMPP; and Nitrapyrin) or N source (urine, dung, slurry, and fertilizer). The NIs were more efficient in situations of high N₂O emissions compared with low; the reduction was 66.0% when EF > 1.5% of N applied compared with 51.9% when

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$EF \leq 0.5\%$. DCD was more efficient when applied at rates $> 10 \text{ kg ha}^{-1}$. NIs were less efficient in urine with lower N content ($\leq 7 \text{ g kg}^{-1}$). NI efficiency was negatively correlated with soil bulk density, and positively correlated with soil moisture and temperature. Better understanding and management of NIs can optimize N_2O mitigation in grazing systems, e.g., by mapping N_2O risk and applying NI at variable rate, contributing to improved livestock sustainability.

Keywords Air pollution · Greenhouse gases · Grassland · Pasture · Manure · Enhanced-efficiency fertilizers

Introduction

Livestock systems are responsible for a large proportion of global greenhouse gas (GHG) emissions, representing *ca.* 18% of all anthropogenic GHG emissions and *ca.* 80% of all emissions from the agricultural sector. Enteric methane (CH_4) from ruminants and nitrous oxide (N_2O) from fertilizer N inputs and excreta applied to or deposited on the soil are the main sources (Steinfeld et al. 2006; Gerber et al. 2013; IPCC 2014). The agriculture sector is the largest source of anthropogenic N_2O emissions, representing 60% (Syakila and Kroeze 2011), with grasslands contributing to 54% of agricultural emissions (Dangal et al. 2019). In addition, N_2O is the main compound causing ozone layer depletion (Ravishankara et al. 2009).

Nitrous oxide emissions from soil are highly variable in space and time because N_2O production and emission are influenced by several factors, including climatic conditions, soil properties, and N management (Mathieu et al. 2006; Chadwick et al. 2014), all of which control the complex biotic and abiotic reactions that produce N_2O (Hayatsu et al. 2008; Spott et al. 2011). A new refinement of guidelines for national GHG inventories was published recently with disaggregated N_2O emission factors (EFs) (IPCC 2019). For example, the default N_2O (EF) for synthetic fertilizer (EF1) has changed from 1.0% (0.3–3.0%) (IPCC 2006) to 0.5% (0.0–1.1%) of N applied in dry climates, and 1.6% (1.3–1.9%) in wet climates (IPCC 2019). For cattle urine and dung deposited on soil by grazing livestock ($EF_{3\text{PRP}}$), the N_2O EF has changed from 2% (0.7–6.0%) to

0.2% (0.0–0.6%) in dry and 0.6% in wet climates (0.0–2.6%).

In addition, specific studies have shown differences in N_2O emissions according to N management, e.g. smaller N_2O emissions from dung than urine in grazing systems (Krol et al. 2017; Chadwick et al. 2018); lower N_2O EF from sheep urine than cattle urine (López-Aizpún et al. 2020); lower N_2O EF from urea fertilizer than calcium ammonium nitrate in temperate climate (Harty et al. 2016; Cardenas et al. 2019), but the opposite in tropical conditions (Degaspari et al. 2020). Understanding the risk of N_2O emissions according to management and edaphoclimatic conditions can help to identify more regional and site-specific mitigation strategies.

There has been an interest in the use of synthetic nitrification inhibitors (NIs) to reduce both direct and indirect N_2O emissions (resulting from NO_3^- leaching) (Misselbrook et al. 2014; Aliyu et al. 2021). The NIs delay microbial oxidation of ammonia to nitrate in soil (Adhikari et al. 2021). Slowing down nitrification in soils without restricting N demand from plants can result in a strong reduction in N loss and an increase in nitrogen use efficiency (NUE) and crop yields (Snyder et al. 2009; Abalos et al. 2014; Li et al. 2017; Cai and Akiyama 2017; Aliyu et al. 2021). The most popular commercially available NIs are Dicyandiamide (DCD), 3,4-dimethylpyrazole phosphate (DMPP), and Nitrapyrin (Adhikari et al. 2021). The mode of action of these inhibitors is to block the ammonia monooxygenase (AMO) enzyme through chelating copper in the first step of nitrification (Subbarao et al. 2006; Trenkel 2010).

According to meta-analysis studies, using DCD and DMPP reduced N_2O emissions by 40–56% in arable systems (Gilsanz et al. 2016; Aliyu et al. 2021), and 45–50% in grasslands (Cai and Akiyama 2017; Chadwick et al. 2018; Li et al. 2021). Whilst no difference was found between the efficacy of DCD and DMPP in the study by Gilsanz et al. (2016), other studies have shown that DCD can be degraded faster in soil and be less efficient than DMPP (Weiske et al. 2001; Marsden et al. 2016). Marsden et al. (2016) showed similar mobility between DCD and DMPP in soil, and concluded that microbial degradation rates may have more influence on NI efficiency than sorption and desorption processes. The half-life of DCD depends on soil properties and temperature, ranging from 7 to 254 days (McGeough et al. 2016).

The efficacy of NIs can be influenced by several factors, including soil, climate, and management characteristics, resulting in a range of N_2O emission reductions of 19% up to 100% following N inputs to agricultural soils (Snyder et al. 2014; Chadwick et al. 2018). For example, DCD applied in urine patches had a greater efficiency in liquid form than zeolite-coated (Cai and Akiyama 2017). Also, DCD was more effective at reducing N_2O emissions when applied at a rate of 30 kg ha^{-1} than 10 kg ha^{-1} (Minet et al. 2018). DMPP reduced N_2O emissions from slurry, but not from ammonium nitrate (Menéndez et al. 2006). Nitrapyrin has been shown to reduce N_2O emissions from slurry by 59% but by 35% from urine (Ward et al. 2018).

Moreover, some studies have reported low efficiency of DMPP, DCD, and Nitrapyrin in reducing N_2O emissions in specific conditions, such as in the dry season or in situations where there was rapid inhibitor degradation (Mazzetto et al. 2015; Marsden et al. 2017; Ward et al. 2018; Pérez-Castillo et al. 2021). Therefore, it is necessary to clarify under what conditions the NIs are most efficient in reducing N_2O emissions in order to improve their effectiveness in grazing systems. The present study differs from previous meta-analysis of NIs in reducing N_2O emissions (Gilsanz et al. 2016; Han et al. 2017; Cai and Akiyama 2017; Aliyu et al. 2021; Li et al. 2021) as it focuses only on grazing systems, considers all N input sources (fertilizer and excreta), and concentrates on the most used NIs worldwide (DCD, DMPP, and Nitrapyrin). The aim of this study was to investigate, through a meta-analysis, the factors that may influence the efficiency of these predominant nitrification inhibitors (NI) in reducing direct N_2O emissions from N input (fertilizer and excreta) in grazing systems.

Material and methods

Data compilation

Original articles were searched on the Web of Science with the terms “ N_2O ”, “nitrification inhibitor”, and “grazing”, resulting in 167 articles from 1996 to 2022. In order to analyze the effects of climatic variables, only studies conducted in the field were included, with laboratory incubations and controlled condition experiments excluded.

The following pieces of information were extracted from each paper and entered into a database: cited reference, agricultural system (pasture, mixed, etc.), the dominant species of pasture plant, country, season, water input in the period (mm), average air temperature ($^{\circ}\text{C}$), average water-filled pore space (WFPS—%), N rate (kg ha^{-1}), N source (urea, ammonium sulfate, calcium ammonium nitrate, slurry, dung, urine), N content (g kg^{-1}), average soil organic carbon (%) (0–10 cm depth), soil texture (% of clay, 0–10 cm depth), average soil temperature (0–10 cm depth, $^{\circ}\text{C}$), bulk density (0–10 cm depth, g cm^{-3}), soil pH (0–10 cm depth), nitrification inhibitor type (Dicyandiamide—DCD; 3,4-Dimethylpyrazole phosphate—DMPP; and Nitrapyrin), NI application rate (kg ha^{-1}), NI mode of application (oral in drinking water, applied separately and mixed with the N source), treatments, number of replicates, N_2O emission (kg ha^{-1}), N_2O emission factor (%) per treatment (mean and standard deviation), days of N_2O measurements, and reduction (%) in N_2O EF due to addition of the nitrification inhibitor. Data not specifically in text or tables was extracted from figures using Web-PlotDigitizer (Rohatgi 2019).

Data organization

Synthetic fertilizers were combined into one category (urea, ammonium nitrate, ammonium sulfate, ammonium sulfate nitrate, and calcium ammonium nitrate) and the N content was not evaluated for this source due to already distinct values. Sheep urine, cattle urine, and synthetic products designed to replicate them were combined into one category. Treatments in which synthetic fertilizers and excreta were combined were excluded. Slurry includes fresh and stored liquid dairy effluent and pig slurry. When not reported, the N_2O EF (% of N applied) was calculated using data on N_2O -N emissions from treatments (N input), discounting background N_2O -N emissions (no N input), and being related to N rate applied. Average air temperature, soil temperature, and WFPS (0–10 cm depth) for the reporting period were used, or calculated from minimum and maximum values when not reported.

A data frame was created to conduct the meta-analysis. Studies without background emissions were excluded. The mean and standard deviation (SD) of N_2O EFs from treatments with and without

(control) nitrification inhibitors were separated from other treatments (no N addition). When a study did not report the SD, the average SD from all studies was considered (Cai and Akiyama 2017). In total, 61 studies were analyzed, with 269 comparisons (control and NIs treatment pairs) from 2164 observations (Table 1). To avoid duplication, we did not include the data from Bell et al. (2015) and Cardenas et al. (2016) repeated in Chadwick et al. (2018). In the only two situations from the 2164 N₂O-EF observations where negative values were reported, values were converted to positive values by adding to all the data the minor value (0.03) + 0.0001 according to van der Weerden et al. (2020).

Meta-analysis

Nitrous oxide EFs from treatments where the inhibitors were used were compared with no inhibitors using the natural log transformation response ratio (RR) (Viechtbauer 2010), following the equation (Eq. 1):

$$RR = \ln \frac{m1i}{m2i} \quad (1)$$

where *RR* denotes the natural log of the response ratio, which we defined as the effect size, and *m1i* and *m2i* are the mean values for the experimental group (containing nitrification inhibitors) and control group, respectively.

The effect sizes for each grouping were calculated from mean N₂O EF, SD, and number of replicates via the weighted random effects model, using the functions *escalc* (measure=ROM) and *rma* (method=REML) of the ‘metafor’ package (Viechtbauer 2010). A heterogeneity test (Qt) was conducted via restricted maximum likelihood estimator. The 95% confidence interval (CI) was generated. The categorical moderator of each grouping was included in the model via ‘mods’ argument in the *rma* function. Comparisons between groups were made using ANOVA ($p < 0.05$). The RR was back-transformed and results were expressed as a percentage (%) of change from control (N treatments without NIs). Publication bias was checked by Egger’s regression test using *funnel* and *regtest* functions of the *metafor* package (Viechtbauer 2010). A multivariate meta-analysis linear model (mixed-effects) was conducted to assess the influence of

environmental factors on the effect size and their non-independence using the function *rma.mv* of *metafor* package (Viechtbauer 2010). Meta-analysis was conducted in R software version 4.0.5 (R core team 2021). Graphics were made in SigmaPlot, version 12.5 (Systat Software 2006).

The efficiency of NIs was evaluated according to classes that may influence it. The following categories (groupings) were analyzed: N₂O emission factor (≤ 0.5 , 0.5–1.0, 1.0–1.5, $> 1.5\%$ of N applied); N source (urine, fertilizer, slurry and dung); NI type (DCD, DMPP, Nitrapyrin); mode of application of NI (separately applied, mixed with the N source, oral intake via drinking water); slurry N rate application (≤ 100 , > 100 kg ha⁻¹); slurry N content (≤ 4 , > 4 g kg⁻¹), urine N rate (≤ 500 , 500–1000, > 1000 kg ha⁻¹), urine N content (≤ 7 , > 7 g kg⁻¹); soil temperature (≤ 10 , 10–15, > 15 °C), soil organic carbon (≤ 4 , 4–8, $> 8\%$), soil bulk density (≤ 1 , > 1 g dm⁻³), WFPS (≤ 50 , 50–75, $> 75\%$). Categories were not divided into classes if the number of data was lower than three comparisons, from only one study, or with small variation in N₂O EF.

Results

Reduction in N₂O emission factor

The N₂O EF for N sources ranged from 0.0001 to 8.25% of N applied (Fig. 1). Dung resulted in a tenfold smaller N₂O EF than other N sources. The median and quartiles (1st and 3rd) of N₂O EFs were: 0.62% (0.21%, 1.31%); 0.42% (0.10%, 1.10%); 0.56% (0.18%, 1.11%); and 0.05% (0.03%, 0.12%) of N applied for urine, fertilizer, slurry, and dung, respectively (Fig. 1).

The average duration of N₂O measurements in the studies was 174 days (20–365), with no difference ($p < 0.05$) in NI efficiency in reducing N₂O-EF between short period (≤ 90 days, $n = 129$), with 54% (45.2–61.4%) of reduction, and long period (90–365 days, $n = 140$), showing 58.9% (51.4–65.1%) of reduction. Overall, the NIs reduced N₂O EF by 56.6%, with a 95% confidence interval of 51.1–61.5%, from all N sources (Fig. 2). The

Table 1 Management of N and nitrification inhibitors (NI) applied in grazing systems from the data analyzed

Reference	Country/ Region	N source ^a	N content (g kg ⁻¹) ^b	N rate (kg ha ⁻¹)	NI type ^c	NI mode of application	NI rate (kg ha ⁻¹)	N ₂ O-N EF (% of N applied) ^d
Ball et al. (2012)	New Zealand	Urine	NA	1000	DCD	Separately	10	0.73–4.66
Balvert et al. (2017)	New Zealand	Urine	NA	600	DCD	Mixed	10	0.02–0.09
Baral et al. (2014)	Denmark	Urine	NA	608	DCD	Mixed	10	0.002–0.03
Barneze et al. (2015)	United Kingdom	Urine	7.98	450	DCD	Mixed	10	0.42–0.66
Bell et al. (2015)	Scotland	Urine	NA	420–480	DCD	Mixed	10	0.06–1.07
Bell et al. (2016)	United Kingdom	AN	NA	320	DCD	Separately	26	0.60–1.34
Cameron et al. (2014)	New Zealand	Dung, Urine	NA	700, 100	DCD	Separately	10	0.05–1.94
Cardenas et al. (2016)	United Kingdom	Urine	NA	405–435	DCD	Mixed	10	0.11–2.96
Chadwick et al. (2018)	United Kingdom	Urine	NA	338–568	DCD	Mixed	6.5	0.01–1.64
Dai et al. (2013)	New Zealand	Urine, Urea	NA	50, 300, 600	DCD	Separately	10	0.18–1.12
de Klein et al. (2011)	New Zealand	Urine	6.1, 10	1000	DCD	Separately	20, 30	0.41–1.38
de Klein et al. (2014)	Australia	Dung, Urine	NA	616–1001	DCD	Separately	10	0.05–3.7
Di and Cameron (2008)	New Zealand	Urea	NA	200	DCD	Separately	10	0.006–0.01
Di et al. (2010)	New Zealand	Urine	NA	1000	DCD	Separately	10	0.3–3.0
Di et al. (2007)	New Zealand	Urine	NA	1000	DCD	Separately	10	0.02–2.0
Dittert et al. (2001)	New Zealand	Slurry	NA	63.9	DMPP	Separately	2	0.7–1.3
Escuer-Gatius et al. (2020)	Estonia	Slurry	26.5	7950	DMPP	Mixed	3	0.02–0.15
Friedl et al. (2017)	Australia	Urea	NA	36.8	DMPP	Mixed	0.2	0.07–0.41
Hoogen- doorn et al. (2008)	New Zealand	Urine	9.0	369	DCD	Mixed	20	0.001–0.21
Giltrap et al. (2010)	New Zealand	Urine	NA	600	DCD	Mixed	7	0.13–0.43

Table 1 (continued)

Reference	Country/ Region	N source ^a	N content (g kg ⁻¹) ^b	N rate (kg ha ⁻¹)	NI type ^c	NI mode of application	NI rate (kg ha ⁻¹)	N ₂ O-N EF (% of N applied) ^d
Kelly et al. (2008)	Australia	Urine	NA	1000	DCD	Separately	10	0.30–0.57
Kim et al. (2014)	New Zea- land	Dung, Urine	NA	700, 770	DCD	Separately	10, 20, 60	0.004–4.1
Krol et al. (2017)	Ireland	Urea	NA	40	DCD	Mixed	1.4	0.02–0.25
Ledgard et al. (2014)	New Zea- land	Dung, Urine	NA, 7.0	691, 1124	DCD	Separately	10	0.01–0.81
Li et al. (2014)	New Zea- land	Slurry	0.9–5.5	100	DCD	Mixed	10	0.01–0.07
Li et al. (2015)	New Zea- land	Slurry	1.6–5.0	53–110	DCD	Mixed	10	0.01–1.87
Luo et al. (2015)	New Zea- land	Urine	7.0	700	DCD	Mixed, Oral	10, 30, 60	0.07–0.21
Luo et al. (2016)	New Zea- land	Urine	7.0	700	DCD	Mixed, Oral	10, 30, 60	0.11–0.23
Macadam et al. (2003)	Spain	CAN, Slurry	NA	80–125	DCD, DMPP	Mixed	1, 25	0.07–5.17
Marsden et al. (2017)	United King- dom	Urine	14.5	725	DMPP	Separately	1	0.63–0.70
Mazzetto et al. (2015)	Brazil	Urine	NA	360	DCD	Separately	10	0.04–0.40
Menéndez et al. (2006)	Spain	ASN, Urine	NA, 4.3	97, 194	DMPP	Mixed, Separately	1	1.41–6.60
Menéndez et al. (2009)	Spain	Slurry	NA	114	DMPP	Mixed	1	0.38–0.64
Merino et al. (2002)	Spain	CAN, Slurry	NA, 4.3	80, 170	DCD	Separately	25	0.004–0.53
Merino et al. (2005)	Spain	Slurry	18.3, 20.4	97, 135	DMPP	Mixed	1	0.30–8.25
Minet et al. (2016)	Ireland	Slurry	NA	96	DCD	Mixed	18	0.1–0.83
Minet et al. (2018)	Ireland	Urine	4.4–7.2	565–959	DCD	Mixed, Oral	10, 30	0.13–1.59
Misselbrook et al. (2014)	England	AN, Urea, Urine, Slurry	2.7–12.5	106–624	DCD	Mixed, Separately	15	0.0001–1.15
Monaghan et al. (2013)	New Zea- land	Urine	6.0	399, 528	DCD	Separately	10	0.40–1.38
O'connor et al. (2016)	Ireland	Urine	6.1	451	DCD	Oral	1	0.14–2.68

Table 1 (continued)

Reference	Country/ Region	N source ^a	N content (g kg ⁻¹) ^b	N rate (kg ha ⁻¹)	NI type ^c	NI mode of application	NI rate (kg ha ⁻¹)	N ₂ O-N EF (% of N applied) ^d
Pérez-Cas- tillo et al. (2021)	Costa Rica	Slurry	0.39	132	Nitrapyrin	Mixed	0.5	1.60–1.69
Qiu et al. (2010)	New Zea- land	Urine	NA	1000	DCD	Separately	10	0.41–1.27
Robinson et al. (2014)	New Zea- land	Urine	7.1	700	DCD	Separately	10	0.08–0.31
Selbie et al. (2014)	Ireland	Urine	5.0, 10.0	500, 1000	DCD	Separately	30	0.04–0.17
Simon et al. (2018)	Brazil	Dung, Urine	NA	516–2560	DCD	Mixed, Separately	8	0.04–0.45
Simon et al. (2020)	Brazil	Urine	9.3	1040	DCD	Mixed	10	0.24–1.33
Smith et al. (2008)	New Zea- land	Urine	5.8	387	DCD	Separately	10	0.65–1.42
Suter et al. (2016)	Australia	Urea	NA	240	DMPP	Mixed	1	0.04–0.16
Thomas et al. (2017)	Canada	Urine	NA	360, 500	DCD, Nitrapyrin	Mixed	2, 10	0.03–0.21
Thorman et al. (2020)	United King- dom	Slurry	2.2	1000	DCD	Separately	10	– 0.03–1.31
Treweek et al. (2016)	New Zea- land	Urine	7.0	700	DCD	Separately	20	0.7–2.1
Vallejo et al. (2005)	Spain	Slurry	4.1	200	DCD	Mixed	10	0.5–2.95
Velthof et al. (1996)	Netherland	AS	NA	80	DCD	Mixed	20	0.1–0.2
Vistoso et al. (2012)	Chile	Urea	NA	40	DCD	Mixed	10	0.021–0.076
Ward et al. (2018)	Australia	Dung, Slurry, Urine	NA	308–1000	Nitrapyrin	Mixed	1	0.0001–0.47
van der Weerden et al. (2016)	New Zea- land	Slurry, Urea	NA	28–65	DCD	Separately	1, 10	0.03–0.94
Zaman and Blen- nerhassett (2010)	New Zea- land	Urine	8.0	600	DCD	Separately	5, 7, 10	0.33–1.71
Zaman and Nguyen (2012)	New Zea- land	Urine	6.8	600	DCD	Separately	10	0.3–2.3
Zaman et al. (2013)	New Zea- land	Urine	NA	600	DCD	Separately	7, 10	0.19–1.08
Zaman et al. (2008)	New Zea- land	Urea	NA	150	DCD	Mixed	10	0.5–1.1

Table 1 (continued)

Reference	Country/Region	N source ^a	N content (g kg ⁻¹) ^b	N rate (kg ha ⁻¹)	NI type ^c	NI mode of application	NI rate (kg ha ⁻¹)	N ₂ O-N EF (% of N applied) ^d
Zaman et al. (2009)	New Zealand	Urine	NA	600	DCD	Mixed	10	0.13–1.87

^aAN, Ammonium nitrate; AS, Ammonium sulfate; ASN, Ammonium sulfate nitrate; CAN, Calcium ammonium nitrate

^bNA, not applicable/available

^cDCD, Dicyandiamide; DMPP, 3,4-Dimethylpyrazole phosphate

^dEF, Emission factor

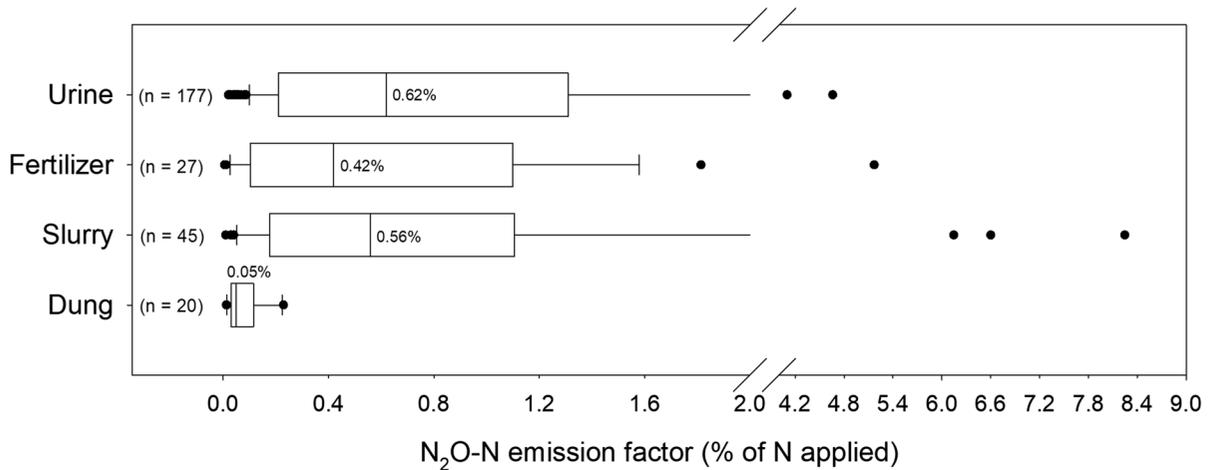
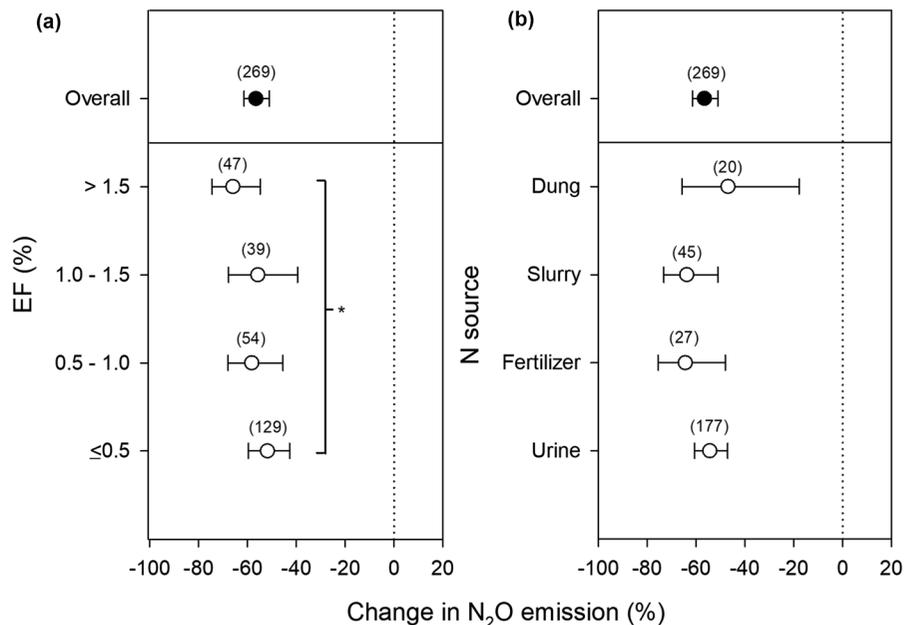


Fig. 1 Boxplot of N₂O emission factors from N input in global grazing systems extracted from the literature. (n) represents numbers of comparisons (control and NIs treatment pairs). Median values are shown in the bars

Fig. 2 Change in N₂O emission factor by the addition of nitrification inhibitors to N inputs in grazing systems, according to emission factor (EF) (a) and N source (b). Mean and 95% confidence intervals are shown. Numbers of comparisons (control and NIs treatment pairs) are indicated in brackets. Significant differences are indicated at *p* < 0.05 (*); 0.01 (**); and 0.001 (***)



reduction in N₂O-EF due to NIs addition ranged from 1.7 to 81.5% (10th and 90th percentiles).

Efficiency of NIs in reducing N₂O

N source and N₂O emission factor

The reduction promoted by NIs was similar between the N sources (Fig. 2b), decreasing N₂O emissions by 54.4% (CI: 47.1–60.6%), 64.4% (48.0–75.6%), 63.8% (51.1–73.3%), and 46.9% (17.7–65.8%) for urine, fertilizer, slurry, and dung, respectively (Fig. 2).

The NIs were more efficient ($p < 0.05$) in situations of high N₂O emissions, with inhibitors reducing N₂O EFs by 66.0% (54.8–74.5%) when the EF was $> 1.5\%$ of N applied, compared with 51.9% (42.8–59.6%) of reduction when the $EF \leq 0.5\%$ (Fig. 2a). The reduction was 58.3% (45.5–68.1%) in N₂O-EF of 0.5–1.0%

and was 55.8% (39.5–67.8%) in N₂O-EF of 1.0–1.5% (Fig. 2a) The N₂O-EF had a negative linear influence on effect size; as the N₂O-EF increased, the reduction effect decreased, increasing the NI efficiency (Table 2).

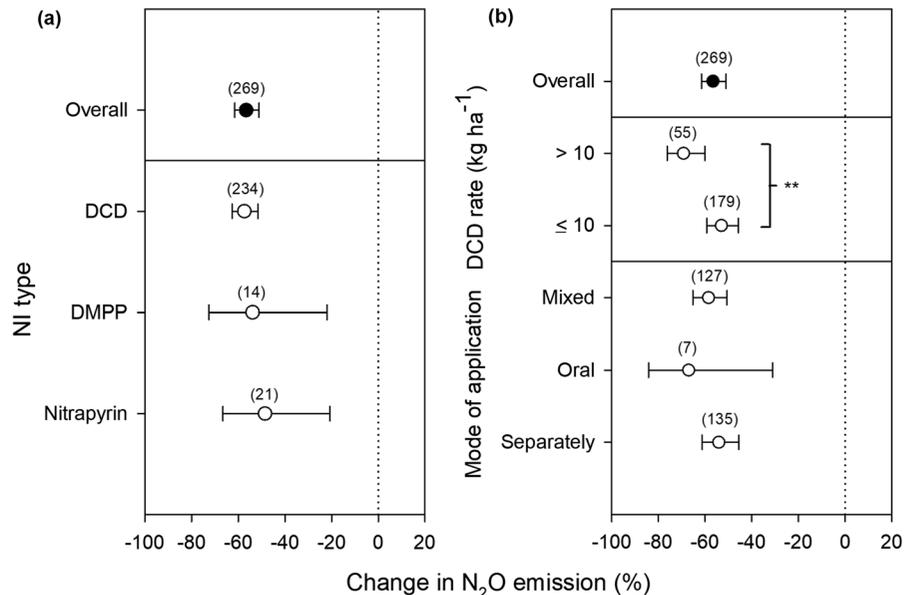
NI type, mode of application and rate

Nitrapyrin, DCD, and DMPP showed similar efficiencies of reduction at their respective rate applications, reducing N₂O EF by 48.5% (20.7–66.6%), 57.4% (51.6–62.6%), and 53.8% (22.0–72.6%) across all N sources, respectively (Fig. 3a). The DCD was more efficient ($p < 0.05$) when applied at a higher rate, with a reduction in N₂O emissions of 69.2% (60.1–76.2%) at a rate $> 10 \text{ kg ha}^{-1}$, and 53% (45.7–59.3%) when applied at a rate $< 10 \text{ kg ha}^{-1}$ (Fig. 3b). The 10th and 90th percentiles of DCD efficiency were 9.8 and 81.5% reduction, respectively. The mode of application of NIs (mixed, separately or oral) resulted in similar ($p < 0.05$) efficiencies of N₂O reduction (Fig. 3). The reductions in N₂O-EF promoted by NIs were 58.5% (50.6–65.2%), 54.1% (45.5–61.2%), and 67.0% (31.0–84.2%) for mixed, separately, and oral application, respectively (Fig. 3b).

Table 2 Influence of environmental moderators in effect size in multivariate meta-analysis linear model

Moderator	Parameter	<i>p</i> -value
N ₂ O emission factor	− 0.2383	<0.0001
Soil temperature	− 0.0163	0.0359
Soil bulk density	0.7631	<0.0001
Soil organic carbon	0.0206	0.0919
Water-filled pore space	− 0.0163	<0.0001

Fig. 3 Change in N₂O emission factor by the addition of nitrification inhibitors (NI) to N inputs in grazing systems, according to NI type (Dicyandiamide—DCD; 3,4-Dimethylpyrazole phosphate—DMPP; and Nitrapyrin) (a), mode of application and DCD application rate (b). Mean and 95% confidence intervals are shown. Numbers of comparisons (control and NIs treatment pairs) are indicated in brackets. Significant differences are indicated at $p < 0.05$ (*); 0.01 (**); and 0.001 (***)



N content and application rate

The N application rate and N content were not assessed for dung and fertilizer due to small variations in the data. However, the effects of N application rate and N content were evaluated for urine and slurry (Fig. 4). The difference ($p < 0.05$) was

only significant for the N content of urine; the NIs were more efficient for urine with higher N content. The reduction in N₂O EF was 46.2% (34.1–56.2%) for urine with N content ≤ 7 mg kg⁻¹, and 64.4% (53.0–73.1%) for urine with N content > 7 mg kg⁻¹ (Fig. 4b). According to urine-N rate, the reductions were 59.7% (49.2–68.1%), 52.6% (46.5–58.0%), and

Fig. 4 Change in N₂O emission factor by the addition of nitrification inhibitors to N inputs in grazing systems, according to slurry (a) and urine (b) application rates and N contents. Mean and 95% confidence intervals are shown. Numbers of comparisons (control and NIs treatment pairs) are indicated in brackets. Significant differences are indicated at $p < 0.05$ (*); 0.01 (**); and 0.001 (***)

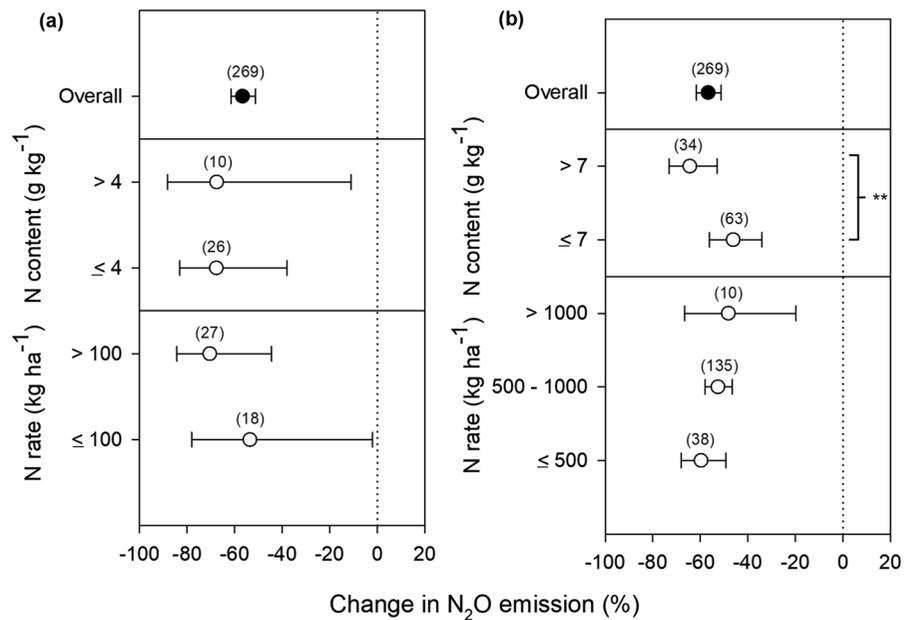
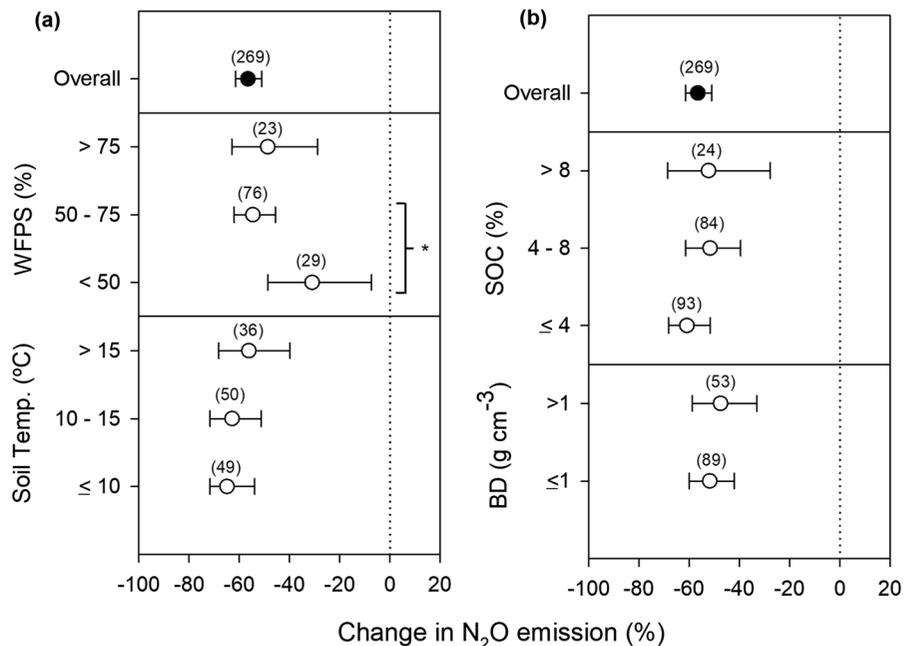


Fig. 5 Change in N₂O emission factor by the addition of nitrification inhibitors to N inputs in grazing systems, according to water-filled pore space (WFPS) and soil temperature (a), soil organic carbon (SOC) and bulk density (BD) (b). Mean and 95% confidence intervals are shown. Numbers of comparisons (control and NIs treatment pairs) are indicated in brackets. Significant differences are indicated at $p < 0.05$ (*); 0.01 (**); and 0.001 (***)



48.2% (19.8–66.6%), for N rates of ≤ 500 , 500–1000, and > 1000 kg ha⁻¹, respectively (Fig. 5b). The NI efficiency in urine ranged from 15 to 74% of reduction (10th and 90th percentiles). In the slurry application, the NIs reduced N₂O-EF by 53.6% (2.0–78.1%), and 70.5% (44.5–84.3%), for N rates of ≤ 100 , and > 100 kg ha⁻¹, respectively (Fig. 5a). The reduction was 67.7% (38.2–83.1%) for slurry N content of ≤ 4 g kg⁻¹, and 67.6% (11.1–88.2%) for N content > 4 g kg⁻¹ (Fig. 5).

Environmental conditions

The NIs were more effective ($p < 0.05$) in soil with intermediate moisture than in dry conditions, with a reduction in N₂O EF of 54.6% (45.7–62.1%) at a WFPS of 50–75%, but 31% (7.3–48.6%) when the WFPS was $\leq 50\%$ (Fig. 5b). In WFPS $> 75\%$, the reduction was 48.7% (28.8–63.0%). Grouping BD, SOC, and soil temperature had no effect ($p < 0.05$) on the efficiency of NIs to reduce N₂O emissions. The reductions were 51.8% (42.0–60.0%), and 47.5% (33.1–58.8%), with soil BD ≤ 1 , and > 1 g cm⁻³, respectively. Considering SOC, the NIs reduced N₂O-EF by 60.9% (51.7–68.3%), 51.7% (39.6–61.4%), and 52.3% (27.7–68.6%), for SOC ≤ 4 , 4–8, $> 8\%$, respectively. With respect to soil temperature, the reductions were 64.9% (53.9–73.3%), 62.9% (51.4–71.7%), 56.25% (39.9–68.2%), for ≤ 10 , 10–15, and > 15 °C, respectively (Fig. 5a).

The environmental variables have a linear influence on effect size, where soil temperature showed a negative coefficient, which means that increasing soil temperature decreased the response ratio (increasing the efficiency of NIs in reducing N₂O emissions); while increasing BD decreased the efficiency of NIs (Table 2). According to the multivariate linear model, increasing WFPS increased the efficiency of NIs, which was similar to results of grouping. The SOC influence was not significant in the model (Table 2).

Discussion

N₂O emission factors for dung and fertilizer were lower than default IPCC values

The median N₂O emission factors found were close to 0.5% of N applied for fertilizer, urine, and slurry;

and 0.05% for dung. The EF for fertilizer and dung were lower than the default value from the new IPCC refinement (IPCC 2019), 1.6% and 0.6% in the wet climate, respectively. However, for urine and slurry, the EF were similar, around 0.6% (wet climate). The smaller N₂O EF from dung than urine is in line with the literature and is attributed to the higher proportion of N in the organic form (Misselbrook et al. 2014). Therefore, in addition to the new IPCC refinement (IPCC 2019), other disaggregated values or developing a country-specific EF (IPCC 2019) may better estimate the N₂O emissions for national inventories, especially for fertilizer and dung, and site-specific conditions.

NIs reduced N₂O emissions in diverse conditions

The reduction of N₂O emissions through NIs added to the N source was on average 56.6%, which is slightly higher than previously reported in meta-analysis studies. Recently, Aliyu et al. (2021) reported a 56% reduction, and Li et al. (2021) observed a 45% of reduction. Cai and Akiyama (2017) calculated an average reduction of 52%, and Gilsanz et al. (2016) showed an average reduction of around 40%. The studies had different focuses related to NIs. Li et al. (2021) evaluated DCD and DMPP in grassland, Cai and Akiyama (2017) studied DCD in urine patches, while Gilsanz et al. (2016) and Aliyu et al. (2021) evaluated the NIs in cropland systems. The present study evaluated DCD, DMPP, and Nitrapyrin for all N sources applied in global grazing systems. To our knowledge, the present study is the first meta-analysis of NIs specifically in grazing systems, exploring all N sources (fertilizer and excreta) and the most widely used NIs.

Contrasting results of NIs efficiencies have been observed in grazing systems. For example, in meta-analysis studies, Gilsanz et al. (2016) reported that DCD was not efficient in reducing N₂O emissions when added to ammonium nitrate in sandy soils, probable due to low emissions in those soil types; on the other hand, Thorman et al. (2020) showed that DCD decreased N₂O emissions to zero when mixed with slurry. In Thorman et al. (2020), slurry was broadcast-applied in spring, where the high NI efficiency may have occurred due to longer NI stability in low temperatures (7 °C). In the present study, the

NIs reduced N_2O emissions between 2 and 83% (10th and 90th percentiles), but on average the NIs were efficient in all conditions evaluated, showing a significant reduction in N_2O emissions in all comparisons (Figs. 2–5); with the lowest average efficiency of 31% of reduction and the highest of 70%.

NIs were more efficient when N_2O emission factors were high

The efficiency of NIs in reducing N_2O EFs varied according to the categories analyzed. The inhibitors were more efficient in situations of high emissions ($EF > 1.5\%$) where they reduced N_2O emissions by 66%, than in situations with low emissions ($EF \leq 0.5\%$) where the average reduction was 52%. The NIs efficiency according to the magnitude of N_2O emissions in grazing systems was not explored in previous meta-analysis studies. However, the higher efficiency of NIs found in some situations was attributed to possible high N_2O emission, such as increasing soil temperature, reported in a recent meta-analysis (Li et al. 2021). It is likely that the lower efficiency of NIs in low EF occurred due to other pathways of N_2O production that were not inhibited by the NIs, e.g. codenitrification (Spott et al. 2011). Cardenas et al. (2016) showed a reduction in N_2O emissions of 58% from NI added to cattle urine in summer when EF was 3%, but no reduction was observed when EF was 0.1% of N applied in autumn. The authors suggested that in summer, nitrification was the main pathway of N_2O emissions, and then DCD was efficient, while in autumn the microbial activity was low, resulting in small N_2O emissions and a lack of efficiency of NI. In this way, mapping the risk of N_2O emissions in grazing systems, such as identifying the hot spots and moments (Misselbrook et al. 2016; Roten et al. 2017; Lush et al. 2018), and applying the NI at variable rate and time, can be a strategic management to optimize N_2O mitigation.

NI efficiency was not affected by NI type or mode of application

In this study, there was no difference in the average NI efficiency according to N source, NI type, and mode of NI application. These results support the meta-analysis of Gilsanz et al. (2016) that reported

no difference between DCD and DMPP. However, in a recent meta-analysis, Li et al. (2021) reported higher efficiency of DCD compared to DMPP, with a reduction of 48 and 33%, respectively. Despite this difference being from aggregated data, there are many more studies with DCD than DMPP (Fig. 3), so to better explore DMPP efficiency and compare it with DCD, more studies are necessary (Gilsanz et al. 2016).

In relation to mode of application, Cai and Akiyama (2017) showed in their meta-analysis study that DCD was more efficient when applied in liquid form than when coated with zeolite. The study of Cai and Akiyama (2017) focused on urine patches, in which DCD in liquid form was probably better mixed with urine, resulting in higher performance than when applied coated with zeolite. On the other hand, the present study involved different N sources (fertilizer, urine, dung, and slurry), and the mode of application resulted in similar efficiency, which suggests that inhibitors were efficiently mixed with the N source, resulting in co-location of inhibitor and NH_4^+ in the soil, and reduced N_2O emissions independently of the mode of application (separate, mixed, or oral). This result demonstrated that NIs could be applied in different ways to N sources, resulting in similar efficacy of N_2O reduction, which can help farmers to plan the best option for management in the field.

NIs were more efficient in urine with high N content

Nitrogen application rate and N content were evaluated for each N source separately, where the difference in NI efficiency was only influenced by N content in urine. The NIs were more efficient in urine with high N content ($> 7 \text{ g kg}^{-1}$) than in lower N content urine. Marsden et al. (2016) showed higher mobility and degradation of NIs in soil when urine was applied; then, the urine with lower N content has a higher C/N ratio, which may increase NIs degradation and movement in soil, resulting in lower efficiency compared with urine containing more N. Higher N manure application rates can also increase N_2O emissions (Han et al. 2017), and can result in higher efficiency of NI to reduce them, as shown here. On the other hand, a higher urine N content may indicate lower NUE, which is undesirable and

can be improved, e.g., by changing the animal diet with more minerals (Singh et al. 2009). In addition, how the urine was stored can be relevant to the composition, despite no difference being observed between urine non-freeze-dried and freeze-dried (Charteris et al. 2021).

Increasing DCD rate increased its efficiency

The efficiency of DCD can be improved by increasing the application rate, with higher efficiency when applied at rates $> 10 \text{ kg ha}^{-1}$ than at lower doses. This result contrasts with the previous meta-analysis studies reported no difference in NI efficiency in reducing N_2O emissions according to their application rate in grassland (Cai and Akiyama 2017; Li et al. 2021). Despite no significant effect, in the study of Li et al. (2021) increasing NI rates (DCD and DMPP) had a tendency ($p=0.07$) to increase their efficiencies in reducing N_2O emissions. It is likely that the greater amount of data of DCD rates in the present study than in the previous meta-analysis allowed a better comparison of effect of DCD dosage in reducing N_2O emission, where a recommendation to apply DCD at a rate higher than 10 kg ha^{-1} can improve its efficiency. However, because nitrification inhibitors can increase NH_3 volatilization losses (Lam et al. 2017), combining them with a urease inhibitor like NBPT (N-(n-butyl) thiophosphoric triamide) may be a better strategy for reducing N_2O and NH_3 losses from urea and urine (Zaman and Blennerhassett 2010; Soares et al. 2012). On the other hand, the DCD can maintain soil pH and ammonium content from urea hydrolysis at high values in soil for longer than the persistency of NBPT, increasing NH_3 losses and offsetting the benefits of NBPT (Soares et al. 2012).

The DCD is commonly applied at a rate of 2–10% of N application, or at 10 kg ha^{-1} in grassland (Trenkel 2010). Increasing DCD rates can also increase the cost of N fertilization and the risk of entry into the food chain (Marsden et al. 2015). The NIs increased price of fertilizer by 30–60%, but their use also increases the profitability of agriculture activity, as it can result in higher NUE, crop yield, and C credits due to $\text{CO}_{2\text{eq}}$ mitigated (IFA 2022). The use of enhanced-efficiency fertilizers, including NIs, has been increased worldwide, corresponding to an annual consumption of 14 Mt of N (Cantarella et al. 2018). However, the

presence of NI in food products can be considered problematic for public perceptions and the industry market (Hoekstra et al. 2020). For example, in New Zealand, the DCD was voluntarily suspended in 2013, due to DCD residues found in milk (MPI 2013). Despite the Codex Alimentarius Commission (FAO-WHO) having not established acceptable residual levels of NIs in food, some regions, such as Europe and New Zealand, have adopted default values (Adhikari et al. 2021). Nevertheless, more studies are necessary to clarify the effect of DCD on animal and human health.

NIs were more efficient in intermediate soil moisture, high soil temperature, and low soil bulk density

Within the constraints of the climate and soil conditions evaluated in the studies in this analysis, the NIs were less efficient in dry conditions (WFPS $< 50\%$) compared to intermediate soil moisture levels (WFPS: 50–75%). Soil moisture was not explored in other meta-analysis of NI efficiency in reducing N_2O emission in grassland, but individual studies reported lower efficiency of nitrpyrin (Pokharel and Chang 2021) and DCD in dry conditions (Mazzetto et al. 2015). Moreover, the lower efficiency of NIs in drier conditions can be an indirect effect of low N_2O emissions from N sources (O'Neill et al. 2021); as we showed here, the NIs were less efficient in situations of $\text{N}_2\text{O}\text{-EF} < 0.5\%$. In general, the highest production of N_2O emissions in soil is expected to occur in WFPS between 50 and 75%, which reflects the more favorable condition for both nitrification and denitrification processes (Del Grosso et al. 2002; Liu et al. 2007).

The efficiency of NIs in reducing N_2O emissions was not affected by grouping soil C, temperature, or bulk density. However, in the multivariate model, the soil temperature had a negative influence on effect size, increasing the NI efficiency as temperature increased. The opposite was observed with soil BD. Similar results were reported in meta-analysis studies with soil temperature (Li et al. 2021) and with BD (Gilsanz et al. 2016), attributing them to possible higher N_2O emissions in high temperatures and in soils with less clay content. In fact, the present study showed the higher efficiency of NIs in situations of higher $\text{N}_2\text{O}\text{-EF}$ (Fig. 2). Interestingly, the

NIs were not affected by increasing temperature and clay content as reported in a laboratory study (McGeough et al. 2016). It is likely that the field N₂O emissions evaluated here occurred in a period when NIs were still efficient, avoiding loss of efficiency due to degradation.

Conclusions

This meta-analysis showed that NIs were able to reduce direct N₂O emissions from N inputs to grazing systems by 50–60%. The present study clarifies important aspects related to NIs efficacy. It is apparent that specific sets of environmental, soil, and N source conditions can influence NI efficiency, suggesting that site-specific recommendations could be used. For example, the NIs were more efficient in situations of high N₂O emissions; at intermediate soil moisture; in urine with high N content; and DCD was more efficient at a rate > 10 kg ha⁻¹. In addition, we showed some conditions where no difference in NI efficiency was observed, which can be useful for guidance to farmers, such as the mode of application of NIs (separately, mixed and oral); NI type (DCD, DMPP, and Nitrapyrin), N input (excreta and fertilizer), and soil organic carbon. Soil bulk density showed a negative correlation with NI efficiency, while soil temperature and moisture showed a positive correlation. Better understanding and management of NIs in grazing systems, e.g., mapping the risk of N₂O emissions and applying NI at a variable rate, can optimize N₂O mitigation, especially when emissions are high, and improve the sustainability of livestock products, a critical issue in the sector.

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writing-reviewing and editing: Rubens A. C. Lamparelli, Gleyce K. D. A. Figueiredo, John J. Sheehan, Lee R. Lynd.

Data availability All data analyzed during this study are included in this published article.

Declaration

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

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