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Using DMPP with cattle manure can mitigate yield-scaled global warming potential under low rainfall conditions

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ABSTRACT

Organic fertilisers can reduce the carbon (C) footprint from croplands, but adequate management strategies such as the use of nitrification inhibitors are required to minimise side-effects on nitrogen (N) losses to the atmosphere or waterbodies. This could be particularly important in a context on changing rainfall patterns due to climate change. A lysimeter experiment with maize (*Zea mays* L.) was set up on a coarse sandy soil to evaluate the efficacy of 3,4-dimethylpyrazole phosphate (DMPP) to mitigate nitrous oxide (N_2O) emissions, nitrate (NO_3^-) leaching losses and net global warming potential from manure, with (R+) and without (R-) simulated rainfall events. Soil water availability was a limiting factor for plant growth and microbial processes due to low rainfall during the growing season. Nitrification was effectively inhibited by DMPP, decreasing topsoil NO_3^- concentrations by 28% on average and cumulative N_2O losses by 82%. Most of the N_2O was emitted during the growing season, with annual emission factors of 0.07% and 0.95% for manure with and without DMPP, respectively. Cumulative N_2O emissions were 40% higher in R-compared to R+, possibly because of the higher topsoil NO_3^- concentrations. There was no effect of DMPP or rainfall amount on annual NO_3^- leaching losses, which corresponded to 12% of manure-N and were mainly driven by the post-harvest period. DMPP did not affect yield or N use efficiency (NUE) while R-caused severe reductions on biomass and NUE. We conclude that dry growing seasons can jeopardize crop production while concurrently increasing greenhouse gas emissions from a sandy soil. The use of nitrification inhibitors is strongly recommended under these conditions to address the climate change impacts.

1. Introduction

The recycling of organic fertilisers derived from livestock plays a central role in the circular bioeconomy framework (Cantzler et al., 2020) and for decreasing the carbon (C) footprint of agriculture. This is because organic fertilisers avoid upstream emissions from the industrial production of synthetic nitrogen (N) (Chen et al., 2022), and have potential to increase soil organic C stocks (Liu et al., 2021). Recent initiatives such as the "*4* per *mille*" (Rumpel et al., 2020) or the "*farm to fork*" strategy (European Union, 2020) have put the replacement of synthetic by organic N sources in the spotlight to meet such environmental objectives. However, the sustainability of organic N fertilisers is challenged by the release of reactive N compounds to the atmosphere (e.g., ammonia, NH_3, or the potent greenhouse gas nitrous oxide, N_2O) or to waterbodies through nitrate (NO_3^-) leaching or runoff. Replacing synthetic with organic N sources can result in lower, similar, or sometimes higher leaching (Wei et al., 2021), volatilisation (Ti et al., 2019) or N_2O losses (Yangjin et al., 2021) as those from synthetic fertilisers, with a high variability depending on the manure composition and environmental and management conditions. Therefore, strategies for the sustainable use of manures and slurries by mitigating N pollution without yield penalties should be identified.

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While the adjustment of N rate and timing is challenging with organic fertilisers due to potential yield declines and technical constraints (Guo et al., 2022), the use of nitrification inhibitors (NIs) can be a highly effective strategy for decreasing NO_3^- and N_2O losses from both organic and synthetic fertilisers (Qiao et al., 2015), leading to potential enhancements of N use efficiency (NUE) and yields (Abalos et al., 2014; Thapa et al., 2016). These compounds deactivate the enzyme responsible for the first step of nitrification (Bozal-Leorri et al., 2022), thus decreasing the availability of NO_3^- susceptible to be leached or stepwise reduced through denitrification. This heterotrophic anaerobic process has been described as the key driver for N_2O emissions when organic fertilisers are applied, even at well-aerated soil conditions, due to the formation of organic hotspots in which oxygen (O_2) is consumed due to the decomposition of organic C and importance of *de novo* NO_3^- production (Petersen et al., 1996). Among commercial NIs, the use of 3, 4-dimethylpyrazole phosphate (DMPP) has been mostly tested with synthetic N fertilisers, whereas field studies evaluating its formulation as a solution to be used with liquid manures (Vizura®) are scarce (Chiodini et al., 2019; Nair et al., 2020).

Previous studies (e.g., Abalos et al., 2017) have shown that the efficacy of NIs to inhibit nitrification and mitigate N_2O emissions from mineral fertilisers depends on environmental conditions (particularly rainfall), with lower performance under environmental/management scenarios that lead to low N losses (Abalos et al., 2014), e.g., under dry conditions. These low-moisture environments involve high O_2 availability and low NO_3^- mobility in the soil, so emissions are strongly dependent on rewetting events (Barrat et al., 2021). In addition, the efficacy of NIs could be also limited under compacted and water-saturated soils with low relevance of nitrification and increased chances for complete denitrification up to dinitrogen (N_2) (Recio et al., 2018). This may be different with organic fertilisers, where an O_2-limited environment can be sustained by intense microbial activity despite well-aerated soil conditions (Markfoged et al., 2011). Thus, N_2O emissions and potential effects of NIs may differ between mineral and organic fertilisers.

In a previous experiment, Vizura® applied with cattle slurry decreased N_2O emissions from a maize crop following grass-clover (Nair et al., 2020), but with no effect of DMPP on crop yield, N uptake, and NO_3^- leaching. This was partially attributed to N immobilisation after the application of C-rich residues, and also to the importance of belowground N sources (from crop residues and soil organic pools) which were not co-located with DMPP. The effectiveness of Vizura® with manure alone (i.e., without the potential interactions with previous crop residues) remains therefore unclear.

The experiment of Nair et al. (2020) evaluated the performance of Vizura® under natural rainfall during an average growing season on sandy soil for a temperate climate, and with simulated extra rainfall events. Climatic projections in North European countries such as Denmark are highly uncertain, with expected increments in annual precipitation but unevenly distributed (Rasmussen et al., 2018). During the growing season, the occurrence of dry periods and heavy rainfall events are expected to increase, with neutral or decreasing tendencies in total rainfall amount. Dry seasons and warmer temperatures can influence N losses (and therefore the mitigation efficacy of NIs) by changing O_2 availability and decreasing NO_3^- mobility (Petersen et al., 1996), by limiting the activity of soil microorganisms (Ussiri and Lal, 2013), or by restricting crop development and plant N acquisition (Miranda-Apodaca et al., 2020). With organic fertilisers such as livestock manure, these effects on bulk soil properties can increase the relative importance of N transformations associated with the organic hotspot, including nitrification and denitrification activity (Wagner-Riddle et al., 2020). To our knowledge, there are no studies evaluating the agronomic (yield, NUE) and environmental (N losses) performance of Vizura® applied with manure under low rainfall conditions.

A lysimeter experiment with coarse sandy soil was set up to test the effectiveness of Vizura® applied with manure to abate direct N_2O emissions and NO_3^- leaching (which is also an indirect source of N_2O) and to affect maize yield and NUE under contrasting rainfall conditions. Together with direct and indirect N_2O emissions, CH_4 emissions were *in situ* measured; while NH_3 volatilisation was modelled to calculate the net global warming potential (GWP). We hypothesised that: i) DMPP applied with manure would effectively mitigate NO_3^- leaching and particularly N_2O emissions, with a higher mitigation potential for low rainfall conditions where nitrification activity near sites of N_2O production may be more important; ii) rainfall amount would affect gaseous and leaching loss pathways with lower leaching losses under drier conditions due to the decreased drainage, and the lower soil moisture as a limiting factor for biochemically-derived N_2O emissions; and iii) the application of extra rainfall and DMPP would result in a positive response in crop yield and NUE indicators.

2. Materials and methods

2.1. Site and experimental design

The experiment was conducted from May 2018 to April 2019 at Aarhus University, Foulum, Denmark (56° 29′ N, 09° 34’ E), where the climate is temperate oceanic with 10-year average values for annual rainfall and mean air temperature of 669 mm and 8.1 °C, respectively. The corresponding values during the typical maize cropping period (May–November) are 410 mm and 13.4 °C, respectively. The trial was carried out in an experimental facility with epoxy-lined-concrete-made drainage lysimeters (size 1.0 m × 1.0 m, and 1.4-m depth) filled in 1992 with a repacked soil whose main topsoil (0–30 cm) characteristics were: 73.8% of coarse sand, coarse sandy texture in the fine fraction, pH (H_2O) = 6, organic matter content = 1.9%. More information about soil properties can be found in Nair et al. (2020). The lysimeters were previously cropped with perennial grasses (2014–2015), grass-clover (2016), and maize (2017).

The experiment comprised two factors (fertiliser and precipitation) in a randomised block design with three replicates. The two factors tested were (1) fertilisation: non-nitrogen application (control) or application of cattle manure without (CM) or with Vizura® (CM + DMPP); and (2) rainfall: natural rainfall (R-) and natural rainfall with simulated extra-rain events (R+). Each block included two lysimeters per treatment; half of them were used for gas and the other half for soil samplings, while maize plants and leachates were collected from all lysimeters. Therefore, a total of 36 lysimeters were set up (Fig. S1).

2.2. Agronomic management

Silage maize (*Zea Mays* L. cv Sunlite FAO 170) was grown according to the traditional management in the area. The soil was manually tilled (20 cm depth) on May 1, 2018. A common dose of phosphorous and potassium through a 0-4-21 NPK fertiliser was applied to all lysimeters on 7th May, thus supplying 30 kg P_2O_5 and 157 kg K_2O ha^{-1}. A rate of 126 kg total N ha^{-1} was applied as cattle manure (8.3% dry matter, 0.23% NH_4^+-N, 0.42% total N) the day before maize sowing (15th May). Vizura® was mixed with manure just before application at the rate recommended by the fertiliser company (i.e., 2.4 L ha^{-1}, 0.45 kg DMPP ha^{-1}). Manure was then incorporated into the topsoil surface (~15 cm) through simulated ploughing immediately after application. For simulated extra-rain event treatments, 10 mm of water were applied on 5th, 6th, and 7th June, 30 mm on 25th June, and 9 mm on 6th and 13th July, using a rain simulator (Nair et al., 2020). It was decided to irrigate the natural rainfall treatments with 4.5 mm of water on 6th and 13th July due to low rainfall, to guarantee the viability of the maize plants. In addition, a water dose of 5 and 4 mm were applied to all lysimeters on 23rd May and 1st June. The total amount of simulated rainfall was 18 mm and 87 mm in R- and R+, respectively. The eight maize plants in each lysimeter were hand-harvested (19th September) to determine the total aboveground biomass and total N content (Dumas method) as explained in Nair et al. (2020).

2.3. Drainage and NO_3^- leaching

Drainage from each lysimeter was collected and automatically measured with a pulse counter (Impulsa AG, Elsterwerda, Germany), with each pulse accounting for a 100 mL volume (Nair et al., 2020). Leachate samples (1.25 mL) were gathered in 1-L bottles at each tipping event. These bottles were emptied eight times during the monitoring period for NO_3^- analysis by a colorimetric method (Autoanalyzer III, Bran + Luebbe GmbH, Norderstedt, Germany). The mass of NO_3^- leaching was calculated as the concentration of NO_3^- multiplied by the leaching volume for a collection period.

2.4. Soil sampling and analysis

The soil was sampled on six dates between 24th May and 30th November. Three samples per lysimeter were randomly collected from 0 to 20-cm depth using a 2-cm diameter auger. Then, a composite sample was made from the samples of each lysimeter. A soil subsample (10 g, sieved at <4 mm) was dried at 105 °C until constant weight to determine the gravimetric water content. Another 10 g of sieved soil were extracted to determine soil mineral N (NH_4^+ and NO_3^-) and pH as explained in detail in Abalos et al. (2020).

2.5. Direct N_2O and CH_4 emissions

Closed static chambers (22.7 L) were placed between maize rows (one chamber per lysimeter). Since gas and soil samples were collected from different lysimeters within the same block, the soil disturbance at each sampling event did not influence the naturally occurring release of N_2O. The samples, which were taken using the same procedure as that described in detail in Nair et al. (2020), were analysed by gas chromatography with an Agilent GC system interfaced with a CTC CombiPal autosampler (Agilent, Nærum, Denmark) and equipped with an electron capture detector (ECD) and a flame ionisation detector (FID) for determining N_2O and CH_4 concentrations, respectively. Gas samplings were performed daily after N fertilisation and the frequency decreased gradually afterwards, but covering all rainfall/irrigation events. Daily N_2O fluxes were obtained using the HMR package (version 1.0.0) through linear and non-linear regressions chosen after user inspection. Cumulative N_2O emissions and N_2O emission factors (EFs) were calculated as explained in Abalos et al. (2017) and Nair et al. (2020), respectively.

2.6. GWP and NUE calculations

The net GWP was calculated considering direct and indirect (from NH_3 volatilisation and NO_3^- leaching) N_2O emissions, and CH_4 emissions. Indirect N_2O emissions associated with NO_3^- leaching were estimated for each lysimeter as the cumulative mass of N lost by this pathway (see section 2.3) multiplied by an emission factor (EF_5) of 0.011 (IPCC, 2019). Total N_2O emissions were the sum of direct as well as indirect N_2O emissions from measured NO_3^- leaching during the whole experiment. Yield-scaled N_2O emissions were calculated as the total N_2O emissions divided by the aboveground biomass yield (Petersen et al., 2012).

Ammonia volatilisation was estimated using the ALFAM model developed by Søgaard et al. (2002). We used the environmental data obtained from the nearby (<1 km) meteorological station and the dry matter and total NH_4^+-N content measured in sub-samples of the manure applied in the field. The same NH_3 emissions were assumed for both rainfall levels since the extra-rainfall episodes in R+ were applied 20 days after manure application while the critical period for NH_3 volatilisation involves the first week after fertilisation (Recio et al., 2018). The same NH_3 emissions were also assumed for CM and CM + DMPP. Several studies have pointed out the potential increase of NH_3 volatilisation – particularly in alkaline soils – when NIs are applied, but the recent meta-analysis of Fan et al. (2022a) indicated that this effect is not significant for DMPP. To estimate indirect N_2O emissions from NH_3 deposition, we used the IPCC EF_4 for humid climates (1.4%). Nitrous oxide (both direct and indirect) and CH_4 emissions were converted to carbon dioxide equivalent emissions (CO_2e) using factors of 273 and 27, respectively (Forster et al., 2021). Net GWP was divided by the biomass yield to calculate the greenhouse gas intensity (GHGI).

Several NUE indicators were calculated using yield and plant N content data (Jones et al., 2021). The Partial Factor Productivity (PFP) and Partial Nutrient Balance (PNB) were calculated as the ratios of biomass yield or N output in aboveground biomass, respectively, to total N applied as manure. Agronomic Efficiency (AE) was calculated similarly as PFP but subtracting the yield in the control to the yield in the rainfall-corresponding fertilised treatment. Accordingly, Crop Recovery Efficiency (CRE) was calculated as PNB but subtracting the N output in the control to the N output in the rainfall-corresponding fertilised treatment. Physiological Efficiency (PE) was calculated as the ratio between yield (subtracting the value of the corresponding control) and N output (subtracting the value of the corresponding control). Nitrogen Surplus was calculated as the difference between manure-N input and N output in aboveground biomass (Quemada et al., 2020).

2.7. Statistical analysis

All analyses were done using R software version 4.0.2. Differences among treatments for crop yield, NUE indicators, NO_3^- leaching, soil mineral N and area-scaled/yield scaled gaseous fluxes and GWP were analysed by type III ANOVAs and multiple comparisons using the Tukey test at 95% probability level. In all tests, block was considered as a random factor and the default level of significance was 0.05. In the case of repeated measurements over time (mass of NO_3^- leaching, soil mineral N content, direct N_2O emission), a repeated measures analysis was performed with a nonparametric analysis of longitudinal data in factorial experiments (package *nparLD*). A Pearson correlation analysis was used to determine the relationship between N_2O fluxes and the rest of the studied parameters (soil NO_3^- and NH_4^+ concentrations and soil temperature.

3. Results

3.1. Environmental conditions

Mean air temperature and cumulative rainfall from May to

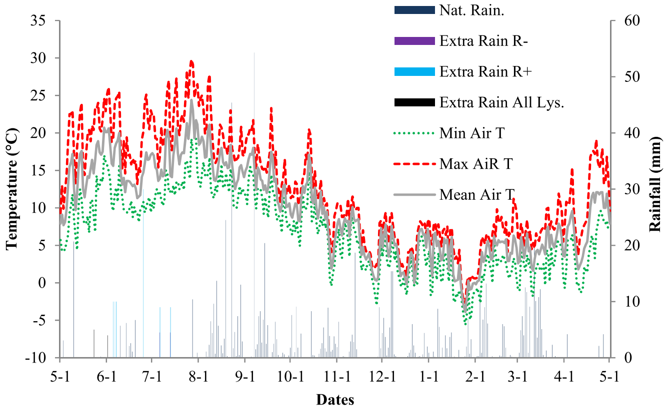

Fig. 1. Daily air temperatures (average, maximum and minimum), daily rainfall and amount of water applied through simulated rainfall events in natural rainfall (R-, 4.5 mm applied on both 6th and 13th July), extra rainfall events (R+, 10 mm of water were applied on 5th, 6th, and 7th June, 30 mm on 25th June, and 9 mm on 6th and 13th July) and both treatments (5 and 4 mm applied to all lysimeters on 23rd May and 1st June).

November were 16.6 °C and 410 mm, respectively (Fig. 1). However, until maize harvesting, the recorded precipitation was only 223 mm (241 and 310 mm considering the simulated extra rainfall events in R- and R+, respectively), which is substantially lower than average values for the region (see section 2.1). Considering the whole experimental period, total precipitation was 775.5 mm, while mean, maximum, and minimum air temperatures were 9.7, 29.8, and −5.6 °C, respectively (Fig. 1).

3.2. Soil mineral N

The highest soil NH_4^+ concentrations were recorded at the first sampling (24th May), reaching 35.8 mg N kg soil^{-1} in CM + DMPP R+ treatment (Fig. 2a). Afterwards, NH_4^+ concentrations sharply decreased until the end of the experiment. In the second soil sampling event (8th June), NO_3^- concentrations peaked in all treatments, with the largest value (22.3 mg N kg soil^{-1}) measured in the CM amended lysimeters (Fig. 2b). A remarkable increase was also observed in the CM R-treatment on 18th July. Average NH_4^+ concentrations decreased in the order CM + DMPP > CM > control ($p < 0.05$, Fig. 2c), and no effects of rainfall were obtained. Average soil NO_3^- concentrations decreased in the order CM > CM + DMPP > control, and were numerically higher in R-than in R+ ($p < 0.10$). The soil pH (Fig. 2d) followed and increasing tendency throughout the experimental period, ranging from 5.4 (average value on 24th May) to 6.1 (average value on 30th November). Differences between fertiliser or irrigation treatments were not significant. Average soil mineral N content and cumulative NO_3^- leaching were correlated ($p < 0.001$, $r = 0.74$). Average NO_3^- concentrations were positively correlated with N_2O emissions ($p < 0.001$, $r = 0.78$).

3.3. Drainage and NO_3^- leaching

During the maize growing season, R+ resulted in significantly higher cumulative NO_3^- leaching than R- (53% increase), while no significant differences were observed between fertiliser treatments (Fig. 3a). However, the contribution of this period to the annual leaching ranged from 10.8% (CM + DMPP R-) to 31.9% (control R-); therefore, most of the annual leaching occurred in the post-harvest period. During the post-harvest period, the only significant differences were observed between the unfertilised control and fertilised treatments, with higher cumulative leaching in the latter. The annual cumulative leaching followed the same pattern as that of the post-harvest period. On average, 12.4% and 12.3% of manure-N was lost through leaching in CM and CM + DMPP, respectively.

3.4. Crop yield and NUE indicators

Both fertilisation and rainfall exerted an effect on biomass yield. Unfertilised control had lower yields than manure-amended lysimeters, with no significant effect of DMPP (Table 1). The extra rainfall significantly increased maize productivity by 20%, compared with R-. Nitrogen concentration in plant biomass was only influenced by the addition of manure, with higher values in CM and CM + DMPP than in control (Table 1). The plants in the control treatment had 45% lower aboveground N uptake than the average value for plants within manure-amended lysimeters, with no differences between CM and CM + DMPP. The extra rainfall increased the N output by 20.6 kg N ha^{-1} (14% increment) compared to R-. The NUE indicators which did not take into account the control lysimeters (i.e., PFP and PNB) were not affected by the nitrification inhibitor, while R+ increased both indicators in

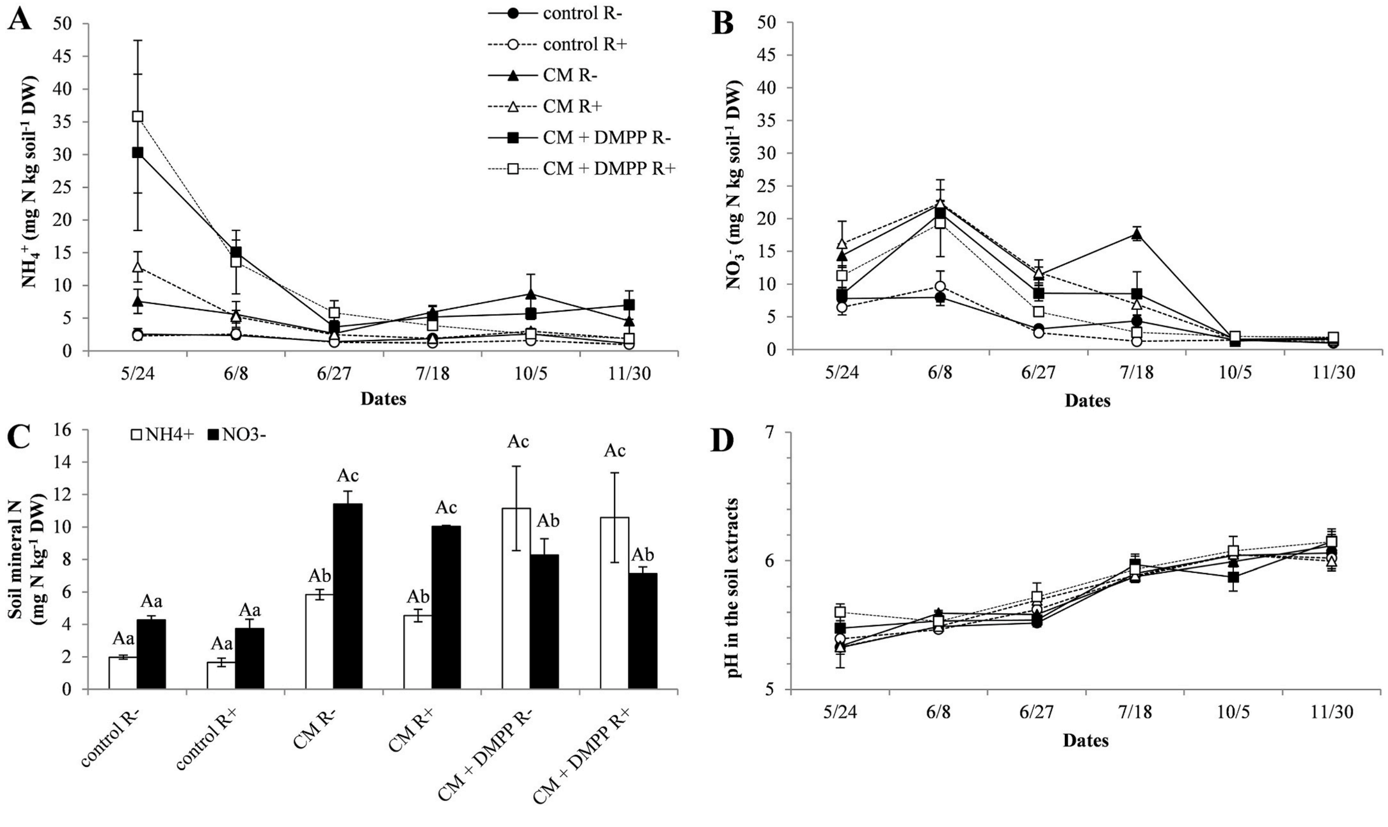

Fig. 2. Temporal dynamics of soil NH_4^+ (A) and NO_3^- (B) concentrations, average values of NH_4^+ and NO_3^- concentrations throughout the experimental period (C) and pH in the soil extracts (C) in the different treatments (control, cattle manure only, CM, cattle manure + Vizura®, CM + DMPP, lower rainfall amount, R-, higher rainfall amount, R+). In subfigure c and for each variable independently, different uppercase letters denote significant differences between rainfall conditions within a fertiliser treatment, while different lowercase letters denote significant differences between fertiliser treatments within the same rainfall condition. Vertical bars indicate standard errors of the mean.

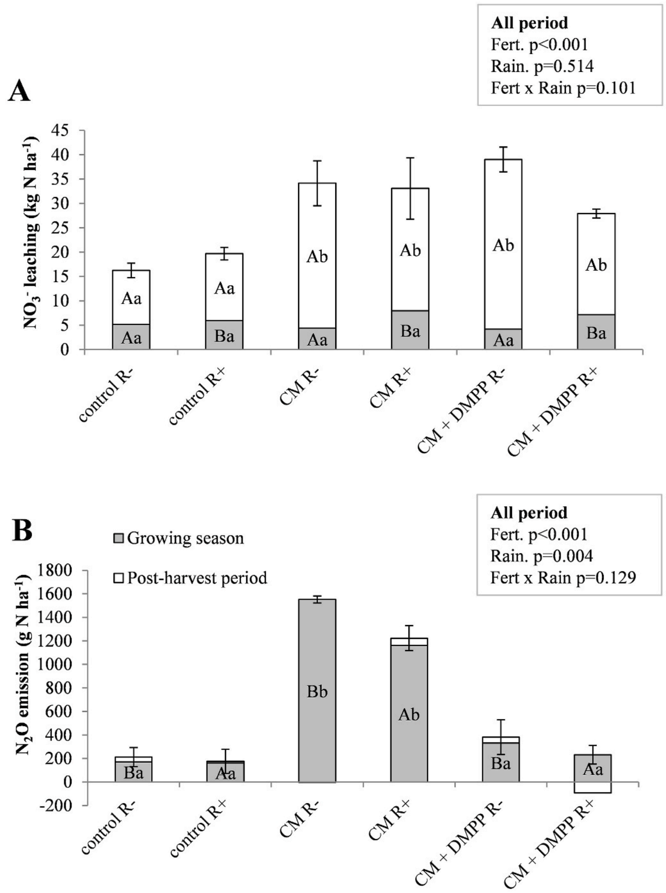

Fig. 3. NO_3^- leaching (A) and cumulative N_2O emissions (B) during the growing (from May to maize harvest) and post-harvest periods for the different treatments (control, cattle manure only, CM, cattle manure + Vizura®, CM + DMPP, lower rainfall amount, R-, higher rainfall amount, R+). Different uppercase letters denote significant differences between rainfall conditions within a fertiliser treatment, while different lowercase letters denote significant differences between fertiliser treatments within the same rainfall condition. Vertical bars indicate standard errors of the mean for the whole experimental period.

comparison with R-by 20% and 16%, respectively ($p < 0.05$, Table S1). A similar result was obtained for CRE (i.e., 31% increment in R+ with respect to R-, Table 1), while neither AE nor PE showed significant differences between treatments (even though R-tended to increase the later indicator with respect to R+) (Table S1). Nitrogen surpluses were negative in all fertiliser and manure combinations, with lower values in R+ than in R- ($p < 0.05$) (Table 1).

3.5. *Yield-scaled N_2O emissions and GWP*

Daily fluxes ranged from −6.9 (control R-) to 152.9 g N ha^{-1} day^{-1} (CM R-) (Fig. S2). Most of the emissions occurred during the maize cropping cycle, and in some treatments net N_2O sinks were measured during the post-harvest period (Fig. 3b). Differences between rainfall and fertiliser treatments were driven by the growing season, with R- and CM increasing cumulative emissions with respect to R+ (average by 40%) and both CM + DMPP and control (average by 6-fold), respectively ($p < 0.05$). The nitrification inhibitor DMPP decreased N_2O emissions to the level of the unfertilised control independent of rainfall (Table 2). Nitrous oxide EFs in the R-lysimeters were 1.06% and 0.14% in CM and CM + DMPP, respectively. The corresponding values in the R+ lysimeters were 0.83% and 0.00% for CM and CM + DMPP, respectively.

Indirect N_2O emissions accounted for 20–56% of total N_2O emissions (Table 2). Measured total N_2O losses (N_2O + indirect from NO_3^-) decreased in the order CM > CM + DMPP > control, and were higher in R-than in R+ for manure-amended lysimeters but not for the control. When scaled to crop productivity, N_2O emissions (direct + indirect) were significantly lower in R+ in comparison with R- (33% mitigation which was no significant for control lysimeters), and also in CM + DMPP (by 64%) and control (by 69%) in comparison with CM-only (Table 2).

For the GWP calculations, *in situ* CH_4 fluxes and estimated NH_3 emissions were also used and converted to CO_2e. No significant differences were found between fertiliser or rainfall treatments with respect to CH_4 fluxes (data not shown), and all treatments were net CH_4 sinks (Fig. 4a). The NH_3 volatilised under the conditions of our study was 4.10 kg N–NH_3 ha^{-1} according to the ALFAM simulation. Total CO_2e emissions were 120.2, 221.2, and 709.9 kg CO_2e ha^{-1} in control, CM + DMPP, and CM, respectively (Fig. 4). In general, rainfall-resulted in a significantly higher GWP (by 23% and 129% in CM and CM + DMPP, respectively) than R+, but not for control treatments. Net GHGI followed a similar trend as area-scaled GWP (Fig. 4b), being higher for CM than for CM + DMPP by an average factor of 3.1. On average, R-increased GHGI by 54.4% in comparison with R+.

4. Discussion

4.1. *Vizura® decreases N_2O emissions but not NO_3^- leaching*

As we hypothesised, the use of DMPP as the active ingredient of Vizura® succeeded in the mitigation of N_2O losses during the maize growing season, which was a consistent finding regardless of rainfall conditions (Table 2). This result illustrates that DMPP was able to inhibit nitrification as indicated by the significant enhancement of soil NH_4^+ concentration (Fig. 2a) and the decreases in those of soil NO_3^- (Fig. 2b), thus limiting the substrate for denitrifiers and therefore the potential N_2O release derived from stepwise NO_3^- reduction (Butterbach-Bahl et al., 2013). The strong and positive correlation between soil NO_3^- and

Table 1

Crop yield, aboveground N uptake, plant N content, Crop Recovery Efficiency (CRE) and N surplus in each treatment (control, cattle manure only, CM, cattle manure + vizura, CM + DMPP, lower rainfall amount, R-, higher rainfall amount, R+). In each column, different uppercase letters denote significant differences between rainfall conditions within a fertiliser treatment, while different lowercase letters denote significant differences between fertiliser treatments within the same rainfall condition. P values and standard errors of the mean (in brackets) are given for each effect and Tukey test ($P < 0.05$) was used for multiple comparisons.

	Crop yield		Aboveground N uptake		Plant N content		CRE		N surplus	
	Mg ha^{-1}		kg N ha^{-1}		%		kg kg^{-1}		kg N ha^{-1}	
Control R-	12.0	Aa	100.6	Aa	0.80	Aa	–		–	
CM R-	17.0	Ab	176.8	Ab	1.40	Ab	0.60	Aa	−50.8	Aa
CM + DMPP R-	16.7	Ab	173.5	Ab	1.38	Ab	0.58	Aa	−47.5	Aa
Control R+	14.5	Ba	105.8	Ba	0.84	Aa	–		–	
CM R+	20.0	Bb	201.1	Bb	1.60	Ab	0.76	Ba	−75.1	Ba
CM + DMPP R+	20.5	Bb	205.8	Bb	1.63	Ab	0.79	Ba	−79.8	Ba
Fert.	<0.001	(0.4)	<0.001	(4.6)	<0.001	(0.03)	0.921	(0.04)	0.921	(4.8)
Rainfall	<0.001	(0.3)	0.003	(3.8)	0.080	(0.02)	0.015	(0.04)	0.006	(4.8)
Fert. × Rainfall	0.453	(0.6)	0.151	(6.5)	0.450	(0.04)	0.579	(0.05)	0.579	(6.8)

Table 2
Annual direct, annual indirect (from NO_3^- leaching), total (direct + indirect from NO_3^- leaching) N_2O emissions, and yield-scaled N_2O emissions in each treatment (control, cattle manure only, CM, cattle manure + Vizura®, CM + DMPP, lower rainfall amount, R-, higher rainfall amount, R+). In each column, different uppercase letters denote significant differences between rainfall conditions within a fertiliser treatment, while different lowercase letters denote significant differences between fertiliser treatments within the same rainfall condition. P values and standard errors of the mean (in brackets) are given for each effect and Tukey test ($P < 0.05$) was used for multiple comparisons.

	Direct N_2O emissions		Indirect N_2O emissions		Total N_2O emissions		Yield-scaled N_2O emissions	
	kg N ha^{-1}		kg N ha^{-1}		kg N ha^{-1}		g N kg^{-1} yield	
Control R-	0.21	Ba	0.18	Aa	0.39	Aa	0.03	Aa
CM R-	1.55	Bb	0.38	Ab	1.93	Bc	0.11	Bb
CM + DMPP R-	0.38	Ba	0.43	Ab	0.81	Bb	0.05	Ba
Control R+	0.18	Aa	0.22	Aa	0.39	Aa	0.03	Aa
CM R+	1.22	Ab	0.36	Ab	1.59	Ac	0.08	Ab
CM + DMPP R+	0.14	Aa	0.31	Ab	0.45	Ab	0.02	Aa
Fert.	<0.001	(0.05)	<0.001	(0.03)	<0.001	(0.04)	<0.001	(0.003)
Rainfall	0.004	(0.04)	0.383	(0.02)	0.001	(0.04)	<0.001	(0.003)
Fert. × Rainfall	0.129	(0.07)	0.202	(0.04)	0.025	(0.06)	0.044	(0.005)

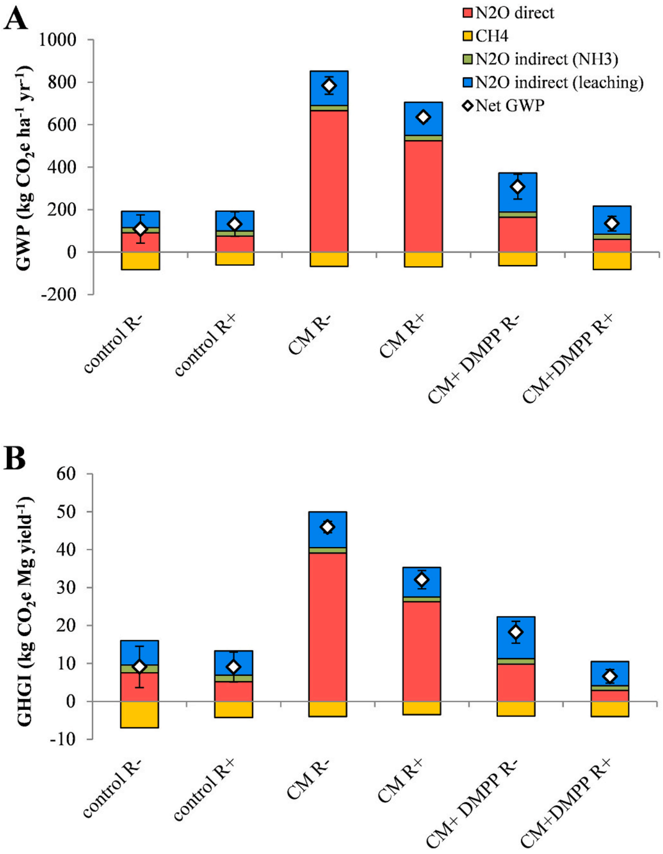

Fig. 4. Global warming potential (GWP, A) and greenhouse gas intensity (GHGI, B) of the different components (direct N_2O emissions, NH_3 volatilisation, leaching and CH_4 emissions) and for the different treatments (control, cattle manure only, CM, cattle manure + Vizura®, CM + DMPP, lower rainfall amount, R-, higher rainfall amount, R+). Net values of GWP and GHGI calculated as the sum of all components (± standard errors) are also represented.

N_2O emissions is evidence that NO_3^- was a limiting factor for N_2O emissions under the conditions of our study. As a result, the use of Vizura® resulted in an effective mitigation of N_2O emissions (by 82% and 94% on average when calculated from cumulative emissions or EFs, respectively). This mitigation efficacy was higher than that reported by Nair et al. (2020) (44% and 67%) with the same soil. These differences could be explained by the wetter conditions during the experiment of Nair et al. (2020), i.e., 51 and 54% higher rainfall than in the current study during the maize growing period with natural and simulated rainfall, respectively. The greater precipitation regimes under Nair et al. (2020) resulted in a much lower N_2O EF (0.54%) than that of this study (0.95%). This unexpected result could be explained by the lower leaching losses in this study (average leaching factor 0.12 *versus* 0.24 in Nair et al., 2020), which increased soil NO_3^- availability and therefore the potential N_2O emissions from denitrification.

In addition to the differences in leaching losses, the lower N_2O EFs under wetter conditions could be due to more complete denitrification (Pilegaard, 2013). Under these conditions, the effect of NIs on denitrification-induced N_2O emissions could be hidden by the enhanced reduction of N_2O to N_2. Although dry cropping seasons could limit the extent and stability of anaerobic hotspots and denitrifying microbial populations, soil wetting after rainfall increases the potential of NO_3^- to reach anaerobic microsites and enhance N_2O emissions (Petersen et al., 1996). Moreover, under low-rainfall conditions N_2O emissions from nitrification of manure-N may become quantitatively more important, which would increase the capacity of NIs to mitigate N_2O emissions. Delaying the accumulation of NO_3^- by using NIs with manures represents, therefore, an effective practice to abate N_2O emissions, particularly in low-rainfall situations. Our results are consistent with previous studies using DMPP with organic fertilisers in incubation (Nair et al., 2021) and field experiments (Chiodini et al., 2019; Guardia et al., 2017), and reinforce that the use of NIs could enhance the environmental sustainability of organic fertilisers through the mitigation of direct N_2O emissions from dry or well-drained soils (Fan et al., 2022a; Tufail et al., 2022).

In contrast to our expectation, Vizura® was not able to decrease cumulative NO_3^- leaching at the end of the experiment. This result is not consistent with global average effects reported for NIs (Quemada et al., 2013; Qiao et al., 2015). However, it is in line with the findings of Nair et al. (2020); these authors found that Vizura® did not decrease leaching losses, and suggested that endogenous N sources from deeper soil layers and therefore not co-located with DMPP (e.g., from roots and soil organic pools) may have accounted for a large fraction of the N lost through leaching. In the current study, the use of Vizura® with manure resulted in numerically lower leaching losses (on average 5.7 kg N ha^{-1} with inhibitor *versus* 6.2 kg N ha^{-1} without) during the maize growing season. We speculate that the limited drainage and NO_3^- leaching due to low rainfall during this period masked the effect of Vizura® at a statistical level. Indeed, adjusting irrigation rate has been described as the most effective N leaching mitigation practice (Quemada et al., 2013) and may cause the effect of DMPP to be limited at field scale (Mateo-Marín et al., 2020). By contrast, most of the annual leaching occurred during the post-harvest period, in which rainfall conditions were normal or even humid considering the average values in the area (see section 3.1). The higher annual leaching losses observed by Nair et al. (2020) for maize after grass-clover compared to the present study (maize after maize) could be an indication that indeed grass-clover residues increased the NO_3^- leaching potential on this coarse sandy soil.

The residual effect of manure-N resulted in lower leaching losses in non-fertilised lysimeters (Fig. 3a), but the effect of DMPP seemed to be temporary and did not last into the post-harvest period. Indeed, the recent study of Chibuike et al. (2022) performed in a pasture system in New Zealand reported a half-life (at 15 °C) of 12–17 days for DMPP, lower than that of nitrapyrin or DCD. The lack of differences in soil mineral N concentrations during the post-harvest period (Fig. 2a and b) is in agreement with the lack of effect of Vizura® after the maize harvest for both direct and indirect N_2O emissions (i.e., from leaching).

4.2. *Rainfall as a complex driver of N losses*

Contrary to our second hypothesis, the low rainfall regime increased N_2O losses in comparison with extra rainfall. Two possible reasons could explain this unexpected result. First, hot moments for N_2O after rewetting can be more intense when previous soil conditions are drier (Barrat et al., 2021; Bergstermann et al., 2011). This enhanced peaking intensity could be a result of the delayed N_2O reductase activity under drier conditions (thus increasing the N_2O:N_2 ratio) and the reconnection of previously isolated biochemical substrates, which are then rapidly consumed by microorganisms thus favouring O_2 consumption and denitrification. Second, the simulated extra rainfall significantly increased NO_3^- leaching during the growing period (Fig. 3a), thus decreasing the availability of this compound in the soil ($p < 0.10$) and limiting the potential losses through denitrification (Velthof and Rietra, 2018). It could be argued that the drier conditions could have restricted the relevance of denitrification. Yet, denitrification was probably the main N_2O-releasing pathway during our study, because this process is stimulated by organic amendments even at medium soil moisture contents (Li et al., 2016) and particularly after rewetting episodes (Montoya et al., 2022). Nair et al. (2020) found that extra simulated rainfall events did not increase N_2O emissions compared to naturally wet conditions, indicating that the regulation of N_2O emissions is complex and not closely linked to rainfall in manure-amended coarse-textured soils.

As expected, the additional rainfall during the maize growing period increased NO_3^- leaching losses (Fig. 3a). However, leaching losses were low even in the lysimeters which received the highest dose of extra rainfall (average of 7.0 kg N ha^{-1}). Since most of the N was leached during the post-harvest period, the effect of extra-rainfall during the maize cycle was diluted at the end of the experiment. The proportion of N leached in this study (28–39 kg N ha^{-1} from manure applied at 126 kg N ha^{-1}) was lower than that in the studies of Hansen and Eriksen (2016), i.e., 74–136 kg N ha^{-1} (N rate of 135 kg N ha^{-1}); or Nair et al. (2020), i.e., 65–162 kg N ha^{-1} (N rate of 145 kg N ha^{-1}). The average leaching factor in this study (0.12) was only half of that assumed for Danish inventories (0.28, Nielsen et al., 2017) or the IPCC default value (0.24, IPCC, 2019). Our results indicate that the amount and distribution of water (through natural rainfall and/or irrigation) could be the most influential factor driving NO_3^- leaching losses. Our study also suggests that the high relative importance of the post-harvest period could limit the possibilities of in-season management of N and water to mitigate this reactive N loss pathway.

4.3. *Agronomic responses and NUE indicators*

Climate change effects in Northern Europe are uncertain, with a potential increase in annual precipitation and heavy rainfall events combined with severe drought episodes (IPCC, 2014), increasing concerns about the potential consequences for plant productivity and NUE (Hu et al., 2019; Shao et al., 2022). Overall, climate projections are favourable for maize cropping in northern countries such as Denmark (Rasmussen et al., 2018), but this could vary depending on the variability in rainfall amount and distribution. As expected, lower rainfall decreased crop yield (as well as absence of fertilisation, Table 1). Therefore, dry conditions in fertilised plots led to significantly lower biomass yield while increasing N_2O losses (with no significant effect on annual leaching), thus increasing total yield-scaled N_2O emissions as an integrating indicator of agronomic and environmental sustainability.

The non-significant effect of Vizura® on yield and NUE indicators (including N surplus), was not in agreement with the overall results of global meta-analyses (e.g., Abalos et al., 2014; Qiao et al., 2015; Sha et al., 2020), but in line with some DMPP trials at field scale (e.g., Nair et al., 2020; Regueiro et al., 2020). It has been argued that the lack of plant response to NIs could be due to N application rates that already meet crop N demand and growth potential without the use of an inhibitor (Rose et al., 2018). As a result, some studies have suggested that the agronomic and economic benefits of enhanced-efficiency fertilisers are more likely to be reached by decreasing fertiliser N rates, through a stimulation of N utilisation efficiency (Fan et al., 2022a; Muller et al., 2022). The N rate used in our study, estimated based on the balance between the foreseeable crop-specific N requirements and considering the N supply from the soil, was lower than that used in earlier studies (Hansen and Eriksen, 2016; Nair et al., 2020). Yet, the dry conditions probably made water availability a limiting factor for yield, thus hiding the potential benefits of DMPP via enhancing N availability. The fact that the highest yield was measured for the DMPP-treated lysimeters with simulated rainfall supports this argument.

None of the NUE indicators were affected by the addition of Vizura®, whereas rainfall amount exerted a significant influence in almost all of them (Tables 1 and Table S1). The lower rainfall led to reductions in all NUE indicators except PE. This implies that under water limiting conditions, the N taken up by plants seems to be preferentially used to develop crop yield rather than for N accumulation (biofortification) in aboveground biomass. Since yields increased with the higher precipitation treatment, PE was not positively related to plant productivity (Isfan, 1993). Synergies between N management (including the use of enhanced-efficiency fertilisers or the management of N timing in a context of weather uncertainty) and other agricultural practices need to be considered to ultimately optimise NUE and crop response to N fertilisation. Values of NUE indicators and N surpluses in our system can be considered as high and low, respectively, in comparison with commonly reported values (Jones et al., 2021; Van Groenigen et al., 2010), thus possibly indicating the high NUE potential of maize at the conditions of the study. In addition, such high NUE values (particularly those of AE and PNB) could denote a relevant contribution of the own system (i.e., the endogenous N from soil or previous cropping seasons to plant N uptake) as a “missing input” (Quemada et al., 2020). Due to the scale of the present study (lysimeters), agronomic and NUE results should be taken with caution and further studies at field scale should be carried out.

4.4. *Global warming potential*

Our GWP assessment revealed that despite the lower NO_3^- leaching in comparison with other studies (see section 4.2), the contribution of this component to total CO_2e emissions through indirect N_2O emissions (i.e., 18%–44%, average 31%, Fig. 4), should not be neglected, which has been often the case in earlier studies (e.g., Adviento-Borbe et al., 2007; Gao et al., 2021; Shen et al., 2018). In fact, indirect N_2O from NO_3^- leaching was the leading contributor to the net GWP in unfertilised and DMPP-treated treatments, and its relevance could be even higher under wet conditions promoting leaching losses. Therefore, studies measuring net CO_2e emissions in temperate or irrigated croplands must include this component, preferably with *in situ* measurements.

Direct N_2O was, on average, the variable which contributed most to net GWP (20%–72%, average 43%, Fig. 4), and therefore the efficacy of DMPP for N_2O mitigation indicates that this strategy should be recommended for reducing the C footprint of forage maize under the conditions of our study. The cost increment from the use of inhibitors has been highlighted as the main barrier for the widespread adoption of these products, unless some incentives are implemented (Sanz-Cobena et al., 2017). Considering the DMPP rate (section 2.2), the price of DMPP (19.65 USD kg DMPP^{-1}, Fan et al., 2022b) and the average GWP mitigation from Vizura® use (489 kg CO_2e ha^{-1}), the GWP-scaled extra cost of the application of DMPP would be 0.02 USD kg abated CO_2e^{-1} at the conditions of our study.

It has been suggested that inasmuch as NIs delay the oxidation of NH_4^+ (first step of nitrification), they could increase the chances for NH_3 volatilisation, particularly under basic-soil pH conditions (Qiao et al., 2015). Recent meta-analyses, however, pointed out that this effect is not significant for DMPP (Fan et al., 2022a; Tufail et al., 2022). The ALFAM simulation under the conditions of our study (e.g., manure incorporation into the soil after application, low wind speed, non-basic soil pH), resulted in a volatilisation factor of 0.033, which is lower than the average value proposed by Pan et al. (2016) for Europe (0.13) or globally (0.18). Fertiliser placement (Zhang et al., 2022) and environmental conditions (Voglmeier et al., 2018) after application exert a critical influence on NH_3 losses, so it could be hypothesised that DMPP could have increased NH_3 losses under high-volatilisation scenarios, thus compromising the environmental efficacy of DMPP. However, to offset the direct N_2O mitigation reached with Vizura® in our experiment, NH_3 volatilisation should have reached 77.4–83.6 kg N ha^{-1} (i.e., 61%–66% of the manure-N applied), which surpasses the upper ranges reported in the literature (Pan et al., 2016) and seems unrealistic when compared to the modelled NH_3 volatilisation by ALFAM (i.e., 4.1 kg N ha^{-1}). Future studies should consider other C footprint components that have not been included in our calculations, such as those derived from manure storage, transport and spreading, farm inputs and operations, or potential changes in soil organic C.

5. Conclusions

The reported findings together with those from previous studies using Vizura® reveal a significant efficacy of this product in the mitigation of yield-scaled N_2O emissions from cattle manure used on sandy soil, and a lack of effect on NO_3^- leaching. Accordingly, the use of DMPP with organic fertilisers offers an opportunity to improve the greenhouse gas balance by lessening direct N_2O emissions, in addition to the potential abatements of upstream emissions derived from the avoidance of synthetic N use or by potentially favouring soil C storage. Our results also suggest that low rainfall cropping seasons threaten the sustainability of maize cropping in North Europe, since they may lead to an increase in the availability of reactive N in the soil and/or rise the intensity of hot moments for N_2O emissions after rewetting. In addition, lower rainfall decreased yield and NUE and increased N surplus, thus boosting yield-scaled emissions and decreasing the agro-ecosystem sustainability. Our results also suggest that the irregular distribution of rainfall could limit the odds for mitigation through N management during the growing season, particularly with regards to leaching losses in well-drained sandy soils. Optimising the agronomic and environmental balance under these conditions will require the combination of different mitigation and adaptation strategies to simultaneously avoid yield penalties and to mitigate N_2O emissions.

Credit author statement

G Guardia: Writing - Original Draft, Writing - Review & Editing, Formal analysis, Visualization, **D. Abalos**: Writing - Review & Editing, Visualization, Methodology, Supervision, Investigation, Formal analysis, **N Mateo-Marín**: Investigation, Formal analysis, Writing - Review & Editing, **D Nair**: Investigation, Formal analysis, Writing - Review & Editing, **SO Petersen**: Writing - Review & Editing, Visualization, Methodology, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.120679.

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