Split N application and DMP based nitrification inhibitors mitigate N$_2$O losses in a soil cropped with winter wheat

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Abstract Nitrogen (N) fertilization to crops might lead to formation and release of reactive N—e.g. nitrate, ammonium, ammonia, nitrous oxide (N$_2$O)—, contributing to eutrophication, atmospheric pollution, and climate change. Use of nitrification inhibitors and splitting of N fertilizer may reduce the N$_2$O emission from arable soils cropped with winter wheat. We tested different N fertilizers treated with 3,4-dimethylpyrazol phosphate (DMPP) and 3,4-dimethylpyrazol succinic acid (DMPSA) by applying 180 kg N ha$^{-1}$ in different N splitting strategies in a full annual field experiment on a loamy soil in Southwest Germany. A threefold split fertilization led to an emission of 2.3 kg N$_2$O–N ha$^{-1}$ a$^{-1}$ (corresponding to a reduction of 19%) compared to a single application of ammonium sulphate nitrate (ASN) ($p=0.07$). A single application rate of ASN with DMPP resulted in an emission of 1.9 kg N$_2$O–N ha$^{-1}$ a$^{-1}$ and reduced N$_2$O emissions from an ASN treatment without NI by 33%. Calcium ammonium nitrate (CAN) with DMPSA reduced N$_2$O emissions during the vegetation period by 38% compared to CAN without a nitrification inhibitor, but this was offset by high emissions after harvest, which was driven by soil tillage with an annual reduction of 26% (CAN: 2.9 kg N$_2$O–N ha$^{-1}$ a$^{-1}$; CAN + DMPSA: 2.1 kg N$_2$O–N ha$^{-1}$ a$^{-1}$; $p=0.11$). Among our tested treatments, a twofold split application of ASN with DMPP efficiently reduced N$_2$O emissions and maintained grain yield when compared to the traditional system with threefold application without nitrification inhibitor. Despite resulting in lower protein contents in the twofold split application, this treatment should be further investigated as a potential compromise between wheat yield and quality optimization and climate protection.

Keywords N$_2$O emission · DMPP · DMPSA · N fertilizer splitting

Introduction

Nitrous oxide (N$_2$O) is a climate-relevant trace gas which also contributes to the depletion of stratospheric ozone (Ravishankara et al. 2009; IPCC 2021). More than half of the anthropogenic N$_2$O emissions are released from agricultural soils (Canadell et al. 2021). It is generally agreed that N$_2$O production in agricultural soils is mainly controlled by the microbiological processes of nitrification and denitrification (Robertson and Groffman 2015), whereas the contribution of other processes such as autotrophic
nitrifier-denitrification to the release of N\textsubscript{2}O is currently under discussion (Wrage-Mönnig et al. 2018).

The use of nitrification inhibitors (NIs) can raise fertilizer N use efficiency (NUE) in agriculture and thus reduce N surpluses. Commercially available NIs are substances capable of retarding the first step of nitrification—the oxidation of ammonia (NH\textsubscript{3}) to hydroxylamine (NH\textsubscript{2}OH). Besides the delay of nitrification, this also results in a reduction of the availability for further nitrite (NO\textsubscript{2}\textsuperscript{-}) oxidation to nitrate (NO\textsubscript{3}\textsuperscript{-}), thus lowering substrate supply for denitrification (Ruser and Schulz 2015). The use of NIs with ammonium (NH\textsubscript{4}\textsuperscript{+})-based fertilizers is supposed to keep NH\textsubscript{4}\textsuperscript{+} for a longer period in its reduced form; NH\textsubscript{4}\textsuperscript{+} is then mainly adsorbed to negatively charged soil particles in upper soil layers for a longer period, reducing NO\textsubscript{3}\textsuperscript{-} leaching losses.

Another way of increasing NUE in wheat (\textit{Triticum aestivum} L.) production is the splitting of N fertilization. Traditional N fertilization strategies for wheat comprise a splitting of fertilizer application in order to adapt N supply to the physiological needs of wheat plants. Wheat breeding schemes in the last decades have developed more flexible cultivars, which are less prone to yield losses through stress events and show a higher influence of N translocation during the grain-filling period than older cultivars (Makary et al. 2020). Due to these traits, wheat N uptake and N utilization of current cultivars is much higher when compared to older cultivars, and new fertilization strategies adapted to regional climatic and soil conditions must be developed. In a series of field experiments on medium to heavy textured soils in South Germany, Schulz et al. (2015) found no differences in grain yield or crude protein content when N fertilization was applied in one, two, or three application rates and soil mineral N (N\textsubscript{min}) contents did not differ after harvest. Since splitting can lead to lower soil N\textsubscript{min} contents throughout the vegetation period (Arregui and Quemada 2006; Guardia et al. 2018)—and since soil N\textsubscript{min} often correlates with N\textsubscript{2}O release (Granli and Bøckman 1994)—lower N\textsubscript{2}O emissions can be expected when N fertilizer is split.

3,4-dimethylpyrazole phosphate (DMPP) is a widely used NI in Europe, which releases 3,4-dimethylpyrazole (DMP) as active compound. Because of its chemical characteristics, DMPP cannot be sprayed on calcium ammonium nitrate (CAN), the most frequently used N fertilizer in Western and Central Europe in 2018 (23% of the N fertilizer market; IFA 2021). 3,4-dimethylpyrazole succinic acid (DMPSA) is a novel NI that also provides DMP as active inhibitor after the microbial degradation of succinic acid (Pacholski et al. 2016). Because of its non-polar chemical nature, DMPSA can be sprayed on CAN, increasing the scope of applicability of DMP (Pacholski et al. 2016). As CAN contains a higher portion of the highly mobile NO\textsubscript{3}\textsuperscript{-}-N than ASN, synchronization of an early high N demand of wheat and N availability right after fertilizer application can be better achieved when compared to ASN. Simultaneously, the NH\textsubscript{4}\textsuperscript{+}-N is stabilized and thus prevented from leaching.

Since weather and soil conditions as well as the presence of fresh organic matter in soil can greatly influence soil redox potential and therefore potential nitrification and denitrification, it seems rather impossible to predict N\textsubscript{2}O emissions induced by different fertilizer types. For example, Lebender et al. (2014a) reported similar N\textsubscript{2}O losses after application of ammonium sulphate (ASN) and CAN at two sites and higher losses in the AS treatment at a third site with different N\textsubscript{2}O emission patterns for the growth and post-harvest period.

In their meta-analysis on the effect of NIs on soil N\textsubscript{2}O emission, Ruser and Schulz (2015) calculated a 35% emission reduction as compared to a conventionally fertilized control treatment without NI. This was the mean N\textsubscript{2}O reduction over all NIs tested. For DMPP, they reported a 38% to 40% reduction. So far only few studies investigated the effect of DMPSA on N\textsubscript{2}O release. Under humid Mediterranean conditions, N\textsubscript{2}O emission from wheat fields was reduced by DMPSA (Huérfano et al. 2016). In an incubation experiment with DMPP and DMPSA, Torralbo et al. (2017) reported similar N\textsubscript{2}O reduction for both NIs.

Most of the field studies investigating DMPSA were conducted under Mediterranean conditions in irrigated systems with mild and rainy winters. In contrast, winter wheat production in South Germany is rainfed, although soils may dry very strongly in the summer months. Rewetting of dry soil in this period after heavy rainfall as well as thawing of frozen soil in winter were shown to induce N\textsubscript{2}O bursts highly relevant for annual balances (Flessa et al. 1995; Guzman-Bustamante et al. 2019). The latter can be significant in the context of NI application because some studies showed N\textsubscript{2}O reduction after DMPP usage a
long time after harvest in winter (Pfab et al. 2012; Guzman-Bustamante et al. 2019).

We aimed to quantify the effect of different N splitting strategies and of the NIs DMPSA and DMPP on N₂O emissions, yield, and N utilization in winter wheat under Southern Germany conditions. We hypothesized that (i) N₂O emissions from a winter wheat field can be decreased in a conventional N fertilization system with three N application rates when compared to a single N application since temporally high mineral N availability which serves as substrate for N₂O production is minimized. (ii) We also assumed lower Nₘᵢₙ concentrations that serve as substrate for N₂O production in the split NI fertilization treatments. Consequently, annual N₂O emissions can be even further mitigated than a single NI application without any decrease in crop yield or protein concentration; lastly, (iii) the NI DMPSA shows similar N₂O reduction under field conditions compared to DMPP, due to the same active compound (DMP).

Material and methods

Field experiment

From a fully randomized block experiment (Guzman-Bustamante et al. 2019) located at the experimental station of the University of Hohenheim “Heidfeldhof”, in Stuttgart, Germany (48° 42’ 59” N; 9° 11’ 42” E), 24 plots of 3 m x 5 m were selected. An overview of climatic conditions and soil properties is given in the supplementary online material. Plots were divided into a sampling and a harvest subplot (1.5 m x 5 m each). Gas and soil samples were taken from the sampling subplot, while the harvest subplot was used for grain and straw yield determination as well as for plant analysis (C and N). The winter wheat variety “Schamane” was sown 6 October 2011 after winter wheat as previous crop. Total fertilizer amount was 180 kg N ha⁻¹, calculated according to the German Fertilizer Ordinance (“good agricultural practice”, DüV, 2006). The first N application took place 29 March 2012 (BBCH 28) as CAN with or without DMPSA ([1]CAN and [1]CAN+DMPSA), or ammonium sulphate nitrate (ASN) with or without DMPP ([1]ASN and [1]ASN+DMPP). For the twofold application treatments, 108 kg N ha⁻¹ was applied as ASN+DMPP on 29 March 2012, with the second application of 72 kg N ha⁻¹ as ASN+DMPP on 23 May 2012 (BBCH 39) or CAN on 31 May 2012 (BBCH 49/51) ([2]ASN+DMPP and [2]ASN+DMPP/CAN). The threefold split treatment ([3]ASN/CAN/CAN) was fertilized on 29 March 2012 with ASN (54 kg N ha⁻¹; BBCH 28), and with CAN on 15 May 2012 (72 kg N ha⁻¹; BBCH 39) and 31 May 2012 (54 kg N ha⁻¹; BBCH 49/51). An unfertilized control treatment was included ([0]control). An overview of the treatments and N fertilization rates is given in the supplementary online material.

Soil N₂O fluxes

Between 6 March 2012 and 14 March 2013, gas measurements took place weekly, with additional sampling during high emission events (after N fertilization, after heavy rain, after tillage, and during freeze/thaw cycles) as recommended by Flessa et al. (2002). N₂O fluxes were determined using the closed chamber method, for which circular PVC bases with an inner diameter of 0.3 m and a height of 11 mm were installed at a depth of ca. 70 mm in the middle of the sampling subplot. In order to account for the growth of the wheat plants inside of the soil rings, additional PVC extensions of 0.3 or 0.6 m height were used during gas sampling in the vegetation period. Closed chambers and extensions were fitted on the rings only when measurements took place. A closer description of the dark chambers fitted with a vent and identical in construction to our chambers was provided by Flessa et al. (1995).

During each gas sampling, four gas samples were taken out of the chamber’s atmosphere with evacuated vials (22.4 mL) through a double cannula inserted into a sampling port with a septum in the chamber’s top at time intervals of 15 min. N₂O gas concentration in the vials was measured with a gas chromatograph (5890 series II, Hewlett Packard) equipped with a⁶³Ni electron capture detector (ECD) and an autosampler (HS40, Perkin Elmer). A linear regression (concentration enrichment over time) was used in order to calculate N₂O fluxes as described by Flessa et al. (1995).

Air temperature and precipitation data was retrieved from weather station “Hohenheim” located 600 m
Soil measurements

Soil samples were taken weekly from a composite sample of eight soil cores (0.3 m depth and 14 mm diameter) per treatment in the sampling subplot outside of the chamber base ring. Soil samples were kept cold in the field and frozen after field sampling until extraction in the lab. 40 g of soil were extracted with 160 mL of 0.5 M K₂SO₄ solution for one hour to determine N_min concentration. NO₃⁻ and NH₄⁺ concentrations in the extracts were measured with a flow injection analyser (3 QuAAtro.AQ2.AACE, SEAL Analytical, UK). Soil moisture was calculated gravimetrically after drying the samples at 105 °C for at least 24 h. Water-filled pore space (WFPS) was calculated after Ruser et al. (1998) using the mean measured bulk density (1.25 Mg m⁻³) in the A_p-horizon during our experimental period.

In order to assess the transport of N_min in deeper soil layers, soil in three depths (0–0.3; 0.3–0.6 and 0.6–0.9 m) was sampled at three dates: before fertilization (6 March 2012), after harvest (8 August 2012), and at the end of the experiment (14 March 2013). At the first date, samples were taken as a composite for each treatment; at the second and third dates, samples were taken separately in each plot. For calculation of N_min amounts, we assumed a bulk density of 1.5 Mg m⁻³ for the 0.3–0.6 m soil layer and 1.6 Mg m⁻³ for the 0.6–0.9 m layer.

The NH₄-N/NO₃-N ratio was calculated in order to follow inhibitory effect of treatments with NI.

Yield and plant analysis

All measurements on yield and yield components took place in the harvest subplot. Spike number per m² was calculated by counting the wheat spikes in a circular area of 0.6 m diameter. Wheat grain was harvested using a plot harvester. Straw and grain samples were taken for each subplot. Samples were dried for 48 h at 60 °C and ground using a cutting mill. C- and N- analyses were conducted with an elemental analyser (vario MAX CN, Elementar Analysensysteme, Hanau, Germany). Thousand grain mass (TGM) was determined gravimetrically after weighting 100 grain subsamples (n=3) counted by a seed counter (Contador, Pfeuffer GmbH, Kitzingen, Germany). N surplus—the balance between N fertilizer input and N removal through N in the harvest—was calculated subtracting grain-N from fertilizer-N.

Seasonal and annual N₂O emission and statistical analysis

Cumulative N₂O emissions were calculated using a step function, i.e. the flux at a given date was assumed to be constant until the next sampling date (Flessa et al. 1995). This was done for each “Season”, which represents the experimental time interval vegetation period (6 March—9 August 2012), tillage (10 August—29 November 2012) and winter (30 November 2012—21 March 2013), and for the whole experimental year.

Statistical analyses were performed with SAS (SAS Institute Inc., Cary, NC, USA). For N₂O fluxes, a repeated measures model was implemented using PROC MIXED with block, season, weekly dates (nested in season) and treatments as fixed effects, weekly date as repeated term with plot as subject and season as grouping variable. A spatial power correlation matrix was used in order to avoid serial autocorrelation and to consider differing sampling dates. For a better distribution of residuals, N₂O fluxes were transformed using the boxcox SAS Macro (Box and Cox 1964; Piepho 2017).

The effect of treatments and seasons on cumulative emissions was assessed using a repeated measures model with block, season and treatments as fixed effects, and season as repeated term, with plot as subject. An autoregressive correlation matrix was used. Effect of treatments on annual emissions and yield parameters were calculated with linear models. A logarithmic transformation was used when necessary to improve residual distribution.

The effect of different variables (soil NH₄, soil NO₃, soil temperature, WFPS and ΔWFPS) on N₂O fluxes was calculated using PROC GLMSELECT and PROC GLM with the Akaike information criterion (AIC value) as selection parameter. To improve residual distribution, all variables were log transformed. The interaction between N_min and use of NI was also assessed by including NI as a dummy variable. The relative importance of variables was calculated dividing the type I sum of squares of each variable by the sum of squares of the model.
Using PROC MIXED the effect of depth, date and treatment on soil NO$_3^-$ content was assessed using a repeated measures model, with depth and date as repeated terms and plot as subject. An autoregressive correlation matrix was used. Because the first soil sampling was done as composite, the model was used with the data of the second (after harvest) and third soil sampling (end of experiment, after winter).

Adjusted means were calculated using the LSMEANS and SLICE statements in PROC PLM, with letter display for pairwise comparisons at $\alpha=5\%$ using the Student–Newman–Keuls method for all linear models (Büchse and Zenk 2013). All graphs were done with the graphical R package ggplot2 (Wickham 2009).

Weather conditions

After sowing, precipitation summed up to 44 mm during October 2011. A dry November was followed by a mild, rainy winter with a median daily temperature of 3.9 °C and a total precipitation of 170 mm during December 2011 and January 2012. From the end of January, temperature dropped down (lowest mean daily temperature: -12.4 °C) without a snow cover for two weeks (S1, supplementary online material). Vegetation period started beginning of March with low precipitation and consequently low soil moisture (Fig. 1). To avoid drought stress due to the lack of rain, the field experiment was irrigated on 29 May 2012 with 17 mm. Precipitation was higher during June and July (172 mm), nevertheless, its clustered distribution led to dry soil conditions by the end of June (30% WFPS) and a rewetting event two days after (42% WFPS after 46 mm of rain). Before harvest, wheat plants showed signs for leaf rust infection—orange-red pustules on leaf surface.

After harvest ("tillage" season), precipitation continued while temperature dropped from 20 °C to 0 °C (end of November 2012), leading to higher soil moisture.
moisture (60% WFPS) at the beginning of the winter season (December 2012). The relatively harsh winter (104 days) was characterized by continuous precipitation with changing temperatures, nevertheless, in a small range (-7 to 11 °C) and with 21 ice days (daily maximum air temperature below 0 °C) (Fig. 1).

Results

N₂O fluxes and cumulative N₂O emission

Temporal dynamics and drivers of N₂O fluxes

Average N₂O fluxes before the first N application were 13 (± 11) µg N₂O-N m⁻² h⁻¹ (Fig. 2). After the first N application, only CAN treatment showed elevated fluxes (41 µg N₂O-N m⁻² h⁻¹) one week after N application. Two peaks with flux rates higher than 100 µg N₂O-N m⁻² h⁻¹ were registered in the CAN treatment in a period of rising temperatures and in conjunction with rainfall on 3 May 2012 and due to the irrigation, which had taken place two days before the second N₂O flux measurements on 31 May 2012. The other fertilized treatments showed rather low fluxes during the vegetation period, ranging from 3 to 68 µg N₂O-N m⁻² h⁻¹ and with a rise of fluxes in June and reduction at harvest.

After harvest, fluxes were high after each tillage event. With 98 µg N₂O-N m⁻² h⁻¹ in the ASN and ASN + DMPP treatment, highest flux in this period was measured after seeding (13 October 2012). During winter fluxes were low, ranging between 0 and 37 µg N₂O-N m⁻² h⁻¹ (Fig. 2).

Soil temperature was a main driver for N₂O fluxes (Table 3). A comparison of soil temperature and fluxes shows a similar course, with higher fluxes during the warmer period between May and September 2012 (Figs. 1 and 2). Soil NH₄-N and NO₃-N were the second and third main drivers for N₂O fluxes,
followed by the weekly change of WFPS (ΔWFPS) and WFPS (Table 3).

**Effect of N fertilization, splitting and N fertilizer type**

N fertilization significantly increased N₂O fluxes during the vegetation period, with treatment [1]CAN showing the highest fluxes (4.3 times higher fluxes compared to [0]control, Table 1). Fertilization significantly increased cumulative emissions only during vegetation period (Table 2).

The type of N fertilizer (ASN or CAN) did not significantly influence fluxes or emission. Nevertheless, higher cumulative emissions were observed during the vegetation period for [1]CAN (Table 2, also supplementary online material). Compared to a single application of CAN, [3]ASN/CAN/CAN treatment lowered N₂O fluxes by 11 µg N₂O-N m⁻² h⁻¹ (Table 1) and consequently also cumulative emission by 38% during the vegetation period (Table 2). Nevertheless, this effect did not have a repercussion on the cumulative annual emissions (p = 0.13).

Splitting of fertilization had a significant influence on the flux behaviour during seasons, with highest fluxes during the tillage period, followed by vegetation and winter period. In the single application treatments winter fluxes were significantly lower than during the rest of the seasons (Table 1).

**Effect of nitrification inhibitor**

Nitrous oxide fluxes were significantly reduced using DMPP and DMPSA, mostly during the vegetation period and, in the case of DMPP, also on an annual basis (Table 1). During the vegetation period [1]CAN+DMPSA reduced fluxes by 12 µg N₂O-N m⁻² h⁻¹, compared to [1]CAN, and [1]ASN+DMPP reduced fluxes by 10 µg N₂O-N m⁻² h⁻¹ compared to [1]ASN. The two highest emission peaks of the [1]CAN treatment (3 and 31 May 2012) were reduced by approx. 60% when DMPSA was used (Fig. 2).

This reduction of N₂O fluxes induced a reduction of cumulative N₂O emissions by 38% in treatments which used DMPSA and DMPP at the single

Table 1 Type 3 tests of fixed effects and back transformed adjusted means of significant effect “treatment” (annual) and significant interaction “season x treatment” for N₂O fluxes

| Effect                  | NumDF | denDF | F-value | p-value |
|-------------------------|-------|-------|---------|---------|
| Block                   | 3     | 799   | 0.27    | 0.8498  |
| Season                  | 2     | 327   | 86.07   | <0.0001 |
| Season × date           | 53    | 1463  | 12.70   | <0.0001 |
| Treatment               | 7     | 456   | 13.76   | <0.0001 |
| Season × treatment      | 14    | 450   | 2.45    | 0.0024  |

| Season¹                  | Vegetation period | Tillage | Winter | Annual |
|--------------------------|-------------------|---------|--------|--------|
| [0]control               | [µg N₂O-N m⁻² h⁻¹] |         |        |        |
| [1]CAN                   | 6.8D b            | 17.7B a | 7.7B b | 8.0D   |
| [1]CAN + DMPSA           | 29.4A a           | 27.7AB a| 10.1AB b| 17.7AB |
| [1]ASN                   | 17.5BC a          | 24.3AB a| 10.2AB b| 13.9BC |
| [1]ASN + DMPP            | 25.7AB a          | 31.5A a | 15.3AB b| 19.9A  |
| [1]ASN + DMPP/CAN        | 15.9C ab          | 20.4AB a| 9.6AB b | 12.3BC |
| [2]ASN + DMPP/CAN        | 17.2C ab          | 25.0AB a| 10.6AB b| 14.1BC |
| [2]ASN + DMPP            | 13.7C a           | 22.2AB a| 7.9B b  | 11.3C  |
| [3]ASN/CAN/CAN           | 18.6BC ab         | 26.9AB a| 11.9AB b| 15.3B  |

1. Adjusted mean N₂O fluxes followed by a common capital letter are not significantly different within treatments (Student–Newman–Keuls; α = 5%)
2. Adjusted mean N₂O fluxes followed by a common small letter are not significantly different within seasons (Student–Newman–Keuls; α = 5%)
Fig. 3  Course of the soil mineral nitrogen (N$_{\text{min}}$) amounts, as soil ammonium content (first three panels), soil nitrate content (panels four to six) and the ammonium to nitrate ratio (ammonium-N/nitrate–N; last three panels). Experimental time periods “vegetation period”, “tillage” and “winter” are represented as coloured blocks. White vertical lines represent N fertilization (solid = all treatments; dashed = only three application rates treatment), grey vertical dotted lines represent tillage events. Note inlets for each panel. The number of application rates is given in square brackets. ASN: ammonium sulphate nitrate; CAN: calcium ammonium nitrate; DMPP: 3,4-dimethylpyrazol phosphate; DMPSA: 3,4-dimethylpyrazol succinic acid

Table 2  Adjusted means for seasonal and annual N$_2$O cumulative emissions as affected by fertilization treatments (n = 4)

| Treatment | Vegetation period | Tillage | Winter | Annual |
|-----------|------------------|--------|--------|--------|
|           | [149 d]          | [119 d]| [112 d]| [380 d]|
| [0]control | 436$^{b\ ab}$   | 605$^{a\ b}$  | 282$^{a\ b}$ | 1322$^{c}$  |
| [1]CAN     | 1564$^{a\ a}$   | 911$^{A\ Ab\ b}$ | 375$^{A\ c}$  | 2850$^{A}$  |
| [1]CAN + DMPSA | 963$^{BC\ a}$ | 821$^{AB\ a}$ | 331$^{A\ b}$  | 2116$^{ABC}$ |
| [1]ASN     | 1223$^{B\ a}$   | 1089$^{A\ a}$  | 508$^{A\ b}$  | 2820$^{A}$  |
| [1]ASN + DMPP | 761$^{C\ a}$   | 765$^{AB\ a}$  | 359$^{A\ b}$  | 1886$^{BC}$ |
| [2]ASN + DMPP/CAN | 914$^{BC\ a}$ | 959$^{AB\ a}$ | 357$^{A\ b}$ | 2230$^{AB}$ |
| [2]ASN + DMPP | 759$^{BC\ a}$  | 805$^{AB\ a}$  | 319$^{A\ b}$  | 1883$^{BC}$ |
| [3]ASN/CAN/CAN | 963$^{BC\ a}$  | 895$^{AB\ a}$  | 414$^{A\ b}$  | 2271$^{AB}$ |

1. Adjusted mean N$_2$O cumulative emissions followed by a common capital letter are not significantly different within treatments (Student–Newman–Keuls; $\alpha$ = 5%)

2. Adjusted mean N$_2$O cumulative emissions followed by a common small letter are not significantly different within seasons (Student–Newman–Keuls; $\alpha$ = 5%)
application rate. At an annual cumulative basis, only ASN + DMPP independent of the number of applications significantly reduced emissions by 33% ([1] ASN + DMPP and [2]ASN + DMPP compared to [1] ASN).

Nitrous oxide fluxes of split treatments which included DMPP were in the same order of magnitude as [1]ASN + DMPP during vegetation period. When compared to [1]CAN and [1]ASN, these treatments significantly lowered fluxes during the vegetation period. Although this effect was not seen during tillage, in the case of [2]ASN + DMPP fluxes were significantly lower compared to [1]ASN also during winter (Table 1). This effect did not translate into lower cumulative emissions for the vegetation period; nevertheless, compared to [1]CAN and [1]ASN, treatments with split N application emitted less N₂O during vegetation period, with the [2]ASN + DMPP treatment emitting 51% less than [1]CAN and 38% less than [1]ASN (Table 2). On an annual basis, [2]ASN + DMPP performed as [1]ASN + DMPP and emitted 34% less than [1]CAN and 33% less than [1]ASN; but it did not differ from the [3]ASN/CAN/CAN treatment (Table 2).

Several logarithmized soil variables influenced N₂O flux rates, with soil temperature and NH₄-N and NO₃-N content being the most influential ones (Table 3). Positively correlated variables were soil temperature, NO₃-N, and ΔWFPS; NH₄-N and WFPS were negatively correlated with N₂O fluxes.

Soil N₉

Highest NH₄⁺ amounts in the upper soil layer (0–0.3 m) were measured after fertilization in the single application treatments with DMPP or DMPSA (Fig. 3). Highest NO₃⁻ amounts were found in the single application treatments and after the third CAN application in the [3]ASN/CAN/CAN treatment on 31 May 2012.

The highest NH₄⁺-N/NO₃⁻-N ratio was found in the [1]ASN + DMPP and [2]ASN + DMPP treatments during most of the vegetation period (Fig. 3). During tillage period the NH₄⁺-N/NO₃⁻-N ratio was < 1 for

| Variable | Estimate | Standard error | t Value | p value | Relative importance (%) |
|----------|----------|----------------|---------|---------|-------------------------|
| Intercept | 89.9 | 24.5 | 3.66 | 0.0003 | 23% |
| Log soil temperature | 3.4 | 1.1 | 3.25 | 0.0013 | 68 |
| Log NH₄ | −4.3 | 0.8 | −5.18 | <.0001 | 14 |
| Log NO₃ | 3.7 | 0.9 | 3.9 | 0.0001 | 12 |
| Log ΔWFPS | 5.4 | 2.2 | 2.42 | 0.0158 | 4 |
| Log WFPS | −22.8 | 6.1 | −3.74 | 0.0002 | 2 |

| Variable | Estimate | Standard error | t Value | p value | Relative importance (%) |
|----------|----------|----------------|---------|---------|-------------------------|
| Intercept | 85.5 | 24.6 | 3.48 | 0.0006 | 24% |
| Log soil temperature | 3.4 | 1.1 | 3.24 | 0.0013 | 65 |
| Log NH₄ × + NI | −3.9 | 1.0 | −3.98 | <0.0001 | 16 |
| Log NH₄ × − NI | −4.0 | 1.3 | −3.12 | 0.0019 | 12 |
| Log NO₃ × + NI | 3.2 | 1.1 | 2.9 | 0.0039 | 12 |
| Log NO₃ × − NI | 4.3 | 1.1 | 3.8 | 0.0002 | 5 |
| Log ΔWFPS | 5.6 | 2.2 | 2.51 | 0.0124 | 5 |
| Log WFPS | −22.1 | 6.1 | −3.62 | 0.0003 | 2 |
Before fertilization, the average NO$_3^-$ amount in the uppermost layer was 7.5 ($\pm$ 1.6) kg N ha$^{-1}$. After harvest, the median NO$_3^-$ amount in the upper layer was 49.8 kg N ha$^{-1}$. Highest NO$_3^-$ amounts after harvest were determined in the uppermost soil layer of the treatments [2] ASN + DMPP and [3] ASN/CAN/CAN (68.2 and 67 kg N ha$^{-1}$, respectively). In this layer, the only significant difference between treatments was found with amounts being higher in the treatments [2] ASN + DMPP and [3] ASN/CAN/CAN when compared to [1] ASN (Fig. 4).

After winter, [1] ASN + DMPP and [1] CAN + DMPSA showed the highest NO$_3^-$ amount in the 0.3–0.6 m soil layer (9.9 and 9.4 kg N ha$^{-1}$) whereas highest amounts in the [0]control and [1] ASN + DMPP treatment were recorded in the 0.6–0.9 m soil layer (17.1 and 17.0 kg N ha$^{-1}$) (Fig. 4).

Yield and yield components

Fertilization was a main driver for yield and yield components, with significant effects on grain and straw yield, spike number as well as on N related variables such as N concentrations in grain and straw (and N amount in these wheat fractions). Among fertilized treatments, the N amount in straw ranged between 25 and 33% of applied N fertilizer and N surplus varied only between 49 and 65 kg N ha$^{-1}$ (Fig. 5). Among the fertilized treatments, a single application of ASN yielded 21.4% more grain and
had 32% more spikes per m² than the traditional [3] ASN/CAN/CAN treatment.

Crude protein content in grain was mainly affected by splitting, with the highest protein content in the [3] ASN/CAN/CAN treatment and decreasing protein content with less N applications (Table 4). Within fertilized treatments, N₂O emission per grain-N in the [2] ASN + DMPP treatment was 38% lower than in treatment [1] CAN.

Discussion

Main drivers for N₂O release

The positive correlation between temperature and N₂O flux rates can be explained not only by a direct effect of temperature on enzymatic activity, but also by an increased soil anaerobiosis after stimulation of soil respiration (Butterbach-Bahl et al. 2013). We found a negative correlation between the N₂O flux rates and the NH₄⁺ contents, which were mainly high after fertilizer application. This might be a hint on nitrification as the main N₂O source in this period. However, we cannot exclude denitrification as another relevant N₂O source, since NO₃⁻, the end product of nitrification, serves as a substrate for denitrification. This was indicated by a positive correlation between N₂O flux rates and NO₃⁻ contents. Using a stable isotope approach, Ruser et al. (2006) reported a contribution of denitrification of up to approx. 66%, at a low soil moisture (40% WFPS) in a soil similar in soil texture and humus content. They found this high ratio of denitrification to the total N₂O flux especially after the rewetting of dry soil, and explained this phenomenon by inferring that increased oxygen consumption and microbial growth after rewetting was due to an enrichment of easily available carbon under dry soil conditions, which induced anaerobiosis even at low soil moisture. This would also explain the positive correlation between N₂O fluxes and the change of soil moisture (ΔWFPS) between two sampling dates.

Soil tillage also stimulated N₂O flux rates. As summarized by Guzman-Bustamante et al. (2019), tillage increases C turnover in soil aggregates, nitrification and denitrification potential and enhances C and N availability of crop residues. Similarly, increased N₂O fluxes after tillage have also been reported e.g., by Mutegi et al. (2010) after winter barley harvest and by Lebender et al. (2014b) after winter wheat harvest.

Use of NI diminished the slope of Log NO₃⁻ (Table 3) indicating that DMP based NIs were

Fig. 5  Mean straw-N and grain-N as affected by N fertilization strategies (n = 4). N balance is given in the bottom of the bars. Error bars indicate standard error of the model mean estimates. Mean values followed by a common letter are not significantly different within variable (Student–Newman–Keuls; α = 5%)
able to lower N$_2$O fluxes by decreasing NO$_3^-$ availability as a substrate for denitrification.

Although moisture plays a predominant role in triggering N$_2$O production, by filling soil pores with water thus limiting oxygen diffusion and consequently stimulating denitrification (Flessa and Beese 1995; Roman-Perez and Hernandez-Ramirez 2021), we found a negative correlation between WFPS and N$_2$O fluxes (Table 3). A negative correlation of these variables during the growing season was also found by Vitale et al. (2013), who hypothesized a limiting effect of high soil moisture on nitrification. Analog to the correlation between NH$_4^+$ and N$_2$O fluxes, WFPS in our study was higher during periods of time when other conditions were limiting, i.e., during winter, when WFPS reached $\approx$70%, but NO$_3^-$ contents ranged only between 0.9 and 7.8 g N kg soil$^{-1}$. Additionally, Guzman-Bustamante et al. (2019) reported a temporal C limitation during the cropping season of winter wheat at the same study site and at the same time as the measurements presented here, overwriting moisture effects on N$_2$O flux rates. As pointed out by Granli and Bøckman (1994), fertilizer application, as a seasonal operation, which takes place when temperature is high and—in the case of South Germany—in periods with most precipitation, can mask the effect of soil-physical variables on N$_2$O fluxes and complicates the interpretation of field studies.

### Effect of N fertilization and N splitting

Annual N$_2$O emissions of [1]CAN and [1]ASN (2.85 and 2.82 kg N$_2$O–N ha$^{-1}$ a$^{-1}$) were in accordance with the range of N$_2$O emissions reported by Kaiser and Ruser (2000) who reported a mean N$_2$O emission of 2.8 kg N$_2$O–N ha$^{-1}$ a$^{-1}$ from 14 field experiments with wheat in Germany. Lebender et al. (2014b) found lower annual emissions at two sites in Germany cropped with winter wheat at the same study site and at the same time as the measurements presented here, overwriting moisture effects on N$_2$O flux rates. As pointed out by Granli and Bøckman (1994), fertilizer application, as a seasonal operation, which takes place when temperature is high and—in the case of South Germany—in periods with most precipitation, can mask the effect of soil-physical variables on N$_2$O fluxes and complicates the interpretation of field studies.
straw biomass then usual (Guzman-Bustamante et al. 2019). The high straw biomass together with enhanced N concentrations in the straw induced relatively high N amounts in the straw remaining on the field after harvest (ASN: 32%; CAN: 27% of applied fertilizer N). Tillage operation promoted mineralization of wheat straw and a rapid nitrification resulted in increased soil NO$_3^-$ amounts in the tillage period, thus, as indicated by the positive correlation between N$_2$O release and NO$_3^-$ availability, stimulating N$_2$O production from denitrification. These high N$_2$O flux rates in the tillage phase were the reason for the high contribution of the emissions during the tillage period to the annual emissions (ASN: 57%; CAN: 45% of annual N$_2$O emissions after harvest).

Although the type of fertilizer can alter N$_2$O emissions (Shcherbak et al. 2014), we did not find statistical differences between the annual emissions of [1] CAN and [1]ASN. Despite lower N$_2$O emissions during the vegetation period for [1]CAN treatment, high fluxes of [1]ASN during tillage and winter offset the N$_2$O mitigation during vegetation period, indicating the need for whole annual measurements for the evaluation of N$_2$O reduction strategies. Similar results were reported from measurements in potatoes (Ruser et al. 2001) and in winter oilseed rape (Kesenheimer et al. 2021). The [1]ASN treatment contained more NH$_4^+$-N than [1]CAN (CAN: 50% as NO$_3^-$N, 50% as NH$_4$-N; ASN: 29% as NO$_3^-$N, 71% as NH$_4$-N). Desorption of higher amounts of fertilized NH$_4^+$ from clay minerals might have postponed N availability and the resulting substrate supply for N$_2$O production into the post-harvest period in the [1]ASN treatment (Lebender et al. 2014a).

Increasing NO$_3^-$ and low NH$_4^+$ amounts during the tillage period indicate a rapid nitrification of mineralized N from N-rich straw. The turnover of easily degradable carbon fractions of the straw such as cellulose and hemicellulose might also have further contributed to O$_2$ consumption, thus increasing anaerobic conditions favouring denitrification and enhancing N$_2$O fluxes after harvest.

A comparison with other field experiments shows that grain yield of our fertilized treatments was rather low, between $-$16% and $-$26% (Pasda et al. 2001; Schulz et al. 2015). The reasons for the low yields might be related to year, as Makary et al. (2020) also reported low grain yields in the same experimental year. They attributed this result to the exceedingly warm winter, which led to an unfavourable high tiller density in spring.

A comparison between the single application in [1]CAN and the traditional threefold application in [3]ASN/CAN/CAN showed significantly decreased fluxes and 38% less emissions during the vegetation period in the traditional split fertilization treatment. Although statistically not significant on an annual basis, the t-test comparison of the annual N$_2$O emission between the treatments with single and three applications was very close to statistical significance ($p=0.056$) and can at least be considered a substantial trend.

Possible reasons for lower N$_2$O emissions with increasing number of N splitting compared to a single application rate were (i) the generally lower soil NO$_3^-$ contents in the treatments with fertilizer splitting and especially during the time of the first N$_2$O peak after rain, and (ii) the fact that fertilizer granules were only slowly dissolved due to relatively low soil moisture following the second and third application. As discussed by Knittel et al. (2007), the later fertilization occurs, the higher the probability that soil might be too dry for fertilizer granules to be dissolved.

The high soil NO$_3^-$ amounts in the split treatments after the second and third application did not induce enhanced N$_2$O fluxes; this might be the result of the low soil moisture and the corresponding good aeration in this period which limited denitrification. Mainly because of different precipitation patterns and the occurrence of heavy rainfall events after N applications, the success of splitting as a N$_2$O reduction strategy can strongly vary as shown by Guardia et al. (2018) and others.

In our experiment, grain yield and quality were influenced by splitting of the N-fertilizer with higher grain yield in the treatment without splitting when compared to the traditional fertilization with three application rates and higher crude protein contents in treatments with split application. Neither Schulz et al. (2015) nor Makary et al. (2020) found differences in yield or N content for split N fertilization on similar study sites in Southwest Germany. Both recommended to consider one single CAN application in a late (shooting) stage when modern wheat varieties are grown on soils with low NO$_3^-$ leaching during the growing season. In contrast, our results recorded under
unusually dry conditions (19% lower rainfall from March to July when compared to the long-term annual mean) seem to be more similar to the ones reported under Mediterranean conditions: we observed slightly higher grain yields with one application rate (Guardia et al. 2020) and a higher N grain content when N fertilizer was split (Ercoli et al. 2013). Yield components such as spike number and TGM followed a similar trend as found by Pasda et al. (2001), with smaller spike numbers and higher TGM when N fertilizer was split. Since results from a previous experiment on the same field showed higher grain yields for a fertilization with three application rates (Guzman-Bustamante et al. 2012) and no difference between protein contents (data not shown), the comparatively milder winter and dryer vegetation period together with the high N amount might have driven spike numbers on [1]ASN and [1]CAN and so elevated competition among wheat plants and decreased grain yield (Maidl et al. 1998). In this sense, split fertilization was not able to contribute to yield formation, since the spike number was too high (Scharf and Alley 1993).

Effect of nitrification inhibitors

Both NIs in our study reduced the mean annual N₂O emission (DMPSA: 26%; DMPP: 33%), with the reduction for DMPP being statistically significant. For [1]CAN+DMPSA the tillage operation after harvest might have masked N₂O reduction during the vegetation period (Corrochano-Monsalve et al. 2020).

Similar reduction potentials for DMPP and DMPSA were reported for field studies by Ruser and Schulz (2015) and by Huérfano et al. (2016). The reduction of N₂O emissions after the application of ammonium containing fertilizers with NIs was explained directly by lower N₂O production during nitrification as well as indirectly by the lower substrate availability for denitrification (Ruser and Schulz 2015). Additionally, Torralbo et al. (2017) detected an increased N₂O reduction during denitrification after NI application which also decreased net N₂O release from soil.

A direct comparison between DMPP and DMPSA cannot be drawn with our dataset, as we used different N fertilizers for the two inhibitors. Differences between the two products (ASN+DMPP vs. CAN+DMPSA) might result either from different efficiencies of the inhibiting compounds or from the different share of NH₄⁺ and NO₃⁻ in CAN and ASN. The latter was reflected by the soil NH₄-N/NO₃-N ratio, which was higher in the ASN+DMPP treatment (vs. ASN) for approximately 3.5 months, whereas it did not differ that clearly for CAN+DMPSA (vs. CAN).

Twofold split application of ASN+DMPP treatment performed similarly to a single application of ASN+DMPP leading to 33% lower annual N₂O emissions compared to a single application of ASN. One of the reasons for this reduction was the same as for the [3]ASN/CAN/CAN treatment: lower soil NO₃⁻ amounts were registered for split treatments during periods with conditions favourable for high N₂O production.

Similarly to our results, splitting NI fertilizers did not further mitigate N₂O emissions compared to a single N application under Mediterranean conditions (Huérfano et al. 2016; Corrochano-Monsalve et al. 2020), because soil conditions during the second fertilizer application were not favourable for N₂O production (WFPS < 48%). Contrarily, if the second NI application occurs when denitrification conditions are optimal (high water content and high soil temperature), high N₂O fluxes might raise emissions to the same level as soil fertilized without NI (Huérfano et al. 2015).

Despite lower N₂O flux rates in the [2] ASN+DMPP treatment during winter, cumulative N₂O emissions from [2]ASN+DMPP and [1]ASN were not significantly different. The lower N₂O flux rates in the [2]ASN+DMPP treatment might hint on long-term effects of NIs on N transformation processes in soil. A significant effect was shown by Pfab et al. (2012) and Guzman-Bustamante et al. (2019) for the same study site as in our experiment. The reasons for possible long-term effects on N₂O emissions as reported by Pfab et al. (2012) and Guzman-Bustamante et al. (2019) from our study site as well as DMPP-induced changes in microbial function diversity in a study site in Italy (Tedeschi et al. 2020) clearly show the need for further verification.

In this regard, determination of the inhibiting compound and metabolites might be interesting, since it was shown that approx. 16% of DMPP were still present in a topsoil under winter wheat
at the end of the vegetation period (Benckiser et al. 2013).

In terms of winter wheat yield and quality, our results agree with Pasda et al. (2001) and Huér-fano et al. (2015), who did not find an effect of split NI on winter wheat grain yield, whereas protein content was increased in a twofold ASN + DMPP application compared to all single application treatments. Since our single application treatments with and without NI were all in a lower crude protein class (≈ 11.2%) compared to the treatments with split application (12.4–14%), splitting seems to be the main factor influencing crude protein in wheat grain as discussed before.

As enhanced-efficiency fertilizers are more expensive than regular mineral fertilizer, its use might not be profitable in a wheat system. From a climate protection point of view, farmers could waive its use when an appropriate N fertilization management is implemented (Li et al. 2018). However, due to expiration of patent protections, NI-containing fertilizers became cheaper on the European market in the last years, and an economical re-evaluation of the use of NIs seems worthwhile.

Conclusion

Our first hypothesis—that a threefold split N application can decrease N₂O emission compared to a single N application—can be partially corroborated (p < 0.1) as [3]ASN/CAN/CAN reduced annual N₂O emission compared to one application of CAN and ASN. The second hypothesis—that split application of a NI fertilizer can further mitigate N₂O emissions, compared to a sole NI application—must be rejected, as N₂O emission levels of both split NI treatments ([2]ASN + DMPP and [2]ASN + DMPP/CAN) showed the same emission levels as a single application of ASN + DMPP. Nevertheless [2] ASN + DMPP contributed to significantly higher grain protein content. Our third hypothesis—that DMPSA used with CAN shows a similar N₂O reduction as ASN + DMPP—must be rejected as well, since a single application of CAN + DMPSA mitigated N₂O emissions from CAN only during the vegetation period but not on an annual basis. Only DMPP was able to lower N₂O fluxes during the vegetation period and winter, thus mitigating annual emissions. Our results support the splitting of N fertilizer in order to achieve high grain quality when appropriate wheat varieties are sown by simultaneously lowering N₂O emissions. As a result of climate change, precipitation patterns (with more heavy rain events during the cropping season) will change more frequently in the future. Such strong rainfall events can trigger N₂O production after N application, and thus the use of DMP-based nitrification inhibitors could be a powerful tool to mitigate N losses in these periods. Future studies should focus on the effects of DMPSA on N transformation in soils, especially after harvest. Determination of long-term effects on nitrification and probably also on denitrifiers may help to improve our understanding in this context.

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Data availability and material Data can be made available on reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest None.

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