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Original Research

Effect of Dicyandiamide on Grassland Nitrous Oxide Emission Rates by a Meta-Analysis

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Abstract

Nitrogen losses of nitrous oxide (N_2O) in grazed grassland are strongly driven by urine deposition by grazing ruminants. There is robust concern about applying nitrification inhibitor to mitigate nitrogen losses in global grasslands. However, the effect of dicyandiamide (DCD) on N_2O emission rates and its driven factors remain unclear due to spatial heterogeneity. In the present study, we synthesized 133 group data from 69 published articles. It was indicated that effect size was -0.784±0.048 (P<0.0001) indicating a reduction of 54.34% in N_2O emission rates. There was significant difference on effect size between different dose of nitrogen fertilization. Moderate dosage of DCD application was the best mitigation effect of 56.09%, which was significantly higher than light dosage of DCD application. Mixed effect model results revealed that precipitation, bulk density and air temperature could explain 16.93%, 12.31% and 3.02% variations in effect size. In addition, DCD application was an effective strategy to mitigate N_2O emission rates in global grazed grasslands.

Keywords: effect size, nitrification inhibitor, N_2O; mixed effects model, bulk density

Introduction

Nitrous oxide (N_2O) is the third most important anthropogenic greenhouse gas, atmospheric levels of N_2O reached new highs at 334.5 ppb in 2022, with a global warming potential approximately 310 times higher than CO_2 [1]. Dung and urine excreted onto grasslands are a major source of N_2O [2]. The application of dicyandiamide (DCD) was an effective way of decreasing N_2O emissions from nitrification process [3, 4].

The N_2O production by the slurry amended soil was twice as that of the mineral amended one [5]. DCD retained soil nitrogen in the ammonium form, which is expected to reduce nitrogen loss [6]. Emission factors were 0.89% and 0.59% for urea fertilizer and urea with DCD in south-west Scotland, this indicated a reduction of 33.7% on N_2O [7]. DCD reduced N_2O emissions by 33.0% in the UK grasslands [3]. DCD treatments decreased alpine meadow N_2O emissions by 36.3% on the Tibetan Plateau [8]. Application of DCD reduced N_2O emissions to 4.8 kg ha^{-1}, representing

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40.0% reductions [9]. DCD reduced N_2O emissions from slurries applied in the spring by 45.0% [10]. To sum up these results, DCD reduction rate ranged from 30.0 to 45.0% [6-10]. Furthermore, DCD was highly effective in reducing N_2O emissions, with the N_2O emission factor of the urine being decreased by 58-63% in New Zealand [11]. The reduction in the N_2O emission factor by DCD ranged from 58% to 83% in New Zealand [12]. Consequently, the incorporation with DCD supplement was a robust method to successfully reduce emissions of N_2O in global grasslands. However, it was also reported that addition of DCD to urine caused no significant reduction in emissions [13]. In addition, strong heterogeneity was reported by aforementioned manipulated results across the global grasslands.

Global N_2O emission rates were affected by soil nitrification and denitrification processes [12]. N_2O emitted in a greater proportion from nitrification rather than from denitrification in the slurry treatment grasslands [5]. The episodic N_2O emissions was a major source of agricultural fields N_2O following heavy rainfall [14]. Numbers of ammonia-oxidizing bacteria amoA gene copy was significantly inhibited by DCD [15]. Ammonia oxidizing bacteria was more greatly inhibited by DCD [8]. DCD appear to be less effective at reducing N_2O emissions due to the higher soil temperature [3]. Soil pH and soil organic matter were considered to be the most important factors influencing the inhibition efficiency of DCD [16]. DCD had no effect on grasslands N_2O at the Southland site, probably due to extremely wet soil conditions [17]. DCD efficiency was positively correlated with soil moisture and temperature, but negatively correlated with soil bulk density [4]. In summary, ammonia-oxidizing bacteria, precipitation, soil temperature, pH and organic matter were referenced as robust driven factors on grassland N_2O emission rates. In addition, the effects of DCD on N_2O emission rates were driven by multifactor across the global grasslands.

Previous studies have focused on the response of N_2O emission rates to DCD in single manipulation, and little study explored its driven factor through meta-analysis. Furthermore, there is a lack of systematic quantitative analyses of the effect size of N_2O rates to DCD application. In this study, the objective of this study was to investigate the impacts of different dosages of DCD on N_2O emission rates. Furthermore, we hypothesized that DCD would significantly reduced N_2O emission rates with the dosage increasing. Meanwhile, precipitation and air temperature and bulk density were the driven factor of effect size. Our study aimed to provide a scientific basis for mitigation N_2O emission rates in grazing grasslands.

Materials and Methods

Data Compilation

We collected published papers using the keywords "nitrification inhibitors" or "dicyandiamide" or "DCD" and "grassland" and "N_2O" or "nitrous oxide" to search web of science and Chinese articles database (China National Knowledge Internet, https://www.cnki.net) from January, 1990, to March, 2023. Total 154 articles were selected, and we screened the full articles to ensure that all the following criteria were met: (1) Concentrations of N_2O rates were measured in both DCD application and the control plots; (2) The concentration of N_2O was determined by gas chromatography. Furthermore, N_2O emission rate was average value during the whole research, and unit was μg/m^2/hour. All used data were collected from published article including DCD dose. In this study, we indicated that DCD dose ranged from 1.7 to 60 kg/hm^2, and the recommended

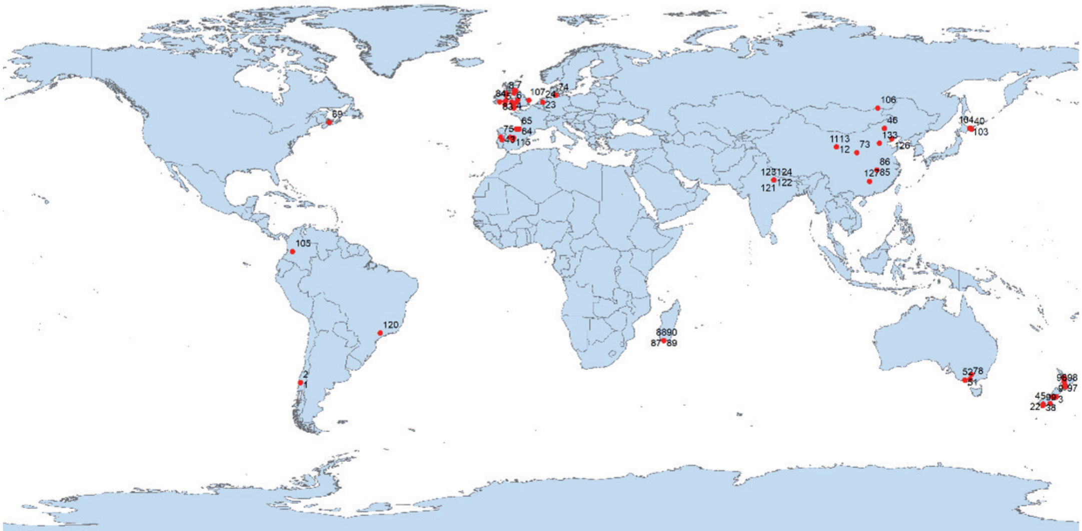

Fig. 1. Geographical locations are showed of the 133 studies in this meta-analysis across the global, and locations of red dots may present multiple effect sizes.

dose of DCD was approximately 10 kg/hm^2. DCD were divided into three groups, including light group (1.7-8.0 kg/hm^2), moderate group (10-15 kg/hm^2) and high group (20-60 kg/hm^2). Partial data were extracted using WebPlotDigitizer software. We collected 133 groups of field experimental results from 69 articles (Fig. 1) and recorded climatic factors, soil and plant characteristics. If the standard error was missed, 1/10 of the average value was used as the default value, if not, then used the average standard error.

A random-effect-model meta-analysis was performed, and log response ratios were calculated as a measure of the effect size. A 95% confidence interval (95% CI) was calculated.

$$\ln R = Ln\frac{x_e}{x_c} = \ln(x_e) - \ln(x_c)$$

where X_e and X_c are the mean values of N_2O emission rates in the DCD treatment and control, respectively.

Variance of lnR was calculated as follows:

$$V_{\ln R} = \frac{S_e^2}{N_e x_e^2} + \frac{S_c^2}{N_c x_c^2}$$

where S_e, N_e, x_e, S_c, N_c and x_c represent the standard deviations, sample sizes, and mean emission values of N_2O rates and controls, respectively.

The effect size of DCD on N_2O rates and confidence interval based on the random effect model were calculated as follows:

Individual field study weight $w_i^* = 1/(v_i + \tau^2)$

where v_i and τ^2 represent the intra- and inter-study variance of N_2O rates.

Effect size $\overline{y} = \dfrac{\sum_{i=1}^{k} w_i^* y_i}{\sum_{i=1}^{k} w_i^*}$

Standard error $SE = \sqrt{\dfrac{1}{\sum_{i=1}^{k} w_i^*}}$

95% confidence interval of effect size: $CI = \overline{y} \pm 1.96\ SE$, y_i was the single study effect size value.

Statistical Analysis

Meta-statistical analyses were performed using R 3.6.2, and we ran a random-effect model of meta-analysis in *metafor*1.9-8 (Benítez-López et al., 2017). Random effects models were conducted to analyse the estimated values and standard errors (rma). The effect of climatic factors and soil characteristics on effect sizes of DCD were analysed using mixed effects models (mods). There are likely biases due to the selection of negative results. In our study, the regression test for funnel plot asymmetry of publication bias was analysed using a mixed-effects meta-regression model (funnel and Egger's test, rma in R 3.6.2).

Publication Bias Analysis of Effect Sizes of DCD on N_2O Rates

Quantitative evaluation of the effect size was achieved from published papers. The bias in the evaluation results was perhaps affected by our selection of the papers. This result indicated that there was not significantly affected by a publication bias using Egger's regression test for funnel plot asymmetry (z = -0.845, p = 0.398). Thus, confirming that this study had no preference for paper publishing, and that the results had full credibility.

Result

Effect Size of DCD Application on N_2O Emission Rates in the Global Grasslands

Overall, DCD application had a significantly negative effect on N_2O emission rates in the global grasslands, and the effect size was significantly different from zero (P<0.0001, Table 1). The estimated value of effect size was -0.784±0.048 (95% confidence interval: -0.877 – -0.691, P 0.0001), indicating a reduction of 54.34% across the global grasslands. Effect sizes varied from -2.430 ± 0.003 to -0.006±0.039 (Fig. 2). Average N_2O emission rates were approximately 70.171±8.659 and 177.645±23.757 μg/m^2/hr in DCD application and control plots.

Effect Size of Different DCD Application Dosages on N_2O Emission Rates

There was a significant difference between different dose of nitrogen fertilization (P<0.05, Table 2). Effect sizes of light, moderate and high dose DCD were -0.587±0.127, -0.823±0.057 and -0.739±0.102, separately. Moderate DCD decreased N_2O emission rates with the best mitigation effect of 56.09%. Decrease ranges of light and high dose of DCD were 44.40% and 52.24%. Meanwhile, other moderators would be involved because tests for residual heterogeneity (Qe = 38289.563, d_f = 130) were also significant (P<0.0001).

Effect of Soil and Climate Factors on the Effect Size Based on Explained Moderator Heterogeneity Statistic

Precipitation, bulk density (P<0.01) and air temperature (P<0.05) significantly influenced effect size based on test of moderators (Table 3). Mixed effect model results indicated that three factors could explain

Table 1. Effect size of DCD on nitrous oxide emission rates in global grasslands.

Items	Effect sizes	Decrease range (%)	95% confidence interval	P	d_f
DCD	-0.784±0.048	54.34	-0.877 – -0.691	<0.0001	132

Note: effect sizes meant average ± stand error.

Table 2. Effect sizes of different DCD dosages on nitrous oxide emission rates.

Fertilization dose	Effect sizes	Decrease range (%)	95% confidence interval	P	d_f
L	-0.587±0.127 b	44.40	-0.836 – -0.337	<0.0001	14
M	-0.823±0.057 a	56.09	-0.935 – -0.710	<0.0001	98
H	-0.739±0.102 a	52.24	-0.938 – -0.539	<0.0001	18

Note: effect sizes meant average ± stand error. Same letters meant no significant differences in the same column. L, M and H meant light DCD dosage (1.7–8.0 kg DCD/ hm^2), moderate nitrogen (10-15 kg N/ hm^2) and high nitrogen (20-60 kg N/ hm^2) fertilization.

16.93%, 12.31% and 3.02% variations in effect size. Furthermore, the precipitation was negative with effect size of DCD, but soil bulk density and air temperature were positive with effect size. Meanwhile, pH, organic matter and altitude showed insignificantly positive effect on the effect size.

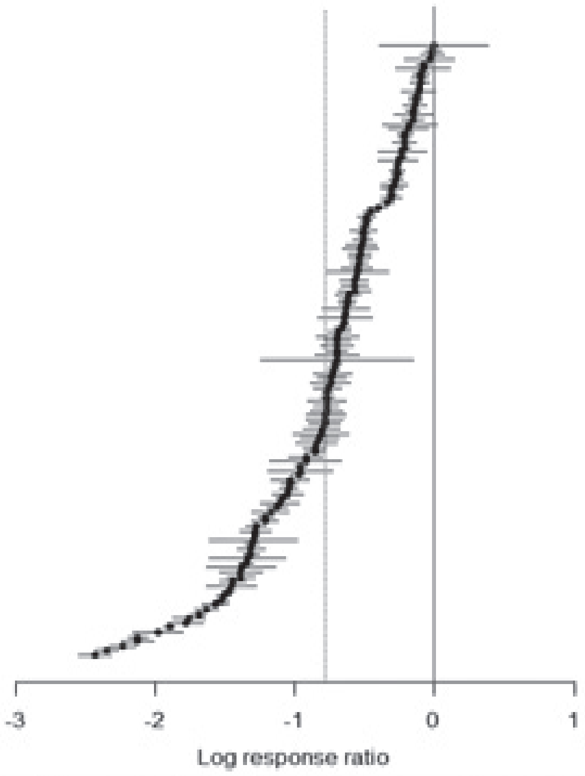

Fig. 2. Forest plots of 133 effects size estimates for responses of nitrous oxide emission rates to DCD. The black dots indicated 95% confidence intervals. Red line and red dashed line meant effect size = 0, and averaged effect size = -0.784 in this study.

Discussion

In grazed dairy pasture systems, a major source of N_2O is the nitrogen returned in the urine from the grazing animal [18]. The N_2O emissions reduction ability of DCD application on urine and dung patches was attracting much concern in global grasslands [19, 20].

In this study, we indicated that dose of nitrification inhibitor DCD significantly reduced global grazed grasslands N_2O emissions rates by approximately 54.34%. Similar studies were also reported for instance, DCD reduced N_2O emissions by 8% and 44% in dairy slurry and urea in Chile grasslands, respectively [21]. N_2O emissions could be reduced by up to 16 % and 51 % through the use of DCD in England and Wales [22]. Addition of DCD to cattle urine also reduced N_2O emission between 23% and 67% [23]. Application of DCD reduced N_2O emissions from a urine patch by 27% in mid-summer and by 47% in mid-spring and in Australia [24]. DCD significantly inhibited the N_2O emission from urine by 33%-72% at a dairy farm in South Otago [25]. In conclusion, our result was comported with these results. Meanwhile, increased DCD from 10 to 30 kg DCD ha^{-1} improved efficacy by significantly reducing N_2O emission from 34% to 64% [26]. DCD application reduced N_2O emissions by 40-50% from both forage species in southern Brazil [19]. DCD reduced the amount of N lost as N_2O by 42% and 60% when applied with calcium ammonium nitrate and cattle slurry, respectively in Spain [27]. DCD significantly reduced cumulative N_2O emissions by 47% and 70% at both Irish grasslands [6]. Average N_2O emission rate in the DCD treatment significantly decreased by 50.7% in Gansu province, China [28]. Furthermore, some extreme inhibition reductions were introduced as following. Application of DCD inhibited the nitrification process, resulting in lower N_2O emissions approximately

Table 3. Analysis of air temperature and altitude and other factors on effect sizes.

Moderators	Test of moderators (Q_M)	P	Model	R^2 (%)	df
Precipitation	10.7699	0.0025^{***}	Y = 0.0750 – 0.0003 x	16.93	89
Bulk density	8.3838	0.0038^{***}	Y = 0.1331 + 0.8308 x	12.31	53
Temperature	3.5277	0.0404^{*}	Y = -0.5405 + 0.0200 x	3.02	81
pH	1.6653	0.1969	Y = -1.3467 + 0.0937 x	0.62	104
Organic matter	1.5007	0.2206	Y= -0.6077 + 0.0002 x	0.58	86
Altitude	0.329	0.5660	Y = -0.5305 + 0.0001 x	0.00	21

Note: Y means effect size. The N and P and K meant nitrogen and phosphorus and kalium. Both * and *** meant there was significant difference at P<0.05 and P<0.01 in the present study.

54%-78% from urine patches [29]. By treating with DCD, N_2O emissions were mitigated by 76%, from 26.7 to 6.4 kg N_2O-N ha^{-1} in the South Island of New Zealand [30]. Total N_2O emissions were reduced from 46 to 8.5 kg N_2O-N ha^{-1} with DCD, representing an 82% reduction [31]. In addition, reduction range of DCD was varied from 8% to 82% across these studies, and we quantitatively evaluated the effect size involving different measurement weights.

In the present results, two reasons were taken into account for this reduction of DCD on grasslands N_2O emission rates. Firstly, DCD had a significant impact in reducing the AOB amoA gene copy numbers especially in the high nitrogen application rates [12]. Secondly, DCD resulted effective in reducing the nitrification rate by 53% in the slurry application [5]. Thus, DCD application increased the length of time mineral nitrogen in the ammonium form and significantly reduced soil nitrate by 47%, which significantly reduced N_2O emissions and leaching nitrogen losses [32].

In this study, we also discovered that precipitation, bulk density ($P<0.01$) and air temperature ($P<0.05$) significantly influenced effect size based on test of moderators. These three factors could explain 16.93%, 12.31% and 3.02% variations in effect size (Table 3). This was because all precipitation, bulk density and air temperature affected nitrification microbial abundance and activity, then controlled N_2O emissions rates. This result was newly concluded under global grasslands ecosystem scale in this study. Previous studies revealed that soil moisture has a major influence on ammonia oxidizing and denitrifying microbial communities [33]. DCD decreased cumulative N_2O emissions especially for the drier and sandier soil [22]. Meanwhile, we collected 90 sets of precipitation and divided into two groups (more or less than 800 mm). The effect sizes were -0.83±0.08 (n = 47) and -0.71±0.07 (n = 43) indicating 56.4% and 50.9% reduction when precipitation was over and lower than 800 mm.

The effect of DCD application on N_2O emissions was not clear during the freeze-thaw event due to significant variation in soil moisture and temperature [34]. To reduce N_2O emissions of both gases, livestock slurry should not be applied on wet soils [35]. Average air temperature ranged from -1.7 to 25.0 ºC in this study, we divided these data into two groups (more or less than 13.4ºC). Both effect sizes were -0.77±0.07 (n = 51) and -0.81±0.10 (n = 43) indicating 53.7% and 55.5% reduction (n = 31). Warming helped to increase the effect of DCD application on inhibiting N_2O emission. Soil bulk density affected grasslands aeration status and redox stat, which was driven factor in controlling N_2O emissions rates [20]. In this study, soil bulks were divided into two groups based on 1.0 g cm^{-3}. Effect sizes were -0.71±0.11 (n = 51) and -0.83±0.10 (n = 43), respectively, indicating 50.8% and 56.4% reduction (n = 31) when bulk was lower or higher than 1.0 g cm^{-3}. In addition, DCD application was proved to be the robust mitigation of grazing grasslands N_2O emission rates, and the reduction effects were better, when precipitation was over 800 mm, or air temperature and soil bulk were over 13.4ºC and 1.0 g cm^{-3}.

Conclusion

In summary, this essay demonstrates that DCD application had a significantly reduction effect on N_2O emission rates in the global grasslands. Effect sizes were showed significantly spatial heterogeneity. There was significant difference between different dose of nitrogen fertilization (*P*<0.05, Table 2). Effect sizes of light, moderate and high dose DCD were different and moderate DCD application has the best mitigation effect. Precipitation, bulk density and air temperature significantly influenced effect size based on test of moderators. Furthermore, soil bulk density and air temperature were positive with effect size, but the precipitation was negative with effect size of DCD. DCD application was the robust reduction strategy of grazed grasslands N_2O emission.

Author Contributions

HY: conceptualization, writing – original draft preparation. YZ and WL: methodology and project administration. QX: methodology and resources, and validation. HZ: formal analysis, investigation, and data curation. YD: writing – review and editing. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no confl ict of interest.

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References

1. WMO. Provisional State of the Global Climate in 2022. World Meteorological Organization, **2022**.
2. CHIBUIKE G., SAGGAR S., PALMADA T. The persistence and efficacy of nitrification inhibitors to mitigate nitrous oxide emissions from New Zealand pasture soils amended with urine. Geoder. **30**, e00541. **2022**.
3. BARNEZE A.S., MINET E.P., CERRI C.C. The effect of nitrification inhibitors on nitrous oxide emissions from cattle urine depositions to grassland under summer conditions in the UK. Chemo. **119**, 122, **2015**.
4. SOARES J.R., SOUZA B.R., MAZZETTO A.M. Mitigation of nitrous oxide emissions in grazing systems through nitrification inhibitors: a meta-analysis. Nutr. Cycl. Agroecos. **125**, 359, **2023**.
5. MERINO P., ESTAVILLO J.M., BESGA G. Nitrification and denitrification derived N_2O production from a grassland soil under application of DCD and Actilith F2. Nutr. Cycl. Agroecos. **60**, 9, **2001**.
6. CAHALAN E., ERNFORS M., MUELLER C. The effect of the nitrification inhibitor dicyandiamide on nitrous oxide and methane emissions after cattle slurry application to Irish grassland. Agricul. Ecosys. Environ. **199**, 339, **2015**.
7. BELL M.J., CLOY J.M., TOPP C.F.E. Quantifying N_2O emissions from intensive grassland production: the role of synthetic fertilizer type, application rate, timing and nitrification inhibitors. J. Agricul. Sci. **154**, 812, **2016**.
8. DU Y.G., XIN Y., SHU K. Effect of nitrification inhibitor on plant biomass and N_2O emission rates in alpine meadows on the Tibetan Plateau. Chem. Eco. **36**, 410, **2020**.
9. QIU W., DI H.J., CAMERON K.C. Nitrous oxide emissions from animal urine as affected by season and a nitrification inhibitor dicyandiamide. J. Soil. Sedi., **10**, 1229, **2010**.
10. THORMAN R.E., NICHOLSON F.A., TOPP C.F. Towards country-specific nitrous oxide emission factors for manures applied to arable and grassland soils in the UK. Fron. Sustain. Food Sys., **62**, e0545, **2020**.
11. BALL B.C., CAMERON K.C., DI H.J. Effects of trampling of a wet dairy pasture soil on soil porosity and on mitigation of nitrous oxide emissions by a nitrification inhibitor, dicyandiamide. Soil Use Manag. **28**, 194, **2012**.
12. DAI Y., DI H.J., CAMERON K.C. Effects of nitrogen application rate and a nitrification inhibitor dicyandiamide on ammonia oxidizers and N_2O emissions in a grazed pasture soil. Sci. Total Environ., **46**, 125, **2013**.
13. BELL M.J., REES R.M., CLOY J.M. Nitrous oxide emissions from cattle excreta applied to a Scottish grassland: Effects of soil and climatic conditions and a nitrification inhibitor. Sci. Total Environ. **50** (8), 343, **2015**.
14. AKIYAMA H., MORIMOTO S., HAYATSU M. Nitrification, ammonia-oxidizing communities, and N_2O and CH_4 fluxes in an imperfectly drained agricultural field fertilized with coated urea with and without dicyandiamide. Biol. Fertil. Soil., **49** (2), 213, **2019**.
15. NING J., AI S., CUI L. Dicyandiamide has more inhibitory activities on nitrification than thiosulfate. Plos One, **13**, 8, e3452, **2018**.
16. CUI L., LI D., WU Z. Effects of nitrification inhibitors on nitrogen dynamics and ammonia oxidizers in three black agricultural soils. Agronom. **12** (2), 711, **2022**.
17. DEKLEIN C.A., CAMERON K.C., DI H.J. Repeated annual use of the nitrification inhibitor dicyandiamide (DCD) does not alter its effectiveness in reducing N_2O emissions from cow urine. Animal Feed Sci. Technol. **166**, 480, **2011**.
18. DUFF A.M., FORRESTAL P., IKOYI I. Assessing the long-term impact of urease and nitrification inhibitor use on microbial community composition, diversity and function in grassland soil. Soil Biol. Biochem. **170**, 108709, **2022**.
19. SIMON P.L., DIECKOW J., ZANATTA J.A. Does Brachiaria humidicola and dicyandiamide reduce nitrous oxide and ammonia emissions from cattle urine patches in the subtropics? Sci. Total Environ. **720**, 137692, **2020**.
20. VARGAS V.P., SOARES J.R., OLIVEIRA B.G. Sugarcane Straw, Soil Temperature, and Nitrification Inhibitor Impact N2O Emissions from N Fertilizer. Bioen. Res. **12** (4), 801, **2019**.
21. ALFARO M., SALAZAR F., HUBE, S. Ammonia and nitrous oxide emissions as affected by nitrification and urease inhibitors. J. Soil Sci. Plant Nutri. **18** (2), 479, **2018**.
22. HARGREAVES P.R., BAKER K.L., GRACESON A. Use of a nitrification inhibitor reduces nitrous oxide (N_2O) emissions from compacted grassland with different soil textures and climatic conditions. Agricul. Ecos. Environ. **310**, 314, **2021**.
23. MORALES S.E., JHA N., SAGGAR S. Impact of urine and the application of the nitrification inhibitor DCD on microbial communities in dairy-grazed pasture soils. Soil Biol. Biochem. **88**, 344, **2015**.
24. KELLY K.B., PHILLIPS F.A., BAIGENT R. Impact of dicyandiamide application on nitrous oxide emissions from urine patches in northern Victoria, Australia. Austral. J. Experim. Agricul. **48**, 2, 156-159, **2008**.
25. DEKLEIN C.A.M., LETICA S.A., MACFIE P.I. Evaluating the effects of dicyandiamide (DCD) on nitrogen cycling and dry matter production in a 3-year trial on a dairy pasture in South Otago, New Zealand. New Zealand J. Agricul. Res. **57** (4), 316, **2015**.
26. MINET E.P., LEDGARD S.F., GRANT J. Feeding dicyandiamide (DCD) to cattle: An effective method to reduce N_2O emissions from urine patches in a heavy-textured soil under temperate climatic conditions. Sci. Total Environ. **615**, 1319, **2018**.
27. MERINO P., ESTAVILLO J.M., GRACIOLLI L.A. Mitigation of N_2O emissions from grassland by nitrification

inhibitor and Actilith F2 applied with fertilizer and cattle slurry. Soil Use Manag. **18**, 2, 135, **2002**.
28. NI H., YANG X., WANG G. Effects of nitrogen application and nitrification inhibitor addition on N_2O emissions in *Medicago sativa* L. grassland. Chin. J. Eco-Agricul. **28**, 317, **2020**.
29. KIM D.G., GILTRAP D.L., SAGGAR S.J. Field studies assessing the effect of dicyandiamide (DCD) on N transformations, pasture yields, N_2O emissions and N-leaching in the Manawatu region. New Zeal. J. Agricul. Res. **57** (4), 271, **2014**.
30. DI H.J., CAMERON K.C. Mitigation of nitrous oxide emissions in spray-irrigated grazed grassland by treating the soil with dicyandiamide, a nitrification inhibitor. Soil Use Manag. **19** (4), 284, **2014**.
31. DI H.J., CAMERON K.C. The use of a nitrification inhibitor, dicyandiamide (DCD), to decrease nitrate leaching and nitrous oxide emissions in a simulated grazed and irrigated grassland. Soil Use Manag. **18** (4), 395, **2002**.
32. SMITH L.C., KLEIN C.A.M., MONAGHAN R.M. The effectiveness of dicyandiamide in reducing nitrous oxide emissions from a cattle-grazed, winter forage crop in Southland, New Zealand. Austra. J. Experim. Agricul. **48** (2), 160, **2008**.
33. DI H.J., CAMERON K.C., PODOLYAN A. Effect of soil moisture status and a nitrification inhibitor, dicyandiamide, on ammonia oxidizer and denitrifier growth and nitrous oxide emissions in a grassland soil. Soil Biol. Biochem. **73**, 59, **2014**.
34. HAMAMOTO T., UCHIDA Y., REIN I. Effects of short-term freezing on nitrous oxide emissions and enzyme activities in a grazed pasture soil after bovine-urine application. Sci. Total Environ. **740**, e140006, **2020**.
35. MKHABELA M.S., GORDON R., BURTON D. Ammonia and nitrous oxide emissions from two acidic soils of Nova Scotia fertilised with liquid hog manure mixed with or without dicyandiamide. Chemos. **65** (8), 1381, **2006**.