DMPP reduced nitrification, but not annual N₂O emissions from mineral fertilizer applied to oilseed rape on a sandy loam soil

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Abstract
Direct field emissions of nitrous oxide (N₂O) may determine whether biodiesel from oilseed rape (Brassica napus L.) fulfills the EU requirement of at least 50% reduction of greenhouse gas emissions as compared to fossil diesel. However, only few studies have documented fertilizer N emission factors (EF) and mitigation options for N₂O emissions from oilseed rape cropping systems. We conducted a field experiment with three N levels (0, 171, and 217 kg/ha), where the N fertilizer was applied as ammonium sulfate nitrate with or without the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP). N₂O fluxes were measured using static chambers technique and soil samples were analyzed for water and mineral N content during a monitoring period of 368 days. The DMPP treatments showed a significantly increased level of ammonium (NH₄⁺) for up to 18 weeks after spring fertilization as compared to the treatments without DMPP. However, this difference did not result in a corresponding decrease in NO₃⁻ soil content, and no differences in cumulative N₂O emissions were found between any fertilized treatments with or without DMPP (mean, 1.26 kg N₂O-N ha⁻¹ year⁻¹). More field experiments are needed to clarify whether DMPP-coated mineral fertilizers could mitigate N₂O emissions under different weather conditions, for example, under conditions where fertilization events concurred with rainfall events increasing water-filled pore space to the assumed 60% threshold for denitrification. Emission factors for mineral N fertilizer were 0.28%–0.36% with a mean of 0.32% across the fertilized treatments. These data concur with recent European studies suggesting that the EF for mineral N fertilizers in oilseed rape cropping systems may typically be lower than the default IPCC value of 1%. Further studies are needed to consolidate an EF for oilseed rape under temperate conditions, which will be determining for the sustainability of Northern European oilseed rape cultivation for biodiesel.

KEYWORDS
biodiesel, DMPP, emission factor, N₂O, nitrification inhibitor, oilseed rape
1 | INTRODUCTION

Savings of greenhouse gas (GHG) emissions from the use of transport biofuels, as compared to fossil fuels, should currently be 50%–65% to comply with the revised Renewable Energy Directive (RED II) of the European Union (European Parliament, 2018). The main GHG emissions from biofuel production are associated with the cultivation step of the biofuel crops, which must be evaluated in order to attest the benefits of replacing fossil fuel. A major crop for biofuel production in the EU is oilseed rape (Brassica napus L.), where the seeds are converted to biodiesel in the form of rapeseed methyl ester (EUBIA, 2019). For this crop, it has been estimated that direct emissions of nitrous oxide (N₂O) in the field represent 33%–55% of total GHGs from the cultivation step as calculated in carbon dioxide (CO₂) equivalents, CO₂eq (Elsgaard, Olesen, Hermansen, Kristensen, & Børgesen, 2013; Malça, Coelho, & Freire, 2014; Queirós, Malça, & Freire, 2015). Such estimates are typically based on the IPCC default Tier 1 emission factor (EF), whereby it is assumed that 1% of the nitrogen (N) introduced in fertilizers and crop residues will be emitted as N₂O-N (IPCC, 2006). However, the RED II directive allows member states to use a country-specific Tier 2 EF, if documented by experimental field measurements of N₂O emissions. This could be important, because N₂O emissions are known to depend on site-specific conditions, such as soil type and climate (Ruser et al., 2017), and because this documentation could promote the implementation of management strategies to mitigate N₂O emissions.

The main processes leading to N₂O emissions from agricultural soils are microbial nitrification and denitrification based on, respectively, ammonium (NH₄⁺) and nitrate (NO₃⁻), although chemical reactions may also result in N₂O (Jones, Peters, Lezama Pacheco, Casciotti, & Fendorf, 2015; Smith et al., 2018). Nitrification is an aerobic autotrophic process where NH₄⁺ is oxidized to NO₃⁻; the first step in the process is the oxidation of NH₄⁺ to nitrite (NO₂⁻) by ammonia-oxidizing archaea and bacteria (Beeckman, Motte, & Beeckman, 2018). During this process, N₂O can be formed by chemical decomposition of the intermediate hydroxylamine (NH₂OH). Denitrification is an anaerobic heterotrophic process typically mediated by facultative anaerobic bacteria, where NO₃⁻ is sequentially reduced to N₂ with N₂O as an intermediate (Butterbach-Bahl, Baggs, Dannenmann, Kiese, & Zechmeister-Boltenstern, 2013). The rates of nitrification and denitrification in soil ecosystems are controlled by several factors, including foremost the oxygen status, the mineral N availability, and the availability of labile organic carbon (C) compounds (Granli & Bøckman, 1994). Sources of NH₄⁺ include mineral and organic fertilizers, decomposition of plant residues, and mineralization of soil organic matter, whereas NO₃⁻ mainly derives from mineral fertilizers and microbial oxidation of NH₂⁺. Thus, inhibition of the nitrification of NH₂⁺ to NO₃⁻ not only will decrease the potential for formation of N₂O during this process but also there will be less NO₃⁻ available for denitrification and associated N₂O losses (Qiao et al., 2015). The synthetic compound 3,4-dimethylpyrazole phosphate (DMPP), like other nitrification inhibitors (NIs), inhibits the first step in the nitrification process (Zerulla et al., 2001). DMPP-coated granular N fertilizers represent an operational way to apply the NI and the resulting effects on N₂O emissions have been addressed in several field studies, yet with variable results (e.g., Huérfano et al., 2018; Menéndez, Barrena, Setien, González-Murua, & Estavillo, 2012; Wallace et al., 2018). Discrepancies between reported effects of DMPP have been attributed to effects of environmental conditions, such as temperature, water-filled pore space (WFPS), and soil NO₃⁻ availability (Huérfano et al., 2016; Menéndez et al., 2012). Studies with DMPP-stabilized mineral N fertilizers showed significant N₂O-mitigating effects of DMPP in subtropical Australian broccoli production (Scheer et al., 2014) and in German cultivation systems with lettuce (Pfab et al., 2012) as well as spring barley, maize, and winter wheat (Weiske, Benckiser, & Ottow, 2001). In contrast, no significant effects of DMPP were found in a subtropical Australian pasture study (Lam et al., 2018) and in a Columbian lettuce cultivation system (Huérfano et al., 2018). To our knowledge, only a single study has documented N₂O emissions after application of DMPP-coated granular fertilizers to oilseed rape cultivation systems (Li et al., 2018). The study tested urea N fertilization in an Australian field trial and reported a 62% reduction in N₂O emissions when using DMPP and urea as compared to urea alone (Li et al., 2018). Hence, the use of DMPP could potentially mitigate N₂O emissions related to oilseed rape cultivation and thereby improve biodiesel sustainability. However, the study by Li et al. (2018) differentiates to typical Northern Europe agricultural conditions on matters like temperature regime, N application rates, and splits, and the effect of DMPP-coated fertilizers still needs to be documented in field experiments under temperate European conditions.

Here, we studied the N₂O-mitigating effect of a commercial DMPP-coated ammonium sulfate nitrate fertilizer (ENTEC® 26) in an annual study of winter oilseed rape cultivation under Danish climate conditions. Full year measurements ensured compliance with the IPCC database requirements (De Klein & Harvey, 2015) and inclusion of potentially important winter N₂O emissions related to frost/thaw events (Smith, 2017). The study was a block designed field experiment (n = 4) including a reference without N and two levels of N fertilizers (171 and 217 kg N/ha) with and without DMPP. The aims of the study were (a) to test the N₂O-mitigating effect of DMPP in an oilseed rape cropping
system, (b) to trace the effects of DMPP in order to evaluate potential reasons for divergent N2O mitigation observed under field conditions, and (c) to quantify the N2O EF for mineral N fertilizer applied to oilseed rape.

2 | MATERIALS AND METHODS

2.1 | Experimental site

The field study was conducted at the Foulumgaard Experimental Station (56°29′41″N, 9°34′04″E) on a soil classified as a Typic Hapludult, coarse loamy, mixed, mesic according to the USDA Soil Taxonomy (Møberg & Nielsen, 1986). The soil contained 36% coarse sand, 41% fine sand, 13% silt, 7% clay, and 1.8% organic C. Long-term mean annual precipitation was 626 mm and mean annual temperature was 7.3°C (Jensen, Plauborg, & Sørensen, 1997) with January and February as the coldest months (−0.5°C) and July as the warmest (15.4°C). Weather data during the study year were obtained from a climate station within 100 m from the experimental site. These data included soil temperatures (diurnal average at 10 cm depth) and daily precipitation (24 hr sum).

2.2 | Experimental design and crop cultivation

Five treatments were included in a randomized factorial block design with four repetitions (i.e., 20 plots in total), where each plot measured 36 m² (6 × 6 m). One half of each plot was randomly selected for gas and soil sampling, whereas the other half was used for the determination of crop yield. The treatments were low and high levels of mineral N fertilizer (171 and 217 kg N/ha, respectively) with and without DMPP and a reference without N fertilizer and DMPP. The DMPP containing N fertilizer was ENTEC® 26 with 26% N (7.5% NO3-N, 18.5% NH4-N) and 13% sulfur (S) on a weight basis (EuroChem, 2018). The DMPP was contained in the coating in amounts corresponding to 0.8% of the NH4-N mass (Huérfano et al., 2016). The corresponding fertilizer without DMPP was a Rosier® NS 26-13 containing 26% N (7% NO3-N, 19% NH4-N) and 12.5% S.

The study was performed in the cropping season 2017–2018. The preceding crop was spring barley (Hordeum vulgare L.), where straw was removed from the field after harvest. Ploughing and seeding (row spacing, 48 cm) of oilseed rape (variety, DK Exclusiv) took place on August 17, followed by herbicide applications of 0.25 L/ha Command® on August 18 and 1.25 L/ha Kerb® on November 30, 2017. The N fertilizer was applied in three splits, with the first split on September 15 (30 kg N/ha in all fertilized treatments). The rest was applied in spring 2018 as two equal splits on March 20 and April 20. Following each NS fertilization, the reference plots (without N application) received a corresponding amount of S in the form of calcium sulfate (CaSO4). Potassium (84 kg K/ha) and phosphorous (26 kg P/ha) were applied to all treatments in early April 2018. Epso Micrōtop® was applied on April 19 to supply the oilseed rape with manganese, magnesium, and boron. Pests were controlled two times in the fall (Sluxx HP® against slugs) and once in the spring (0.3 L/ha Biscaya OD 240® against pollen beetles; April 22). On July 10, the oilseed rape was mown for drying, which is common practice in Denmark, and on July 19, it was harvested. During harvest, the fresh weight of straw from each plot was recorded by the combine harvester (Haldrup plot harvester; J. Haldrup A/S, Løgstør, Denmark), and the seed and a representative straw sample from each plot were collected and stored for subsequent analyses.

2.3 | Gas flux measurements

GHG fluxes from all 20 plots were measured by use of opaque static chambers in 55 campaigns completed during the study year. The static chambers, made of polyethylene, had inside dimensions of 56.8 cm × 36.8 cm × 31.9 cm (L × W × H) and were isolated by ALUthermoQUATTRO® (Adflexion Aps). The chambers were equipped with a pressure equilibration tube (De Klein & Harvey, 2015) and a manifold connecting three outlet tubings (inner diameter, 2 mm) from the chamber headspace. This design was adopted to obtain representative samples of headspace gas without using a battery-driven fan. During gas flux measurements, the chambers were placed on metal collars (55.5 cm × 35.5 cm) permanently inserted to approximately 15 cm soil depth between crop rows. The collars had a water-holding groove for airtight sealing between the collar and chamber. Details of chambers, collars, and manifolds are shown and described in Figures S1–S5 and Table S1. To calculate the effective chamber headspace volume, the mean height of the collar groove above soil surface was measured after collar insertion (and after reinstallation following field operations). During chamber deployment, five gas samples (10 ml) were taken at regular intervals from each chamber during a deployment time of 60–90 min. Samples were stored at overpressure in 6 ml pre-evacuated Exetainers (Labco Ltd.). Concentrations of N2O were measured on an Agilent 7890 gas chromatograph system (Agilent) configured and calibrated as described in Petersen et al. (2012). The general frequency of GHG flux measurements was once per week during the growth season and once per 2 weeks during late autumn, winter, and early spring. The frequency was increased to two to three campaigns per week following fertilization and major rainfall events. Measurements were usually completed between 10 and 13 (and always between 8 and 14).

Flux calculations were performed using the HMR package for R (Pedersen, Petersen, & Schelde, 2010). The HMR script analyses and compares fluxes using a linear
approach, as well as a nonlinear approach, which is a regression-based extension of the Hutchinson and Mosier model (Hutchinson & Mosier, 1981). The HMR default selection between linear and nonlinear fluxes is based on minimizing a single combined mean-squared error criterion, and both linear and nonlinear fluxes are calculated with standard error, 95% confidence limits, and p-value for the null hypothesis of no flux. The default model selection was supported by visual inspection of individual concentration-time series, and in some cases, a recommended nonlinear flux estimate was judged to result from random measurement error, especially when low or no flux was indicated by linear regression. To deal with this in a standardized way, we applied a threshold of \( p < .01 \) for the specified \( p \)-value of the fit, meaning that only nonlinear fits meeting this additional requirement were accepted (otherwise linear regression was used for flux calculation). The choice of the threshold \( (p < .01) \) was based on visual examination of the entire flux dataset \( (>1,000 \text{ fluxes}) \).

Cumulative fluxes were estimated by linear interpolation of flux rates between measuring dates following the trapezoidal principle.

EFs for the applied mineral N fertilizer were calculated from the differences between annual \( \text{N}_2\text{O} \) emissions in fertilized and reference plots. The treatment-specific EFs were calculated by block, meaning that cumulative emissions from the treatments minus reference were calculated within each block and the results were expressed as the mean ± standard error of the mean (SEM) of the four blocks.

2.4 | Soil sampling and analyses

Soil for measurement of pH, gravimetric water content (for WFPS determination), and mineral N (i.e., \( \text{NO}_3^- \) and \( \text{NH}_4^+ \)) was sampled once a week in the growing season, and once every 2 weeks in late autumn, winter, and early spring. Samples were collected from 0 to 20 cm depth in all 20 plots using a 2 cm diameter soil corer, with six samples per plot being pooled and stored at \(-18^\circ\text{C}\) for subsequent analysis. After thawing, each soil sample was sieved (<6.3 mm) and mixed. Subsamples were dried to constant weight at 105°C for determination of soil moisture. Ten gram portions of field-moist soil were extracted in 40 ml of 1 M KCl for 30 min (end over end, 32 rev per min), and pHKCl was measured by glass electrode before the soil suspension was filtered (VWR European Cat. No. 516-0867). Filtrates were frozen \((-18^\circ\text{C})\) for later analyses of \( \text{NO}_3^- \) and \( \text{NH}_4^+ \) using a QuAAtro39 AutoAnalyzer (SEAL Analytical, Inc.).

Total concentrations of soil C and N were determined for each plot in top soil (0–20 cm) sampled in the autumn 2017. Sampling was done as for mineral N analyses, and the soil was air-dried and sieved (<2 mm) before C and N analyses by combustion using a vario MAX cube CN analyzer (Elementar Analysensysteme GmbH).

Intact soil samples were collected for measurement of bulk density on three occasions, that is, September 25 (2017) and May 6 and August 22 (2018). These soil samples were collected in 100 cm\(^2\) metal rings (diameter, 6 cm; height, 3.5 cm) from 3 to 8 cm soil depth at five randomly selected places across the entire field. The dry weights were determined after drying at 105°C (24 hr).

2.5 | Seed and straw analyses

Moisture content, oil content, and protein content in seeds were measured using a FOSS Infratec™ 1241 Grain Analyser (FOSS). The N content was calculated from the protein content assuming a protein-to-N mass ratio of 6.26 (Hansen, Munkholm, Melander, & Olesen, 2010). For use in dry matter (DM) calculations, four representative straw samples were collected following harvest and an average straw moisture content of 34.2% was determined and used for all plots. Subsamples of straw from each plot for total C and N analyses (by combustion) were grinded using a RETSCH SM 2000 heavy-duty cutting mill with a mesh size of 1 mm.

2.6 | Statistical analyses

Statistical analyses were performed using the R software (R Core Team, 2018). Tests of treatment effects on cumulative \( \text{N}_2\text{O} \) emissions and yields were performed in a mixed model, using the \textit{lme} function in the nlme package (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2018), with treatment as fixed factor and block as random factor, followed by pairwise comparisons using the \textit{glht} function in the multcomp package (Hothorn, Bretz, & Westfall, 2008), which performed a Tukey’s test. The second test of treatment effects on cumulative \( \text{N}_2\text{O} \) emissions was performed with the levels of N (171 and 217 kg N/ha), DMPP (with and without), and their interaction as fixed factors and block as random factors. Tests for treatment differences in soil mineral N concentrations (\( \text{NO}_3^- \) and \( \text{NH}_4^+ \)) on individual sampling days were performed using a mixed model (\textit{lme} function) with corAR1 as an argument to include the autocorrelation in repeated sampling, and with treatment, day, and their interaction as fixed factors and plot as random factor. Soil mineral N concentrations were log transformed in order to obtain variance homogeneity. The \textit{emmeans} function in the \textit{emmeans} package (Lenth, 2019) was used for pairwise comparison of soil mineral N content between treatments for each sampling day using the \textit{lme} model parameters and a significance level of \( p < .05 \).
3 | RESULTS

3.1 | Soil and climate conditions

Soil bulk density was consistent across the field site and seasons with a mean ±SEM of 1.36 ± 0.02 g/cm³ (n = 15). Total C and N concentrations (measured in fall 2017) likewise were similar among plots (n = 20) with means ±SEM of 19.2 ± 0.2 mg C/g and 1.54 ± 0.02 mg N/g. Water-filled pore space was 50%–60% at the time of crop establishment (August 17, 2017), and consistently around 60%–70% from October 2017 to April 2018 (Figure 1). From April, WFPS dropped steeply to 15%–20% in June and only increased again after August. This pattern coincided with a summer

**FIGURE 1** Soil temperature (10 cm depth), water-filled pore space (WFPS), and precipitation during the study year. Data for WFPS are the mean of the five experimental treatments; coefficients of variation were less than 10%

**FIGURE 2** Dynamics of soil ammonium (NH₄⁺) concentration during the study year. (a) Reference treatments without nitrogen (N) fertilization. (b) Low N treatments (171 kg N/ha) with and without nitrification inhibitor, DMPP. (c) High N treatments (217 kg N/ha) with and without DMPP. Solid arrows indicate fertilization events; dashed arrow indicates harvest of oilseed rape. Plowing and sowing of oilseed rape were performed in mid-August 2017, that is, 2 weeks before the first soil sampling. Data are shown as means with standard error bars (n = 4). Asterisks indicate soil sampling days with significantly higher NH₄-N concentration in treatments with DMPP than without DMPP (p < .05). Between mid-February and mid-March soil sampling was hampered due to frozen top soils.
that was unusually dry and hot for Danish conditions, with soil temperatures at 10 cm depth increasing from around 5 to 22°C from April to August (Figure 1). Soil temperatures during the foregoing winter were typically 0–5°C, but frozen top-soil hindered soil sampling in some periods of February and March. Mean annual soil and air temperature during the study year was 9.9°C and 9.2°C, respectively, and the total rainfall was 646 mm. Major rain events occurred in September 2017 (43 mm), in late April 2018 (23 mm), and in mid-August 2018 (60 mm during 6 days).

**FIGURE 3** Dynamics of soil nitrate (NO$_3^-$) concentration during the study year. (a) Reference treatments without nitrogen (N) fertilization. (b) Low N treatments (171 kg N/ha) with and without nitrification inhibitor, DMPP. (c) High N treatments (217 kg N/ha) with and without DMPP. Solid arrows indicate fertilization events; dashed arrow indicates harvest of oilseed rape. Plowing and sowing of oilseed rape were performed in mid-August 2017, that is, 2 weeks before the first soil sampling. Data are shown as means with standard error bars ($n = 4$). Asterisks indicate soil sampling days with significantly lower NO$_3^-$-N concentration in treatments with DMPP than without DMPP ($p < .05$). Between mid-February and mid-March soil sampling was hampered due to frozen top soils.

**FIGURE 4** Dynamics of nitrous oxide (N$_2$O) fluxes during the study year. (a) Reference treatments without nitrogen (N) fertilization. (b) Low N treatments (171 kg N/ha) with and without nitrification inhibitor, DMPP. (c) High N treatments (217 kg N/ha) with and without DMPP. Solid arrows indicate fertilization events; dashed arrow indicates harvest of oilseed rape. Plowing and sowing of oilseed rape were performed in mid-August 2017, that is, 2 weeks before the first soil sampling. Data are shown as means with standard error bars ($n = 4$).
3.2 | Dynamics of soil ammonium and nitrate

Reference plots had consistently low background levels of ammonium, typically 1–10 kg NH₄-N/ha with a mean of 4.3 kg NH₄-N/ha during the study year (Figure 2a). Autumn fertilization with 30 kg N/ha in all fertilized treatments caused temporary increases in soil ammonium concentrations to 15–27 kg NH₄-N/ha, but without significant differences (p > .05) between treatments with and without DMPP (Figure 2b,c). Following the spring fertilization events, peak concentrations were 40–60 kg NH₄-N/ha and 60–90 kg NH₄-N/ha in the low N and high N treatments, respectively, with highest NH₄⁺ concentrations in the DMPP treatments for an extended period (Figure 2b,c). This DMPP effect was significant on 16 sampling days for the low N treatments, and on 18 sampling days for the high N treatments. Thus, for both the low and high N treatments, the increased NH₄⁺ content in DMPP treatments persisted for several months after the last fertilizer application, that is, from around mid-April to mid-August.

Nitrate in reference plots ranged from 1 to 22 kg NO₃-N/ha (mean 5.8 kg NO₃-N/ha) with periods of highest NO₃-N concentrations in autumn after crop establishment and in summer after crop harvest (Figure 3a). Autumn fertilization caused small temporary increases (~10 kg NO₃-N/ha), which were followed by low winter concentrations of around 3 kg NO₃-N/ha in all treatments, that is, with similar dynamics in treatments with and without DMPP (Figure 3b,c). The highest NO₃⁻ levels after spring fertilizer events were around 40 and 50 kg NO₃-N/ha in low N and high N treatments, respectively, with generally higher concentrations in treatments without DMPP, although this was statistically significant only for one sampling occasion in mid-April (Figure 3b,c). After mid-July, the soil NO₃⁻ concentration increased gradually for several weeks in all treatments (including the reference), but still to levels that reflected the N fertilizer levels, that is, to 25 and 40 kg NO₃-N/ha in low and high N treatments, respectively, and these levels were unaffected by the DMPP treatment.

3.3 | Nitrous oxide emissions

Nitrous oxide fluxes were consistently low during large parts of the monitoring period independent of treatment, with levels typically between 0.00 and 0.02 mg N₂O-N m⁻² hr⁻¹ (Figure 4). In the unfertilized reference plots, two notable emission peaks were recorded, that is, following crop establishment and especially at the time of final soil thaw in late March (Figure 4a). Peaks of N₂O emissions in the fertilized treatments (Figure 4b,c) were observed (a) following the ploughing event (before crop establishment), (b) after the autumn fertilizer event, (c) at the time of final soil thaw and first spring fertilization event, (d) after the second spring fertilization event, and (e) after the crop harvest.
concurrent with the end of the long dry summer. Emission peaks in the fertilized treatment had similar magnitude with and without DMPP with highest emissions representing 0.10–0.15 mg N₂O-N m⁻² hr⁻¹.

Cumulative annual N₂O emissions ranged from 1.12 to 1.43 kg N₂O-N h⁻¹ year⁻¹ across the four fertilized treatments, which showed no significant differences (Table 1). The N₂O emission in the reference treatment (0.64 kg N₂O-N h⁻¹ year⁻¹) was significantly lower than in the fertilized treatments, except for the low N treatment without DMPP (p = .06). Testing the N application rate and DMPP as explanatory variables of cumulative N₂O emissions (using block as random factor) showed firstly that there was no significant interaction (p = .48) and secondly that without the interaction term, the effect of N application rate was significant (p < .001), whereas the effect of DMPP was not (p = .81).

Nitrous oxide EFs for the added mineral N fertilizer ranged from 0.28% to 0.36% across all fertilized treatments, resulting in an overall average EF of 0.32% (Table 1). There were no significant treatment differences in EFs neither in relation to N fertilizer levels nor to DMPP. Likewise, yield-scaled N₂O emissions were not significantly different between fertilized treatments (Table 1).

### 3.4 | Plant and oil yields

The average yield data of all fertilized treatments were 5,784 kg straw DM/ha (excluding 8 cm stubble), 2,668 kg seed DM/ha, and 1,354 kg oil/ha, with no significant difference between fertilized treatments (Table 1). This means that no significant yield or oil increases were derived from the additional 46 kg N/ha in the high N treatments, and further showing that DMPP did not affect the yield of oilseed rape.

### 4 | DISCUSSION

#### 4.1 | N₂O emission dynamics

Peaks of N₂O emissions in the mineral N fertilized treatments were associated with soil tillage, spring thaw, fertilization, and post-harvest residue degradation. The first N₂O peak occurred after油seed rape establishment, which redistributed the preceding barley stubble and roots in the soil, potentially also breaking up soil aggregates. The resulting fresh organic matter availability expectedly stimulated microbial mineralization (see Figure S6 for CO₂ flux dynamics) and oxygen consumption, thereby creating anoxic microsites facilitating heterotrophic denitrification. Such stimulated N₂O production due to organic matter decomposition was previously found in soil with moisture contents as low as 30% WFPS (Li, Hu, & Shi, 2013), confirming that residue decomposition can support N₂O emissions across a wide range of soil water contents.

Mineral N fertilization was followed by N₂O emission peaks in both autumn and spring. However, the first emission peak in spring could not be explicitly attributed to the N application, because a similar peak was recorded in the reference treatment. Instead, a factor other than N availability must have controlled N₂O emissions at this time. The soil was thawing around the time of first spring fertilization, and freeze–thaw cycles have been acknowledged to contribute substantially to the overall N₂O emissions when winter fluxes are included in N₂O quantification (Smith, 2017). Suggested mechanisms leading to frost/thaw-related N₂O peaks include liberation of protected C substrates from physically protected soil organic matter, and reduced N₂O diffusivity during soil frost periods (Kim, Vargas, Bond-Lamberty, & Turetsky, 2012). Although the low background emission measured before the frost period questions the relevance of the latter mechanism in our study, the present data does not allow further mechanistic interpretations.

After the second spring fertilization, distinct N₂O emission peaks emerged, which had a longer duration compared to the thaw-related N₂O emission. These N₂O emissions were dependent on N fertilization, but were apparently triggered by the rain events in late April, which increased WFPS to 57%, that is, close to the presumed 60% threshold for bulk soil anaerobic conditions (Huérfan et al., 2018). At the time of the N₂O peak event, the top-soil NO₃⁻ concentrations corresponded to 10–31 kg NO₃-N/ha in the fertilized treatments as compared to the somewhat higher concentrations of 28–57 kg NO₃-N/ha few days after fertilization. Thus, the timing of the N₂O emission peak was not controlled by NO₃⁻ concentration alone, but rather by the interaction between N availability and impeded oxygen diffusion due to high WFPS. It follows that, if the rainfall event had occurred at the time of the highest soil NO₃⁻ concentrations, the N₂O emission might have reached higher levels and potentially created a significant difference between treatments with and without DMPP. The haphazardness of precipitation (and the resulting increase in WFPS) in relation to NO₃⁻ availability following fertilization is known as a contributing factor to diverging cumulative N₂O emissions between years (Ruser et al., 2017), but could also explain contrasting results between studies with respect to the apparent effects of DMPP in mitigating field-scale N₂O emissions. Future field studies addressing this topic should aim at experimentally stimulating N₂O fluxes in periods of high NO₃⁻ soil concentrations by manipulation of soil water content, in order to quantify the latent emission risk under field conditions in addition to quantifying the emissions under the actual weather conditions prevailing during the study year.

The observed post-harvest N₂O emission peak occurred at a time of increased organic matter availability, as expected due to decaying root and harvest residues. Nitrification was evident from the net accumulation of 20–30 kg NO₃-N/ha, which was generally unaffected by the two N fertilizer levels (low and high) and DMPP treatment within each of the
N levels. Despite the nitrification and increased NO$_3^-$ levels, emission peaks of N$_2$O clearly depended on the rainfall event of mid-August, which increased the WFPS to 48%. This finding indicates that nitrification as such did not cause increased N$_2$O emissions in the dry period, and that N$_2$O resulted from denitrification in anaerobic microsites promoted by organic matter decomposition (oxygen consumption) at soil moisture levels considerably below the approximate 60% threshold for bulk soil anaerobiosis (Duan et al., 2018; Li, Sørensen, Olesen, & Petersen, 2016). Despite the lack of N$_2$O emissions before the rainfall event, we cannot exclude as an option that nitrification could potentially contribute to the N$_2$O emission peak due to an increased N$_2$O/NO$_3^-$ product ratio as aeration became restricted (Granli & Bockman, 1994). Oxygen depletion in microsites with organic matter is caused by respiratory oxygen demand exceeding the oxygen diffusion rate, which is increasingly impeded at higher water contents that are typical of organic matter microsites as compared to the bulk soil (see Duan et al., 2018). Furthermore, N$_2$O produced in anaerobic hot spots may preferentially be transported and emitted to the atmosphere since the surrounding aerobic bulk soil is not conducive of N$_2$O reduction to N$_2$. The importance of the post-harvest emissions are in line with the results of Walter et al. (2015), who indicated that oilseed rape post-harvest N$_2$O emissions tended to exceed the emissions during the growing season. Walter et al. (2015) suggested that the post-harvest emissions were residue driven and not dependent on N fertilizer. Likewise, the post-harvest emissions in our study could be residue driven, and indeed, cumulative post-harvest N$_2$O fluxes from July 12 to September 4 (2018) were not significantly different between the low N and high N treatments.

### 4.2 Effects of DMPP

This study found similar N$_2$O emissions for mineral N fertilizer with and without DMPP when applied under field conditions to oilseed rape on a sandy loam soil. DMPP treatments had higher concentrations of NH$_4^+$ on several soil sampling days following the fertilization events, which was consistent with an inhibitory effect of DMPP on microbial nitrification under field conditions. However, this did not lead to a corresponding lower soil NO$_3^-$ concentration in the DMPP treatments. In a meta-analysis, Qiao et al. (2015) documented increased soil NH$_4^+$ content following fertilizer application with DMPP (and other NIs) as compared to treatments without DMPP, and reported a corresponding decrease in soil NO$_3^-$ concentration. The lack of clear DMPP effect on NO$_3^-$ concentrations in our study aligns with the lack of N$_2$O mitigation, since reduced NO$_3^-$ availability could be the main reason for N$_2$O-mitigating effects of DMPP (Kong, Eriksen, & Petersen, 2018). It is uncertain why the impeded NH$_4^+$ oxidation was decoupled from significantly decreased NO$_3^-$ concentrations, but for one thing, the applied mineral fertilizer consisted of 29% NO$_3^-$-N (and 71% NH$_4^-$-N), thereby to some extent diluting the effect of reduced nitrification. Further experiments should examine whether the use of a solely NH$_4^+$-N containing DMPP fertilizer could mitigate N$_2$O emissions while at the same time maintaining N-scaled crop yields.

The persistence of DMPP and DMPP efficiency in soil ecosystems is believed to depend on complex ways on soil moisture and temperature (Menéndez et al., 2012), but more studies have indicated relatively rapid degradation of DMPP, that is, within 1–7 weeks (Dittert, Bol, King, Chadwick, & Hatch, 2001; Merino et al., 2005; Scheer et al., 2014). In the present study, significant effects of DMPP on soil NH$_4^+$ concentrations were traced 16–18 weeks after the DMPP fertilizer application. Nevertheless, substantial nitrification took place within this period, as discussed below, which questions the apparent long-term effect of DMPP.

The post-harvest increase in soil NO$_3^-$ concentrations from mid-July to early August (20–30 kg N/ha), which indicated nitrification of mineralized NH$_4^+$, was similar with and without DMPP, but occurred during the prolonged period of significantly larger soil NH$_4^+$ content in treatments with DMPP (4–18 kg NH$_4^+$-N/ha). This finding might indicate that the applied DMPP was active in relation to remaining NH$_4^+$ from the fertilizer application, but that the DMPP was not able to inhibit the nitrification of mineralized N from crop residue degradation. One hypothesis, although speculative, could be that accumulated NH$_4^+$ (and/or NH$_3$) in nitrifying bacteria, which are inhibited by DMPP, could act as a kind of living or dead storage of NH$_4^+$, which reappears in extractions of mineral N from the soil samples. Reconciling this hypothesis and our findings would presuppose a long-term persistence of the DMPP activity; yet, this persistence could be limited to DMPP attached to transmembrane ammonia monooxygenases (Beeckman et al., 2018), which may lead to structural protection from degradation. Another suggestion for the long-term maintained difference in NH$_4^+$ concentrations could be limited substrate (NH$_4^+$) diffusion due to the dry soil conditions (Stark & Firestone, 1995), which potentially could cause the long-term effect despite that DMPP activity ceases during the dry period. In any case, the duration and underlying mechanism of DMPP activity should be further clarified, and also consideration should be given to the role of the N source in relation to the spatial distribution of NH$_4^+$ and DMPP in the soil.

### 4.3 EFs for mineral N applied to oilseed rape

This study found N$_2$O-N EFs for mineral N applied to oil seed rape (0.28%–0.36%) that were lower than the IPCC default EF of 1% (IPCC, 2006). Likewise, other recent studies in Europe found EFs related to oilseed rape cultivation to be lower than 1%. Vinzent, Fuß, Maidl, and Hülsbergen (2018) reported
EFs of 0.19–0.50 for different fertilizer treatments across 3 years of oilseed rape cultivation in Germany, and Ruser et al. (2017) suggested an EF of 0.6% based on a range of N fertilization rates in five German study sites across 3 years. Furthermore, a preceding meta-analysis of seven European sites (German and one British) with oilseed rape field trials estimated a fertilizer-related emission of 1.27% based on an averaged nonlinear relationship between N fertilizer application rates and measured N2O emissions (Walter et al., 2015). Based on the data presented by Walter et al. (2015), we deduced an EF of 0.73% for an N fertilizer level of 200 kg N/ha.

We expect the present emission results to be representative for oilseed rape on Danish nonirrigated sandy soils, although conclusions based on one experimental year are inherently uncertain and the unusual dry summer calls for discussions on data representativeness. The harvested seed yield in the experimental plots was 2,668 kg/ha (DM), which was 14.5% lower than the average national oilseed rape yield in 2018 (3,121 kg/ha; Statistics Denmark, 2019), which included both nonirrigated and irrigated soils. However, due to the dry and warm summer, yields on nonirrigated soils expectedly were lower than on irrigated soils, and therefore, our yield data seem representative for nonirrigated soils. In terms of microbial processes, the dry and warm summer could have influenced the cumulative N2O emissions, although in various ways. For example, due to low WFPS, denitrification could be decreased and thus lead to less N2O emissions. Conversely, the high temperature could stimulate N mineralization and turnover, which in combination with transient plant drought stress could increase the microbial N availability, potentially leading to higher N2O emissions. In another study at the experimental site (Baral, Learke, & Petersen, 2019), annual emissions of N2O from festulolium (Festulolium braunii L.), tall fescue (Festuca arundinacea L.), and maize (Zea mays L.) were determined between April 2017 and April 2018 with resulting fertilizer N EFs of 0.23, 0.32, and 0.54%, respectively. These crops received NPK fertilizer with 60% NH4-N and 40% NO3-N in a study year with 752 mm total precipitation and soil temperatures (10 cm depth) not exceeding 18°C. This suggests that EFs below 1% are typical for sandy loam soils in this region. Furthermore, Ruser et al. (2017) showed a persistent increase in median N2O emissions with increasing annually rainfall across five sites in 3 years. Despite extended summer drought, the 646 mm of rainfall in this study year was comparable with the 626 mm long-term mean. Thus, considering annual rainfall as driver of cumulative N2O emissions, the present study year was not unusual. Finally, our EF results are in line with recent German field studies covering multiple years (Ruser et al., 2017; Vinzent et al., 2018) and substantiate that typical EFs for mineral N fertilizer in oilseed rape cropping systems may be lower than 1%.

Nitrogen supply to crops and microorganisms in the reference plots could result from the regional atmospheric N deposition (13 kg N ha−1 year−1; Ellermann et al., 2018), biological N fixation, and mineralization of crop residues and soil organic matter. Although the present study focused on the role of mineral N fertilizers, EFs for other N sources, such as crop residues, are likewise important to assess in order to evaluate the overall sustainability of biodiesel derived from oilseed rape (Ruser et al., 2017). A tentative estimate of the N input from the preceding barley crop indicated an input of 42 kg N/ha (Supplementary Materials). Assuming an EF of 0.32% for this N pool would correspond to an emission of 0.13 kg N2O-N ha−1 year−1, that is, much lower than the measured emission of 0.64 kg N2O-N ha−1 year−1. Thus, although N supply could come from additional sources, these data indicated that an EF of 0.32% for the crop residues would be too low. Indeed, an EF of 1% would seem more appropriate, resulting in an emission of 0.42 kg N2O-N ha−1 year−1 from barley crop residues in the reference treatments. However, more experimental studies are needed to firmly differentiate EFs for mineral fertilizer N and N originating from organic material such as crop residues.

The lack of seed yield increase in the high N treatments as compared to the low N treatments makes N fertilizer reduction a straightforward N2O mitigating option. However, due to the summer drought, we find it too speculative to suggest this option. In addition, the nonsignificant differences between yield-scaled N2O emissions point at no additional emissions because of the higher N rate.

To conclude, significant and long-term inhibition of nitification by DMPP was found in field studies of mineral fertilized oilseed rape on sandy loam soil. However, corresponding long-term effects on NO3 concentrations could not be detected, which likely caused the lack of DMPP reduction of cumulative N2O emissions at the two levels of N fertilization (171 and 217 kg N/ha). The temporal relationship between increased NO3 soil content following fertilization and precipitation events was considered as an important contributing factor to the cumulative N2O emissions and maybe also, in this case, to the nonsignificant effect of DMPP, since the major rainfall events occurred when there was no difference in NO3 soil concentrations between treatments with and without DMPP. Based on comparison to an unfertilized reference treatment, the applied mineral N fertilizer had an average N2O EF of 0.32%, which is in line with recent multiyear European studies of oilseed rape cropping systems, collectively indicating that the EF for mineral N fertilizers is typically below the default IPCC value of 1%. We conclude that DMPP in mineral fertilizers at least in some cropping years may not lead to mitigation of field-scale N2O emissions in oilseed rape cultivation systems and thereby does not increase the sustainability of biodiesel production. However, further studies are encouraged, which cover multiple cropping years and alternative N fertilization scenarios.
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