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Effect of nitrification inhibitor (DMPP) on nitrous oxide emissions from agricultural fields: Automated and manual measurements

Azeem Tariq a,c,⁎, Klaus Steenberg Larsen b, Line Vinther Hansen a, Lars Stoumann Jensen a, Sander Bruun a

a Department of Plant and Environmental Sciences, University of Copenhagen, Denmark
b Department of Geosciences and Natural Resource Management, University of Copenhagen, Denmark
c School of Environmental Sciences, University of Guelph, Ontario N1G2W1, Canada

HIGHLIGHTS

• N₂O mitigation effect of DMPP was tested in spring barley and spring oilseed rape.
• Manual and automatic chambers were used to capture spatial and temporal variability.
• Application of DMPP tended to reduce the cumulative N₂O emissions.
• Crop yield and crop N uptake were not affected by the use of DMPP.
• Continuous measurements capture greater temporal variation in fluxes than sporadic.

ABSTRACT

Nitrogen fertilisation contributes significantly to the atmospheric increase of nitrous oxide (N₂O). Application of nitrification inhibitors (NIs) is a promising strategy to mitigate N₂O emissions and improve N-use efficiency in agricultural systems. This study investigated the effect of NI, 3,4-dimethylpyrazol phosphate (DMPP) on N₂O mitigation from spring barley and spring oilseed rape. Manual and automatic chamber methodologies were used to capture spatial and temporal variability in N₂O emissions. In a second experiment, we study the effect of N fertiliser levels without NI (0 %, 50 %, 100 %, 150 % and 200 % of recommended amount of N fertiliser), as well as 100 % of N with NI on N₂O emissions in spring barley. The automated chamber measurements showed dynamics of N₂O changes throughout the season, including positive and negative peaks that were unobservable with manual chambers due to low temporal resolution. Although not significant, application of NI tended to reduce N₂O emissions. The reduction was on average 16 % in spring barley and 58 % in spring oilseed rape in manual chamber measurements. However, N₂O reduction was 108 % in continuous automatic chamber measurements in spring barley. The N₂O EFs for the growing season were very low (0.025 % to 0.148 %), with a greater reduction in EF in spring oilseed rape (76 %) than in spring barley (32 %) with NI application. A positive correlation (R = 80 %) was observed between N fertiliser levels and N₂O emissions. Crop yield and crop N uptake were not significantly affected by the use of NI. This study highlighted that NI can reduce N₂O emissions, but the reduction effects are plot, crop and microclimate specific. Long-term experiments with continuous plot-scale measurements are needed to capture and optimise N₂O mitigation effect of NIs across wide variability in soils and microclimates in agroecosystems.
1. Introduction

Nitrogen (N) fertilisation is important to ensure a high crop yield to meet the food demand of the growing world population, which is expected to be over nine billion by 2050 (United Nations, 2019). Crops only take up about 35 % to 70 % of the N applied, and N is therefore lost to the environment through nitrate (NO3−) leaching and gaseous emissions in the form of elemental nitrogen (N2), ammonia (NH3) and the potent greenhouse gas (GHG) nitrous oxide (N2O) (Bodirsky et al., 2012). With a 100-year global warming potential 273 times higher than CO2 (carbon dioxide), N2O accounts for 6.4 % of the total global radiative forcing (IPCC, 2021). In order to decrease the climate impact of agricultural emissions, it is important to develop new technologies and management practices to ensure high crop production with optimum utilisation of nutrients and minimal N2O emissions.

Globally, agricultural soils account for 66 % of anthropogenic N2O emissions, with an estimated emission of about 6.8 Tg N2O-N year−1 of which 4.2 Tg N2O-N year−1 are emitted as a consequence of N fertilisation, either as direct emissions from the field or indirect emissions through losses of reactive nitrogen from agricultural systems (Davidson and Kanter, 2014; IPCC, 2007). In Denmark, agriculture accounted for 89 % of total national N2O emissions in 2015 (Albrektsen et al., 2017). The Intergovernmental Panel on Climate Change (IPCC) methodology assumes a linear relationship between N application rates and N2O emissions (De Klein et al., 2006); however, a nonlinear relationship between applied N and N2O emission is more realistic as N2O emissions are driven by any excess soil N that is not taken up by the crops (Snyder et al., 2009). The nonlinear relationship means that there is a greater potential for reduction of N2O emissions in regions with higher N application rates than in regions with low N application rates (Jia et al., 2019).

It is generally assumed that the main microbial pathways responsible for N2O production and emissions in agricultural soils are nitrification and denitrification (Braker and Conrad, 2011), although other microbial pathways can also produce N2O (Butterbach-Bahl et al., 2013). Microbial processes involved in N2O production and emission depend on soil oxygen conditions and the availability of carbon (C) and N substrates. Fertiliser management and soil water conditions are therefore crucial factors controlling N2O emissions from agricultural soils (Abalos et al., 2016; Chen et al., 2014; Lebender et al., 2014).

A potential way to reduce N2O emission is to use nitrification inhibitors (NIs), which have been recommended as a potential N2O mitigation strategy by the IPCC (IPCC, 2014). Since the 1950s, different chemical compounds have been identified which actively inhibit the nitrification and stabilise ammonium (NH4+) in soil after fertilisation. Among the NIs, 3,4-dimethyl pyrazolphosphate (DMPP) is the most commonly used in Europe due to its high efficiency, the small amounts needed and its low ecotoxicity (Zerulla et al., 2001). DMPP deactivates the ammonia monoxygenase (AMO) enzyme by indiscriminately binding to membrane-bound proteins (Chaves et al., 2006) or by making a complex with copper cations (a cofactor of nitrification pathways) (Corrochano-Monsalve et al., 2021; Ruser and Schulz, 2015). The deactivation of the AMO enzyme inhibits or delays the oxidation of NH4+ (Cassman et al., 2002; Subbarao et al., 2006), which may potentially reduce N2O emissions and NO2− leaching and improve the crop N-use efficiency (Akiyama et al., 2010; Recio et al., 2019; Ruser and Schulz, 2015). The potentially increased crop N-use efficiency with the application of NIs may provide an opportunity to reduce N fertiliser application. However, the use of NIs in crop fertilisation has shown variable efficiency in reducing N2O emissions without having a negative effect on crop yield, as demonstrated for example by Akiyama et al. (2010) (31–48 %), Nair et al. (2020) (46–67 %) and Menéndez et al. (2012) (3–45 %).

Agricultural N2O emissions are extremely variable over time, with a considerable proportion of total N2O emissions often occurring during a small number of events, known as hot moments (McDaniel et al., 2017). To date, closed static manual chambers are the dominant technique for field measurements of N2O emissions from agricultural soils. This method is simple, easy and inexpensive to implement in field and laboratory settings to study the treatment effects and specific processes involved in GHG emissions. Manual chambers are widely accepted for GHG flux measurements in agriculture, but they are labour intensive and this limits the temporal resolution of the flux measurements, and compromises accurate estimates of cumulative emissions (Barton et al., 2015; Savage et al., 2014). In contrast, automatic chamber techniques with frequent sampling in time overcome the uncertainty associated with temporal variability. The high-frequent flux measurements with automatic chambers provide better understanding of soil flux responses to management (ploughing and fertilisation) and precipitation events, which may be missed with the manual chamber approach (Savage et al., 2009; Smith and Dobbie, 2001). Furthermore, continuous N2O measurements with automatic chambers are important in order to be able to focus on the short lived processes, which are often missed with manual chamber measurements, for example N2O peaks that does not occur in conjunction with fertiliser events and N2O uptake by soils (Hénault et al., 2012). However, automatic chamber technologies have limitations in terms of covering spatial variability, since automatic chamber systems are expensive and implementing them in remote areas with no power supply (Brown et al., 2018) or for field experimental trials with distant plots is challenging. Therefore, a combination of high-frequency automated measurement and spatially distributed manual chamber measurements are required to capture both hot moments and hotspots from cropland (Parkin, 2008; Savage et al., 2014), grassland (Smith and Dobbie, 2001) and forest (Savage and Davidson, 2003). In this study, we used a combination of automatic chamber and manual chamber measurements to capture the temporal and spatial variability of N2O emissions after application of DMPP with mineral fertiliser in an agricultural field.

The objectives of this study were: i) to compare spatial and temporal patterns of N2O emissions based on manual and automatic chamber measurements, ii) to determine the potential of DMPP to reduce N2O emissions in soils cropped with spring barley and spring oilseed rape, and iii) to examine the effect of increasing N fertiliser levels on total N2O emissions during spring season.

2. Material and methods

2.1. Experimental site

The field experiment was conducted at the University of Copenhagen research facility in Højbakkegård, Taastrup in Denmark (55°40′ N, 12°17′E), which is situated about 20 km west of Copenhagen. The soil type is a sandy loam and the soil physical and chemical properties are presented in Table 1. Data on temperature and rainfall were collected from University of Copenhagen Climate station close to the experimental field (Fig. 1).

2.2. Experimental design and treatments

2.2.1. Experiment 1

The experiment was a fully randomised block design with three replicates. There were a total of 12 plots each being 10 m × 12 m (Fig. 2). Each plot was divided into two sampling plots (each 10 m × 4.5 m) and
a central harvest plot (10 m × 3 m). Sampling plots were used for gas and soil sampling. Harvest plots were used to measure crop yield and to collect plant material for crop C and N analysis (Fig. 2).

The treatments included two crops (spring barley \( \textit{Hordeum vulgare L.} \) cv. KWS Irina) and spring oilseed rape \( \textit{Brassica napus cv. Silver Shadow} \)), which received mineral ammonium sulphate nitrate (NS, 26/13) fertiliser (containing 7.5 % \( \text{NO}_3^- \)-N, 18.5 % \( \text{NH}_4^+ \)-N and 12.5 % sulfate-S) with and without NI coating (ENTEC®, BASF, Ludwigshafen am Rhein, Germany). The active NI ingredient used in this fertiliser was 3,4-dimethyl pyrazolphosphate (DMPP ~1 % relative to the content of \( \text{NH}_4^+ \)-N). The fertiliser was applied on 24 April 2019 at the recommended economically optimal rate of 145 kg N ha\(^{-1}\) in spring barley and 121 kg N ha\(^{-1}\) in spring oilseed rape. Phosphorus (P), potassium (K) and sulfur (S) were applied according to Danish agronomic recommendations at rates of 21, 59 and 15 kg ha\(^{-1}\) in spring barley and 22, 51 and 25 kg ha\(^{-1}\) in spring oilseed rape respectively. Spring barley was seeded at the rate of 165 kg ha\(^{-1}\) on 1 April and spring oilseed rape was seeded at the rate of 2.4 kg ha\(^{-1}\) on 2 April 2019. The zero-N fertiliser control plots (2 m × 2 m ) were established in spring barley plots only. To capture the background emissions during the growing season (see Fig. 2).

**Fig. 1.** Daily total rainfall (mm day\(^{-1}\)) and daily average air temperature (°C) of the study area.

**Fig. 2.** Layout of the experiment treatments and chambers (manual and automatic) in Experiment 1 and Experiment 2. In Experiment 1, spring barley and spring oilseed rape, respectively, were grown in plots (10 × 12 m\(^2\)) with and without addition of nitrification inhibitor (NI) in a block design with 3 replicates, i.e. \( N = 12 \). Zero N fertiliser control treatments were established in spring barley plots only. In Experiment 2, different N level treatments were applied in small plots (1 × 1 m\(^2\)) inside the 3 plots of Experiment 1 with spring barley without NI. To capture the temporal variability, six automatic chambers were placed in spring barley plots with and without NI (three in each plot) in block 1. To capture spatial variability, a collar for manual chamber measurements was installed inside all 12 plots. A central harvest plot (10 × 3 m\(^2\)) was kept undisturbed to record crop yield and N uptake at harvest.
2.2.2. Experiment 2

A short-term experiment was carried out for eight weeks to determine the effect of increasing N application rate. Six small plots (1 m × 1 m) were established in each plot of spring barley without NI (Fig. 2). Six fertiliser treatments were tested in these small plots: i) unfertilised N control (0 kg N ha⁻¹), ii) 50 % N (72.5 kg N ha⁻¹), iii) 100 % N (145 kg N ha⁻¹), iv) 150 % N (217.5 kg N ha⁻¹), v) 200 % N (290 kg N ha⁻¹) of the recommended N rate (145 kg N ha⁻¹) without NI, and vi) 100 % N + NI (145 kg N ha⁻¹) of recommended N with NI in spring barley. Experiment 2 received the same type of N fertiliser (NS, 26/13) and same amounts of P, K and S as Experiment 1. During N fertiliser application in the plots in Experiment 1 (24 April 2019), the small plot areas of Experiment 2 were covered with plastic tarps mounted on wooden frames. Any N fertiliser that landed on the frames was removed. Subsequently, each plot in Experiment 2 was fertilised by hand on 25 April 2019.

2.3. Nitrous oxide gas sampling

Gas fluxes were measured with both manual static chambers and automatic dynamic chambers, as shown in Figs. 2 and 3.

Six automatic chambers were installed in spring barley plots (three chambers in each spring barley plot with and without NI) in block 1 to capture the temporal variability of the N₂O fluxes (Fig. 2). Further spatial coverage by the automatic chambers was not possible due to restrictions in terms of tube lengths between chambers and gas analyzers. There was no automatic chamber in spring oilseed rape plots. To cover the spatial variability, a soil collar for large manual chamber measurements was installed inside each plot of Experiment 1 (i.e. 3 replicates, N = 12). In addition, 6 small collars were installed within each spring barley plot without NI in Experiment 2 covering further overall spatial heterogeneity (N = 18) (Fig. 2). In total 3 large manual chamber measurements for each treatment of spring barley in Experiment 1, and 3 small manual chamber measurements for related spring barley treatments in Experiment 2 were compared with automatic chamber measurements.

2.3.1. Manual static chambers

Fifteen large manual chambers (internal dimension = L 0.75 m × W 0.75 m × H 0.25 m) were used for periodic flux measurements in Experiment 1 to cover the 12 plots and three additional zero N addition plots (Fig. 2, Fig. 3c and d). While eighteen small manual chambers (internal dimension = L 0.35 m × W 0.25 m × H 0.25 m) were used in Experiment 2. The small chambers were used due to small plot areas in Experiment 2 (1 × 1 m²) (Fig. 3a and b). Stainless steel collars were inserted at approximately 15 cm soil depth on 15 April 2019 in Experiment 1 and on 24 April 2019 in experiment 2, i.e. one day before the first flux measurement campaign, and remained in the field throughout the season. White PVC chambers (20 cm height) equipped with an internal battery to power a fan for headspace mixing as well as a butyl rubber septum were used for gas sampling in Experiment 1. Intersections 40 cm in height were used to extend the PVC chambers when the crops became too tall to fit inside the chambers (Fig. 3e). Both PVC chambers and intersections were equipped with a sponge rubber seal 10 mm wide and 4 mm thick to ensure they were tightly closed. During the deployment of the chambers, they were placed on the stainless-steel collars, and clamps were placed on opposite sides of the chamber to ensure proper closure between chamber and collar (Fig. 3d and e). Grey plastic boxes sealed with a multilayer insulation sheet (Aluthermo Quattro®) and equipped with a butyl rubber septum for gas sampling were used for sampling in Experiment 2 (Fig. 3b).

Headspace samples were taken from the chambers immediately after chamber deployment, and at four consecutive times with at least 15-min intervals. The 10 ml gas samples were extracted with a syringe (B. Braun 10 ml syringe with a Luer lock) equipped with a needle (BD Microlance - 0.6 × 25 mm) through the septum in the chambers. Immediately after extraction, the gas samples were injected into 3-ml evacuated vials closed with butyl rubber septa (12.5 mm diameter, Exetainer Labco Ltd., UK). After gas sampling, the vials were stored at room temperature until further analysis. The N₂O concentrations in the collected samples were measured by a gas chromatograph (Bruker 450-GC 2011) equipped with an electron capture detector. Argon (99.99 %) was used as the carrier gas for N₂O at a flow rate of 60 ml min⁻¹. The oven temperature was set at 50 °C.

Fig. 3. Manual static chambers and automatic dynamic chambers used for gas flux measurements: a) small stainless steel collars (0.35 m × 0.25 m) used in small plots in Experiment 2, b) chambers placed on the small collars during gas sampling in Experiment 2, c) large stainless steel collars (0.75 m × 0.75 m) used in the main plots in Experiment 1, d) white PVC chambers (20 cm height) equipped with a battery and fan placed on the large collars during gas sampling in Experiment 1, e) chambers extended with 40 cm intersection, f) ECO₂Flux automatic chamber closed in “light mode” with inner polyurethane tube closed, g) automatic chamber closed in “dark mode” with both inner polyurethane tube and outer aluminium tube closed, h) inner view of auto chamber equipped with fan, PAR sensor and soil probe.
Gas sampling commenced a week before fertiliser application in Experiment 1. The most frequent gas sampling was carried out immediately after fertiliser application in both Experiments 1 and 2 at one to three-day intervals for two weeks. For the following six weeks, gas samples were collected weekly and then biweekly until crop harvest. In Experiment 2, gas samples were only collected until eight weeks after fertilisation.

2.3.2. Automatic dynamic chambers

ECO2Flux chambers (Prenart Equipment ApS (www.prenart.dk), Copenhagen, Denmark) were installed on 24 April 2019 in the spring barley plots with and without N. They were installed in block 1 only, acting as pseudo replicates (Fig. 2) owing to the need to avoid long tubing and cabling to the central multiplexer and analyser units. The automatic chambers have circular base frames covering a ground area of 0.31 m² and a volume of 250 L (a mean height of 80 cm). The automatic chambers were equipped with a fan for proper headspace mixing in the relatively large chamber volume, a photosynthetically active radiation (PAR) sensor, an air temperature sensor, and a soil probe measuring soil temperature and moisture (Fig. 3h). The ECO2Flux automatic chambers have a unique feature of transparent and dark chambers, which allows for direct measurement of net ecosystem exchange (transparent mode) and ecosystem respiration (dark mode), and thereby also the gross rate of photosynthesis estimated as the difference between the transparent and dark measurements. The automatic chambers consist of two flexible, moveable tubes and a lid (Fig. 3 i and g); the inner transparent tube made of polyurethane acts as an airtight gas flux chamber together with a lid, while the outer aluminium-coated tube becomes active during dark measurement.

The ECO2Flux automatic chambers are controlled by a LI-8100/LI-8150 Multiplexer CO₂/H₂O infrared gas analyser system (LI-COR Biosciences, www.licor.com). To measure continuous N₂O fluxes, a PICARRO, G-2508 analyser (PICARRO, www.picarro.com) was connected in a parallel loop (according to LI-COR App. Note 138). The flow rate for the PICARRO G-2508 analyser was kept below 1 standard litre per minute (slpm), which was lower than the flow rate between the LI-8100 and the LI-8150 multiplexer (1.5 slpm) to ensure the recirculation of air in chambers (Fig. S1). Fig. S1 gives a schematic overview of the setup with six automatic chambers, analysers (LI-8100 and PICARRO G-2508) and the multiplexer (LI-8150). The automatic chambers were set up to close two times per turn, first with the transparent tube only and second with both the transparent and dark tubes. Chambers were programmed to close for 5 min during dark measurements to protect the plants from rising temperatures. During dark measurements, chambers were closed for 30 min to allow more observational time for N₂O flux estimation. A pre- and post-purge time of 5 min was used between measurements to ensure the circulation of ambient air between adjacent measurements and to allow plots enough time to re-equilibrate steady-state diffusion after the last measurement. Only the dark measurements were used for N₂O flux calculations. Each chamber ran a full measurement cycle four times a day. Continuous N₂O measurements were recorded throughout the season, starting immediately after fertilisation, until harvest.

2.4. Calculations of N₂O fluxes, cumulative N₂O emissions and emission factors

Nitrous oxide fluxes from manual chambers were calculated from the measured N₂O concentrations using the HMR method (Pedersen et al., 2010). The automatic selection of the flux calculation method was used, i.e. linear, non-linear or no flux according to the HMR package implemented in R (R Core Team, 2020), which is based on variance and statistical significance level. The N₂O fluxes were calculated using the mean air temperature and pressure collected during sampling from the nearby climate station. N₂O fluxes from automatic chambers were calculated with the HMR method using R software in the same way as with the manual measurements.

The cumulative N₂O emissions for the manual and automatic chambers were calculated using linear interpolation by multiplying the mean flux of two consecutive fluxes with the time interval between the two sampling dates, and summing these values (Eqs. (1) and (2)).

\[ F_{\text{end,new}} = \left( t_b - t_a \right) \times \left( F_{\text{t_a}} - F_{\text{t_b}} \right) / 2 \]

where \( F_{\text{t_a}} \) is the emission taking place between the two adjacent measurement times \( t_a \) and \( t_b \) and \( F_{\text{t_a}} \) and \( F_{\text{t_b}} \) are the fluxes of N₂O on the two adjacent measurement times.

Cumulative emission of N₂O over the growing period were calculated using the following equation:

\[ \text{Cumulative emission} = \sum \text{flux}_{\text{all}} \]

The emission factors (EFs) for the growing period were calculated by subtracting the cumulative N₂O-N emissions of the zero-control receiving no N fertiliser from the cumulative N₂O-N emission in each N-treated treatment, and dividing it by the corresponding total amount of applied N (Eq. (3)).

\[ \text{Emission Factor (EF̂)} = \left( \text{N₂O-N}_{\text{treatment}} - \text{N₂O-N}_{\text{zero N-control}} \right) / \text{N applied} \times 100 \]

where N₂O-N_{treatment} is the cumulative N₂O-N emission in the N-treated treatment in spring barley and spring oilseed rape and N₂O-N_{zero N-control} is cumulative N₂O-N emissions of the zero-control receiving no N fertiliser in spring barley. Our experiments did not include a zero-control plot for spring oilseed rape, and we had to assume that the emission of the zero N plot of spring barley was similar to the background emissions in the spring oilseed rape, although we cannot be sure about that. Similar assumptions have been used in other studies, e.g. Baral et al. (2022).

2.5. Soil sampling and analysis

Soil samples were collected on each gas sampling day in the sampling plots, except when gas sampling was more frequent than once a week (with soil samples still taken just once a week). On each day of soil sampling, six individual soil cores (20 mm diameter, 0–20 cm depth) were collected randomly from each sampling plot to make one composite sample per plot. The composite soil samples were transported from the field to the laboratory in a cooler with cooling elements, and subsequently stored at −20 °C until further analysis. Soil samples were analysed as soon as possible within two months after collection. However, some soil samples were stored for up to six months.

The samples were analysed for soil water content, soil pH, NO₃⁻ and NH₄⁺. The soil samples were defrosted at 4 °C and passed through a 5 mm sieve to remove stones, roots and crop residues. Then 10 g sieved soil was weighed in a pre-weighed container and oven-dried at 100 °C for 24 h for volumetric moisture calculation. Another 10 g sieved soil and 40 ml 1 M KCl solution were added to a 50 ml extraction tube with a screw cap, and shaken for 1 h on an end-over-end shaker. Subsequently, the samples were centrifuged at 1000 rpm (revolutions per minute) for 5 min, and then filtered through 2.5 μm Whatman filter papers (Whatman, Z240702). The soil pH of the extracts was measured with a standard pH meter (radiometer PHM210 Meter-Lab). The extracts were stored in a freezer at −20 °C until further NO₃⁻ and NH₄⁺ analysis using a flow injection analyser (Foss FLStar Analyst 5000).

Soil bulk density was measured four times a year at three-monthly intervals. Three random cores of 100 cm³ were collected per plot at 4 cm soil depth to measure bulk density. Water-filled pore space (WFPS) was calculated based on the average bulk density of the study site, i.e. 1.7 g cm⁻³ (Table 1), the volumetric moisture content which was calculated from gravimetric water content measured on each sampling day and assuming soil particle density of 2.65 g cm⁻³.
2.6. Crop harvest and analysis

The central 10 m × 1.5 m strips of the harvest plots were harvested at the beginning of August 2019 with an experimental combine harvester and the fresh matter yield of grain and straw was recorded. A subsample of the grain and straw was dried at 60 °C for 48 h to determine the dry matter contents. Dried grain and straw samples were ground for total N content analysis using an elemental micro-analyser (vario MICRO cube, Elementar, Germany). The spring oilseed rape was poorly established with hardly any grain yield. Therefore, the harvested yield is presented as the total aboveground biomass harvested for both spring barley and spring oilseed rape.

2.7. Statistical analysis

Statistical analysis was performed with the software R version 4.0.2 (R Core Team, 2020). Homogeneity of variance and normality of residuals were inspected using diagnostic plots and a Shapiro-Wilk normality test respectively. The N₂O emission data were transformed using the sqrt function in R to achieve normal distribution of residuals, before performing the statistical test. Least-square means and pairwise comparisons were performed at p ≤ 0.05 level using the R packages lsmeans and multcomp (Hothorn et al., 2008; Lenth, 2016).

For Experiment 1, the linear mixed-effect model (lme) with repeated measurements was used to analyse the effect of crop type and NI addition (fixed effects) on N₂O fluxes, soil NH₄⁺, soil NO₃⁻ and soil pH over time, with day of measurement used as the repeated factor. The model was specified with the exponential correlation function (corExp) to account for the correlation between consecutive flux measurements. Block and plot were included as random factors in the analysis. For Experiment 2, N fertiliser levels were included as the fixed effect in a linear mixed-effect model (lme), with block as the random factor.

Cumulative N₂O emissions and emission factors in Experiments 1 and 2 and aboveground biomass and crop N contents in Experiment 1 were assessed using a linear mixed-effect model (lmer), with block as the random factor.

3. Results

3.1. Soil and climate conditions

During the experimental period (April–July 2019), the daily average air temperature ranged from 4 °C to 23 °C (Fig. 1). The total rainfall during the experimental period was 176 mm, with the most rainfall (64 mm) in the month of June. The total rainfall during the experimental period at the study site was 46 mm lower than the average rainfall for the period from 2011 to 2017. Soil water content varied between 6 % and 19 % during the experimental period.

3.2. N₂O emissions with and without NI in spring barley and spring oilseed rape

Regardless of NI treatments, N₂O-N emissions tended to increase rapidly after fertiliser application on 24 April, peaking on 29 April in spring barley (0.21 (±0.16) mg N₂O-N m⁻² day⁻¹ with NI and 0.43 (±0.30) mg N₂O-N m⁻² day⁻¹ without NI) and on 27 April in spring oilseed rape (0.16 (±0.06) mg N₂O-N m⁻² day⁻¹ with NI and 0.25 (±0.10) mg N₂O-N m⁻² day⁻¹ without NI) (Fig. 4a and b). Negative N₂O fluxes were observed in all treatments on 7 May. After 7 May, the spring oilseed rape treatments generally showed higher N₂O-N fluxes compared with the spring barley treatments, except for 11 June when spring oilseed rape with NI showed a negative flux (−0.37 (±0.45) mg N₂O-N m⁻² day⁻¹). The second peak of N₂O-N fluxes was observed from 11 June to 1 July in spring barley and from 20 May to 24 June in spring oilseed rape treatments. During the second peak, N₂O-N fluxes were 65–89 % lower in spring barley treatments than in spring oilseed rape treatments. During the first four weeks of fertilisation, N₂O-N fluxes were higher in spring barley without NI than in spring barley with NI. N₂O-N fluxes from spring oilseed rape without NI remained higher throughout the season compared with spring oilseed rape with NI, except on 24 June. However, there were no significant (p > 0.05) differences in N₂O-N fluxes between NI treatments in spring barley and spring oilseed rape.

3.3. Dynamics of soil mineral N, soil moisture and soil pH

Before application of N fertiliser on 24 April, NH₄⁺-N concentrations in the soil were around 1 mg N g⁻¹ dry soil in all treatments, and NO₃⁻-N concentrations were between 9 and 10 mg N g⁻¹ dry soil. The subsequent dynamics of NH₄⁺-N and NO₃⁻-N in spring barley and spring oilseed rape treatments reflected the fertiliser application and effect of NI (Fig. 4c, d, e and f). Soil NH₄⁺-N and NO₃⁻-N concentrations rapidly increased after fertiliser application and continued increasing slightly for three weeks. NH₄⁺-N concentrations were significantly higher (p ≤ 0.05) in the NI treatments compared with the treatments without NI in both spring barley and spring oilseed rape (Table S1). During the first five weeks after fertilisation, soil NO₃⁻-N concentrations remained higher in the treatments without NI, although the differences were small. As the plants grew, soil NO₃⁻-N concentration dropped below the pre-fertiliser level, i.e. ~ 2 mg N g⁻¹ soil at the beginning of June in spring barley and after mid-June in spring oilseed rape. Soil NO₃⁻-N concentrations were significantly (p ≤ 0.05) higher in spring oilseed rape than in spring barley, probably due to the poor growth of rape (Table S1).

The dynamics of soil moisture are presented as water-filled pore space (WFPS) for the complete growing season (Fig. 4g and h). At the start of the experiments in April 2019, WFPS was in the range of 50 % to 60 % in all treatments, peaking on around 27 May with 77 % WFPS in spring barley and 85 % WFPS in spring oilseed rape treatments. WFPS remained above 50 % in all treatments until the end of June, then decreased to 37 % towards the end of the growing season. Generally, soil pH(NO₃) remained constant throughout the growing season at a range of between 4 and 6 (Fig. 4i and j), with consistently higher pH in block 3 (6–7) compared to other two blocks (4–5). The NI treatment showed no effect on soil pH in spring barley, while spring oilseed rape without NI showed consistently lower (non-significant) soil pH than spring oilseed rape with NI.

3.4. Comparison of automatic and manual chamber measurements

Measurements from the three automatic chambers in the spring barley with and without NI treatments in block 1 and the mean measurements of the three replicates of the small and large manual chambers showed good agreement of measured N₂O fluxes throughout the sampling period (Fig. 5). The N₂O fluxes measured with the manual and automatic chambers were quite similar at the start of experiment, i.e. immediately after fertilisation and during the intensive period of manual sampling (Fig. 5 and 6). However, some peaks were missed with the manual chamber measurements, e.g. the peak observed with automatic chamber no. 4 immediately after fertilisation application and with automatic chamber no. 3 a very high peak on around 22 May in connection with a rainfall event were not observed with the manual chambers. The maximum N₂O fluxes measured with the manual and automatic chambers in spring barley were 1.24 mg N₂O-N m⁻² day⁻¹ and 3.65 mg N₂O-N m⁻² day⁻¹ respectively. Similarly, negative N₂O fluxes that were quite extensive were mostly missed by the manual chamber measurements during the season. The minimum N₂O fluxes were in the range between −0.26 mg N₂O-N m⁻² day⁻¹ (manual chambers) and −0.59 mg N₂O-N m⁻² day⁻¹ (automatic chambers). In automatic chambers no. 2, 5 and 6, negative N₂O fluxes were consistently observed between the end of May and mid-June that were not captured with manual chambers. The mean N₂O peaks from manual chambers were not consistent with all automatic chamber measurements within same treatment, for example in spring barley without NI, the manual chambers measurements were very much correlated with automatic chamber no. 1 and 3 compared to autochamber no. 2 (Fig. 5). The total variability in manual chamber measurements (58.2 standard deviation) was higher than that in the automatic chamber measurements (34.9 standard deviation).
may be a random chance that placement of some of the manual chambers captured hotspots missed by the placement of the automatic chambers. However, the high frequency measurement with automatic chambers also captured a few “hot moments” missed by the less frequent sampling with manual chambers (Fig. 5 and Fig. 6).

3.5. Effect of N fertiliser levels on nitrous oxide emissions

The effect of N fertiliser levels on N2O-N emissions was significant ($p \leq 0.05$). Although the unfertilised control, 50 % and 100 % fertilisation rates were not significantly ($p > 0.05$) different throughout the sampling period,

Fig. 4. Nitrous oxide fluxes ($\text{mg N}_2\text{O}-\text{N m}^{-2} \text{ day}^{-1}$), concentration of soil ammonium (mg NH4-N g$^{-1}$ soil) and nitrate (mg NO3-N g$^{-1}$ soil), soil water-filled pore space (WFPS, %), and soil pH (0.01 M CaCl2) in the plots planted with spring barley (left) and spring oilseed rape (right) with and without DMPP (NI) in Experiment 1. Values represent the mean of three blocks ± standard error. ↓ indicates time of fertilisation.
Fig. 5. Nitrous oxide fluxes (mg N$_2$O-N m$^{-2}$ day$^{-1}$) measured using the automatic chambers from a plot with spring barley without NI (left) and with NI (right) in Experiment 1. The solid lines represent the fluxes from each of the three individual (replicate) automatic chambers. The filled circles represent the mean nitrous oxide fluxes (mg N$_2$O-N m$^{-2}$ day$^{-1}$) from three large (●) and three small (●) manual chambers from the three blocks with the same treatments as the automatic chambers with which they are depicted. Error bars indicate standard error. ↓ indicates time of fertilisation.

Fig. 6. Nitrous oxide fluxes (mg N$_2$O-N m$^{-2}$ day$^{-1}$) measured using the manual chambers and daily average fluxes measured with automatic chambers in spring barley without NI (a) and spring barley with NI (b) during the growing period. Manual chamber values represent the mean of three replicates of each large and small chambers ± standard error. Automatic chamber values represent the daily mean of all fluxes measured by three auto chambers placed in each treatment plot ± standard error.
the N₂O-N fluxes for higher N levels (150 % N and 200 % N) were significantly (p ≤ 0.05) higher than the optimal and suboptimal N levels from 21 May onwards, reaching a maximum (0.51–0.56 mg N₂O-N m⁻² day⁻¹) on 20 June (Fig. 7). The total N₂O-N emissions were significantly (p ≤ 0.05) higher from the 200 % N fertiliser level than from the 0, 50 % and 100 % N levels. As in Experiment 1, no significant (p > 0.05) difference was observed in N₂O-N emissions with and without NI application.

3.6. Cumulative N₂O emissions and emission factors

Application of NI showed no significant (p > 0.05) reduction in cumulative N₂O-N emissions, irrespective of whether they were determined by manual or automatic chambers (Tables 2, 3 and 4). Application of NI in spring barley resulted in a 108 %, 16 % and 52 % reduction in cumulative N₂O-N emissions than from spring barley without NI from automatic chambers (Table 3), large manual chambers in Experiment 1 (Table 2), and small manual chambers in Experiment 2 (Table 4) respectively. Cumulative N₂O-N calculated from the automatic chambers was lower than from the manual chambers: 36–90 % lower in spring barley without NI treatment and 66–133 % lower in spring barley with NI. Application of NI in spring oilseed rape showed a tendency to reduce cumulative N₂O-N emissions. Although this was not significant. On average the emissions were 58 % lower than for spring oilseed rape without NI in the large manual chambers.

The EFs were generally very low. There were no significant differences in EF between NI treatments, crops and N fertiliser levels. The highest EF was observed in the spring oilseed rape without NI, with EF of 0.148 (± 0.047) %. The lowest EF was observed in the spring barley with NI (0.025 (± 0.027) %). Application of NI resulted in a 32 % and 76 % reduction in EF in spring barley and spring oilseed rape respectively. Although not significant there was a tendency for the EF to be reduce with the NI treatment at the optimal level (100 % N) of N application in spring barley measured in Experiment 2. On average the reduction was 65 %.

Table 2
Cumulative N₂O emissions (g N₂O-N ha⁻¹) and emission factor (%) for crop growing period with manual chamber measurements, total aboveground crop biomass (Mg ha⁻¹) and N contents (kg N ha⁻¹) for the NI (nitrification inhibitor, DMPP) treatments in Experiment 1 with spring barley and spring oilseed rape. Values represent the mean of three replicates ± standard error. Lower case letters indicate a significant difference (p ≤ 0.05) between treatments.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cumulative N₂O-N (g ha⁻¹)</th>
<th>Emission factor (%)</th>
<th>Total aboveground crop biomass (Mg ha⁻¹)</th>
<th>Crop N contents (kg N ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring barley zero N control</td>
<td>53.99 (± 19.59)</td>
<td>0.037 (± 0.024)</td>
<td>14.22 (± 0.56)</td>
<td>156 (± 3.3)</td>
</tr>
<tr>
<td>Spring barley without NI</td>
<td>107.15 (± 34.59)</td>
<td>0.025 (± 0.027)</td>
<td>15.54 (± 1.04)</td>
<td>166 (± 5.1)</td>
</tr>
<tr>
<td>Spring barley with NI</td>
<td>90.08 (± 39.43)</td>
<td>0.148 (± 0.037)</td>
<td>3.83 (± 0.45)</td>
<td>39 (± 3.9)</td>
</tr>
<tr>
<td>Spring oilseed rape without NI</td>
<td>233.36 (± 56.68)</td>
<td>0.036 (± 0.030)</td>
<td>3.88 (± 0.45)</td>
<td>37 (± 5.8)</td>
</tr>
<tr>
<td>Spring oilseed rape with NI</td>
<td>97.40 (± 36.51)</td>
<td>0.015 (± 0.007)</td>
<td>156.47 (± 52.98)</td>
<td>0.048 (± 0.016)</td>
</tr>
</tbody>
</table>

Table 3
Cumulative N₂O emissions (g N₂O-N ha⁻¹) for crop growing period with automatic chambers for the NI (nitrification inhibitor, DMPP) treatments in Experiment 1 with spring barley. Values represent the mean of three replicates (chambers) ± standard error.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cumulative N₂O-N (g ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring barley without NI</td>
<td>39.52 (± 15.45)</td>
</tr>
<tr>
<td>Auto chamber # 1</td>
<td>40.90</td>
</tr>
<tr>
<td>Auto chamber # 2</td>
<td>12.09</td>
</tr>
<tr>
<td>Auto chamber # 3</td>
<td>65.56</td>
</tr>
<tr>
<td>Spring barley with NI</td>
<td>3.04 (± 18.07)</td>
</tr>
<tr>
<td>Auto chamber # 4</td>
<td>32.07</td>
</tr>
<tr>
<td>Auto chamber # 5</td>
<td>13.20</td>
</tr>
<tr>
<td>Auto chamber # 6</td>
<td>27.98</td>
</tr>
</tbody>
</table>

Table 4
Cumulative N₂O emissions (g N₂O-N ha⁻¹) and emission factor (%) for the spring growing period with manual chamber measurements for different N levels and NI (nitrification inhibitor, DMPP) treatments in Experiment 2 with spring barley. Values represent the mean of three replicates ± standard error. Lower case letters indicate a significant difference (p ≤ 0.05) between treatments.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cumulative N₂O-N (g ha⁻¹)</th>
<th>Emission factor (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero N</td>
<td>(0 kg N ha⁻¹)</td>
<td>15.91 (± 5.75)</td>
</tr>
<tr>
<td>50 % N</td>
<td>(72.5 kg N ha⁻¹)</td>
<td>57.21 (± 5.59)</td>
</tr>
<tr>
<td>100 % N</td>
<td>(145 kg N ha⁻¹)</td>
<td>77.01 (± 16.77)</td>
</tr>
<tr>
<td>150 % N</td>
<td>(217.5 kg N ha⁻¹)</td>
<td>129.74 (± 17.02)</td>
</tr>
<tr>
<td>200 % N</td>
<td>(290 kg N ha⁻¹)</td>
<td>156.47 (± 52.98)</td>
</tr>
<tr>
<td>100 % N + NI</td>
<td>(145 kg N ha⁻¹)</td>
<td>37.16 (± 3.64)</td>
</tr>
</tbody>
</table>
3.7. Crop yield and N uptake

Crop yield is presented as total biomass, since the spring oilseed rape did not grow properly and only green biomass was harvested at the end of season. Application of NI tended to increase the crop yield and N uptake, but there was no significant (p > 0.05) effect of NI on crop yield and N uptake in spring barley and spring oilseed rape (Table 2).

4. Discussion

4.1. Spatial and temporal patterns of N₂O fluxes

Spatiotemporal variability of N₂O fluxes is a major source of uncertainty when assessing the efficiency of GHG mitigation strategies in agricultural crop production (Paustian et al., 2016). While many experiments have assessed the effect of nitrification inhibitors, this study is the first attempt to monitor the spatial and temporal dynamics of N₂O emissions simultaneously, and how they are affected by NI, by using manual and automatic chamber techniques. Many authors have encouraged the use of automatic chamber systems to determine high-frequency seasonal and annual N₂O fluxes from agricultural soils to obtain a higher temporal resolution and better resolved peaks (Barton et al., 2015; Savage et al., 2014; Smith and Dobbie, 2001). The experimental setup in the present study provided a cross-check to characterise N₂O fluxes from four treatments in a spatially extensive area and two treatments with high temporal resolution using automated measurements. While it would be optimal to measure all treatments at a high temporal and spatial resolution, the strategy used here was to cover the spatial variability with six soil collar (3 large and 3 small) per spring barley treatment installed over three blocks (2 collars, 1 large and 1 small per block) and the temporal variability with three automatic chambers in each spring barley plot in block 1. One large soil collar per spring oilseed rape treatment installed per plot. There was no automatic chamber in spring oilseed rape plots.

The primary objective for deploying the automatic chambers was to test whether some important peak events in N₂O fluxes might be missed by manual chamber measurements. The comparison of manual and automatic measurements showed that the peak in emissions following fertilisation was captured well with the intensive manual sampling frequency occurring at this time (three samplings per week, Fig. 5, and Fig. 6). However, after two weeks, the peaks in N₂O fluxes were missed with the manual chambers, because samplings were conducted too sporadically during these periods (weekly or biweekly). Similar estimates of N₂O fluxes with manual and automatic sampling strategies have been reported with high manual sampling frequency (3–7 days intervals) from corn/soybean fields in USA (Parkin, 2008) and intensively managed ryegrass in Scotland (Smith and Dobbie, 2001).

The pattern of N₂O fluxes from individual automatic chambers in each treatment showed considerable variability between the three automatic chambers under the same treatment (Fig. 5 and Fig. 6). Moreover, the manual chamber measurements were always in agreement with at least one of the three automatic chamber measurements for both treatments (Fig. 5). The differences observed in the automatic chamber measurements within the same treatments can be explained in several ways. First, the spatial variability at the experimental site could be substantial, even within a few metres. Spatial variability is caused by small differences in topography that affect water dynamics and cause inhomogeneous distribution of crop residues that again lead to spatial variability in soil moisture levels, soil mineral N contents and microbial activities (Ruser et al., 2006; Velthof et al., 1995). The differences could also be influenced by the fact that the automatic chambers were not measuring at the exact same time during the day (each measurement cycle was completed in 7 h) and there is substantial diurnal variation in the emissions of nitrous oxide (Ferrari Machado et al., 2019). Standard deviations (SD) are considered more appropriate for comparison of variabilities between measurements when fluxes range from negative to positive, particularly if mean values are negative (McDaniel et al., 2017; Webster, 2001), as in the case of the automatic chamber measurements during some periods (Table 3). The variability in measurements was high during extreme flux periods in both manual (SD between 0.05 and 0.39) and automatic chambers (SD between 0.06 and 0.31), i.e. immediately after fertiliser application and during rain events. During the periods of low emissions the variability was also lower in both manual (SD between 0.04 and 0.14) and automatic chamber (SD < 0.06) measurements. These findings are in line with other studies, where periods of high fluxes show greater spatial variability in N₂O fluxes from agricultural fields (Bellingrath-Kimura et al., 2015; Konda et al., 2010; McDaniel et al., 2017). The comparison of manual and automatic measurements indicates high heterogeneity in N₂O fluxes both temporally (hot moments) and spatially (hotspots) in agricultural fields. Savage et al. (2014) recommended a N₂O flux measurement system with numerous, less frequent manual chambers and a few automatic chambers with high temporal resolution to capture both hotspots and hot moments of N₂O emissions.

4.2. Negative N₂O fluxes

Substantial periods with negative N₂O fluxes were observed with the automatic chamber measurements, which were only rarely observed with the manual chamber measurements (Fig. 5). Uptake of N₂O in agricultural soils has been reported in several studies (Chapuis-Lardy et al., 2007; Flechard et al., 2005; Nefelt et al., 2010; Ryden, 1981). N₂O uptake is generally believed to be a consequence of microbial N₂O reduction (Bremner, 1997), which is the last step of the microbial denitrification where N₂O is converted to N₂. Denitrifiers utilise NO₃, NO₂ and NO as an electron acceptor under anaerobic conditions. Complete denitrification, i.e. conversion of N₂O to N₂, occurs predominantly when only N₂O is available as an electron acceptor (Jones et al., 2011). Studies have found that low NO₃ concentrations, i.e. < 1 µg N g⁻¹ (Ryden, 1981) and < 5 kg ha⁻¹ (Goossens et al., 2001), stimulate N₂O uptake in soil. A low NO₃ concentration (< 4 mg NO₃-N g⁻¹ soil) five weeks after fertilisation (Fig. 4e) and only sporadic rain events (Fig. 1) may have created the optimal conditions for N₂ formation from N₂O as well as for N₂O diffusion in the soil, to allow for negative fluxes to take place until mid-June (Fig. 5). At the beginning of June, negative N₂O fluxes were measured with automatic chambers, but very few negative fluxes were found with the manual chambers. Therefore, all data quality control measures were followed for the automatic chamber measurements, e.g. observing individual measurements for N₂O with CO₂ (Fig. S3) and correlation of N₂O with H₂O fluxes (Fig. S2), and no reason could be found to reject the negative N₂O fluxes. Since all the automatic chambers were placed in the N-fertilised plots of spring barley, there was no background N₂O flux calculation based on continuous measurements. However, Crill et al. (2000) conducted the continuous measurements in fertilised and zero control plots and found an increase in N₂O emissions by the factor of 3.6 with N application in corn, which was mainly due to high N₂O peaks around N-fertiliser events. The consistent negative fluxes in the automatic chambers were observed around near zero N₂O fluxes in manual chambers (Fig. 5 and Fig. 6). In certain cases, when N₂O emissions are near zero, it is hard to determine whether the negative N₂O fluxes are real or an artifact of instrumental noise (Gowan et al., 2014). The N₂O fluxes observed in this study are frequent and cannot simply be rejected as experimental noise (Chapuis-Lardy et al., 2007). Negative N₂O fluxes was twice as common in automatic chambers (47 %) compared to manual chambers (22 %), indicating that there was a period with negative N₂O fluxes that was missed in manual chamber measurements. The largest negative fluxes measured were −0.26 mg N₂O-N m⁻² day⁻¹ (manual chambers) and −0.59 mg N₂O-N m⁻² day⁻¹ (automatic chambers), which were higher than the maximum negative fluxes (−0.132 mg N₂O-N m⁻² day⁻¹) reported by Gowan et al. (2014) who interpreted them as a result of instrumental noise from the different agricultural fields across the UK. However, maximum negative fluxes reported by Butterbach-Bahl et al. (2002) (−0.29 mg N₂O-N m⁻² day⁻¹) from pine plantations in sandy cambisols and by Ryden (1981) (−0.5 mg N₂O-N m⁻² day⁻¹) from grassland in a loam soil are within the range of negative N₂O fluxes observed in this study (Fig. 5). Especially, Ryden (1981) reported bi-directional N₂O fluxes.
from fertilised grassland, with brief N₂O fluxes around fertiliser events and frequent low N₂O fluxes or N₂O uptake outside the fertiliser events, which is comparable to seasonal N₂O pattern observed in this study (Fig. 5 and Fig. 6).

4.3. N₂O emission and its relationship with soil moisture, mineral N and pH

Soil nitrification and denitrification processes are highly dependent on N substrate and oxygen (O₂) availability in the soil. In addition, electron donators are important for the denitrification process, and is highly related to available C. The concentration of O₂ is rarely measured in soils, and instead WFPS is often used as a proxy for indicating soil O₂ status (Chen et al., 2008; Linn and Doran, 1984). High WFPS > 90% will usually result in depletion of soil O₂ (Bateman and Baggis, 2005). Hu et al. (2015) and Ruser et al. (2006) reported denitrification as a principal source of N₂O emissions at a higher WFPS > 70 %, while nitrification predominates at WFPS < 70 %. However, reduction of N₂O to N₂ has been observed to be strongest when the soil is cleaved into saturated conditions, so an optimum level of WFPS for maximum N₂O emission is suggested to be between 65 % and 85 % (Davidson, 1991; Ruser et al., 1998; Skiba and Smith, 2000).

The typical short-lived N₂O fluxes that were observed after fertilisation have been reported in numerous studies (Clayton et al., 1997; Jones et al., 2007; Leahy et al., 2004). Higher N₂O emissions attained when neither mineral N substrate, oxygen content and available C are limiting nor climate (rainfall, temperature) and soil variables (soil moisture, soil pH) are restricting nitrification and denitrification (Smith et al., 2003). The initial N₂O peaks were observed when soil NO₃⁻ was high and soil moisture was favourable (WFPS between 75 % and 79 %) for denitrification (Fig. 4). However, as NH₄⁺ levels were also high, nitrification could play a significant role during this peak as well. da Silva Cardoso et al. (2020) also reported high N₂O emissions related to the denitrification process due to high NO₃⁻ concentration and WFPS > 70 % under a grassland system. Decreasing N₂O fluxes were observed two weeks after fertilisation, which corresponded to decreasing soil NH₄⁺ and NO₃⁻ (Fig. 4c and f) and low ambient temperature in the range of 4–5 °C (Fig. 1) and soil temperature < 10 °C (measured in automatic chambers, data not shown), below which denitrification and nitrification have been reported to be low in soil (Stanford et al., 1975). Wertz et al. (2013) reported downward shift in nitrifier and denitrifier communities with decreasing soil temperature, but found unexpected increments in N₂O at ~1 °C when C and NO₃⁻ was not a limiting factor for denitrification temperate soil.

The second peak of N₂O fluxes was higher in spring oilseed rape than in spring barley during the period between 20 May and 17 June, which could be related to higher soil NO₃⁻ concentration in the soil under the spring oilseed rape treatments compared with spring barley (Fig. 4f and Table S1) as there was no considerable difference in WFPS between spring barley and spring oilseed rape treatments during that period (Fig. 4g and h). The most likely explanation for the significantly higher NO₃⁻ concentration in the soil under spring oilseed rape compared with spring barley is that the poorly growing spring oilseed rape was not able to take up N effectively (Table 2). Furthermore, N₂O fluxes from mid to late June in the spring barley treatments was clearly not a consequence of high NO₃⁻ concentrations (soil NO₃⁻ concentrations were mostly below 5 mg NO₃⁻·N g⁻¹ soil) during that period. These relatively low soil NO₃⁻ concentrations did not appear to be enough to limit N₂O production in the situation with rather high and fluctuating soil WFPS, indicating a poor relationship (p-value 0.085–0.39) between NO₃⁻ and N₂O emission (Table S1 and S2).

NI application caused higher soil NH₄⁺ concentrations (Fig. 4c and d), but these did not result in significant differences in N₂O emissions (Fig. 4a and b and Table S1). The consistently high soil NH₄⁺-N concentrations could be related to restricted substrate diffusion due to dry soil conditions during the application period (Stark and Firestone, 1995). The persistence and efficiency of NI e.g. DMPP in the soils depends on soil moisture and temperature (Menéndez et al., 2012). Most studies has reported rapid degradation (1–7 weeks after application) of DMPP in the soil (Dittert et al., 2001; Scheer et al., 2014). In the present study, we observed consistently higher soil NH₄⁺-N concentrations throughout the season in NI treatments, which supports previous observations of long-term effects of DMPP by Thers et al. (2019). Therefore, year-round studies including off-season emission measurements are important to explore the potential of NI to reduce N₂O emissions.

The spring barley crop grew better than the spring oilseed rape and the soil NO₃-N concentration tended to decrease faster over time after fertiliser application in the spring barley crop resulting in no difference in pH between NI treatments. However, since the spring oilseed rape was not able to take up soil NO₃⁻-N (37–39 kg N ha⁻¹) as effectively as spring barley (156–166 kg N ha⁻¹) due to poor crop growth (Table 2), and because of nitrification in the treatment without NI that resulted in the production of more protons (Padhi et al., 2017), which could be the cause of the lower soil pH (by 0.59 units) in treatments without NI compared with NI treatments in spring oilseed rape. Qiao et al. (2015) also reported an increase in soil pH by 0.23 units with NI application. The increased pH in NI treatments corresponds to a comparatively smaller release of protons due to the inhibition of nitrification process (Qu et al., 2014).

4.4. Effect of nitrification inhibitors on N₂O emissions and emission factor during the crop growing period (experiment 1)

Application of NI tended to reduce N₂O-N emissions, although not significantly so. The reduction was 58 % and 16 % in spring oilseed rape and spring barley respectively. The higher N₂O-N reduction with NI in spring oilseed rape than in spring barley could be related to poor crop growth and less N uptake by spring oilseed rape. The N₂O-N emission reductions observed with NI application in the present study were in the range of N₂O-N mitigation by field application of DMPP in other studies. Weiske et al. (2001) found a 49 % reduction in N₂O-N emissions with DMPP application together with mineral fertiliser in southern Germany, and in a meta-analysis Gilsanz et al. (2016) reported a 38 % reduction in N₂O-N emissions with application of DMPP in cropland.

Based on comparisons with the unfertilised control treatment, the fertilised treatments in spring barley and spring oilseed rape with and without NI resulted in N₂O-N EFs in the range of 0.025 % to 0.148 % (Table 2), which is in line with other studies, indicating that the EFs for mineral N fertilizers in northern Europe are often far below the default IPCC EF value of 1 % when measurement was only performed during the growing period (Baral et al., 2017; Baral et al., 2020; Dobbie et al., 1999; Migliorati et al., 2014). Baral et al. (2020) reported EFs of 0.40 % and 0.53 % for the spring barley growing period without NI and with NI application respectively. Conversely, Migliorati et al. (2014) reported the lower EFs for wheat and corn during the growing period with NI (~ 0.005 % and 0.24 %) compared to without NI (0.19 % and 0.93 %). Finally, Dobbie et al. (1999) reported comparatively higher EFs for growing periods of spring barley (0.6–0.7 %) and spring oilseed rape (1 %). According to recent IPCC updates, the aggregated EF for mineral N in managed soil is 0.010 (0.001–0.018) and disaggregated EF for wet climates is 0.016 (0.013–0.019) (IPCC, 2019). The results of present study are not directly comparable with IPCC EFs because the present study presented the EFs only for crop growing period and not for entire year. However, application of NI tended to reduce the EF of mineral fertiliser applied in spring oilseed rape and spring barley by 76 % and 32 % respectively. In addition, although not significant the EF based on short-term spring season measurements tended be reduced with NI application in the spring barley (Table 4) on average by 65 %. The N₂O-N emissions and EFs for the crop growing period showed that the N₂O reduction effect of NI application with fertiliser was higher when N₂O emissions were higher as well.

4.5. Effect of fertiliser amount on N₂O emission and emission factor during the spring growing period (experiment 2)

In this study, the amount of fertiliser application was positively correlated (r = 80%) with cumulative N₂O-N emissions (Table S2). A positive linear relationship between N application and N₂O emissions indicated
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that the concept of emission factors worked quite well within the range of N application from 0 to 200 % of normal recommended N application that was tested here. Several studies have found a linear response between N application rates and cumulative N₂O emissions in arable cropping systems (Lebender et al., 2014; Liu et al., 2012). Other studies have reported a non-linear response of N fertiliser rates and N₂O emissions (Walter et al., 2015). N application could be considered a limiting factor for N₂O-N emissions, as lower N₂O-N emissions with less N application were identified as having a higher rate of N application. This could correlate with substrate supply for microbial nitrification and denitrification, which increases N₂O production in the soil and thereby enhances N₂O emission (Guzman-Bustamante et al., 2019; Slicherbak et al., 2014).

The difference in EFs for different N fertiliser levels was non-significant, but the cumulative N₂O emissions indicated a clear pattern with significant differences along the N addition gradient (Table 4, Table S1). The EFs for different N levels were in the range of 0.042 % (145 kg N ha⁻¹) to 0.057 % (72.5 kg N ha⁻¹), which were lower than the EFs reported by Abdalla et al. (2010) for 140 kg N ha⁻¹ (0.63 %) and 70 kg N ha⁻¹ (0.42 %) in spring barley from sandy loam soil in Ireland.

4.6. Cumulative N₂O emissions from manual and automatic chambers

The lower estimates of cumulative N₂O emissions with automatic chamber measurements (63–103 %) compared with manual chamber measurements were surprising (Tables 2 and 3). The main reason for this difference was the greater number of negative fluxes (47 %) with automatic chamber measurements, which were not captured in the manual chamber measurements (22 %). This could be due to the fact that automatic chambers covered the temporal variability better (3–4 measurements per chamber per day) and was able to capture some hot moments, which were missed by manual chambers with less intensive sampling (2–3 times per week for two weeks after fertilisation, weekly until eight weeks of fertilisation, and biweekly until harvesting) at the specific period of the day (10:00 and 12:00 AM). One of the major disadvantages of the manual chamber method is the inability to assess potential diurnal and day-to-day variability in fluxes (Reeves et al., 2016). Different studies have reported differences in N₂O emission estimates between manual and automatic chambers, e.g., Yao et al. (2009) reported that manual chambers overestimate cumulative N₂O emissions by 18 % in a rice-wheat rotation ecosystem because they neglect diurnal variations in N trace gas fluxes. Malajan et al. (2002) also reported 60 % higher N₂O emissions with manual chamber measurements from boreal organic soils due to a high variation in diurnal temperature. In contrast, some studies have reported no differences in manual and automatic chamber measurements due to diurnal variability when manual chamber measurements were conducted at intervals of 2–9 days in a fertilised tree plantation (Weitz et al., 1999) or 3–7 days in fertilised grassland (Smith and Dobbe, 2001). While, Tallae et al. (2019) reported 38 % higher N₂O emission estimate with automatic chambers compared to manual chambers in irrigated maize crop.

4.7. Effect of nitrification inhibitor on crop yield and N uptake

Previous studies have shown very variable effects of NI on crop yield and crop N uptake. The non-significant effects of NI on yields in this study align with the findings of Guzman-Bustamante et al. (2019) and Migliorati et al. (2016), who found no yield-increasing effect of DMPP in winter wheat and sorghum respectively, while Abalos et al. (2014) and Thapa et al. (2016) reported in their meta-analyses an increase of 7 % and 7.5 % in agricultural crop yield with application of DMPP. Likewise, Pasda et al. (2001) reported higher crop yields, but lower N uptake by crop with DMPP application in various agricultural and horticultural crops in western and southern Europe. It is well known that the efficiency of NI for increasing crop uptake depends greatly on the crop. In Denmark, the strongest effect is seen in maize and much less pronounced effects are seen in cereals and crucifers (Hansen, 2021). The fact that the NO₃⁻N contents did not differ between treatments with and without NI can be attributed to more NO₃– being taken up by the growing crop. These findings are in line with Huerfano et al. (2015) and Polychronaki et al. (2012), who found no effect of DMPP on crop yield and N uptake in winter wheat and cotton.

5. Conclusions

This study combined spatially extensive, infrequent manual measurements and highly frequent automated measurements to study the effect of DMPP as a nitrification inhibitor on emissions of N₂O from an agricultural field. The comparison of manual and automatic measurements revealed that some N₂O peaks related to rain events and in particular negative N₂O fluxes during the growing period were missed during periods when manual chamber measurements were being performed on a weekly or bi-weekly basis. Application of NI resulted in consistently higher soil NH₄⁺-N concentrations and slightly lower NO₃–-N concentrations in the soil. The reduction in N₂O emissions and EF with NI were higher in spring oilseed rape (58 % and 76 %, respectively) compared with spring barley (16 % and 32 %, respectively). This could be due to the fact that spring oilseed rape was well established in this study, consequently it could not take up N from soil as efficiently as spring barley. The accumulated N₂O emissions and EF calculated over the growing season indicated that the N₂O mitigation effect of NI was greater when N₂O emissions were also higher. The rate of fertiliser application was positively correlated (r = 0.798, p = 0.00036) with N₂O emissions. NI application has no significant effect on crop biomass yield and N uptake. Therefore, long-term field studies are required, including measurements during the postharvest season, to optimise the N₂O mitigation potential of NI.

CRediT authorship contribution statement

Azeem Tariq: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Visualization; Writing - original draft preparation.
Klaus Steenberg Larsen: Conceptualization; Formal analysis; Methodology; Writing - review & editing. Line Vinthø Hansen: Writing - review & editing. Lars Stoumann Jensen: Conceptualization; Investigation; Methodology; Writing - review & editing. Sander Bruun: Conceptualization; Investigation; Methodology; Writing - review & editing.

Data availability

Data will be made available on request.

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.

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Appendix A. Supplementary data

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