The scarcity and distribution of rainfall drove the performance (i.e., mitigation of N oxide emissions, crop yield and quality) of calcium ammonium nitrate management in a wheat crop under rainfed semiarid conditions

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ABSTRACT

Selection and management of synthetic nitrogen (N) fertilizers, including nitrification inhibitors, are considered an effective strategy to mitigate N oxide emissions, but depends strongly on soil characteristics and climatic conditions. We evaluated the effect of the use of a nitrification inhibitor (2-(3,4-dimethyl-1H-pyrazol-1-yl) succinic acid isomeric mixture (DMPSA)) or splitting calcium ammonium nitrate (CAN) on the N oxides and CH₄ emissions, yield, N use efficiency (NUE) and bread-making quality in a winter wheat (*Triticum aestivum* L.) crop. During a typical rainfall cropping season, neither splitting CAN nor using DMPSA reduced significantly the area-scaled or yield-scaled emissions in comparison to a single application of CAN without inhibitors. This could be explained by the low emissions in CAN amended plots. Conversely, in the subsequent extremely dry cropping season, higher N oxide peaks occurred after soil rewetting and the effectiveness of DMPSA was significant in these conditions (e.g. 83% mitigation for NO). No improvement in the NUE, yield or bread-making quality was achieved with a split application or DMPSA added to the fertilizer. Under the conditions of the study, splitting CAN should not be recommended from a yield-scaled emissions viewpoint, while the use of DMPSA may be encouraged due to large variability in the amount and distribution of rainfall.

Introduction

Low nitrogen (N) efficiency in agricultural soils is an issue of economic and environmental concern (Sutton et al. 2013). The N lost from the agroecosystems pollutes groundwater resources via nitrate leaching (NO₃⁻) and the atmosphere via volatilization of ammonia (NH₃) and emission of N oxides (nitrous oxide, N₂O, and nitric oxide, NO). Nitrogen oxides have an important effect on direct radiative forcing, directly (N₂O, which is a potent greenhouse gas, GHG) or indirectly (NO, as a precursor of tropospheric ozone, O₃) (Menon et al. 2007; IPCC 2014). Furthermore, N₂O is

considered the main stratospheric anthropogenic O₃ depleting emission (Ravishankara et al. 2009), whereas NO is also involved in the formation of acid rain through its oxidation (Pilegaard 2013). The production of NO also has negative effects on human health because it causes an increase in the concentration of atmospheric aerosols (Compton et al. 2011).

The pivotal target of decreasing the emissions of N oxides from agricultural soils requires the choice of cost-effective mitigation strategies adapted to each specific cropping region (Sanz-Cobena et al. 2017; Carswell et al. 2019). In this sense, the management of N fertilization has been identified as one of the most promising strategies (Xia et al. 2017; Winiwarter et al. 2018). Semi-arid regions (e.g. Mediterranean) are characterized by low N₂O fluxes (Cayuela et al. 2017), particularly due to the limited weight of N₂O emissions from denitrification (Guardia et al. 2018a). This condition is caused primarily by a low soil moisture content, whereas a low organic carbon (C) concentration and common basic/calcareous soils have also been proposed as significant drivers (Mørkved et al. 2007; Aguilera et al. 2013). Thus, the use of calcium ammonium nitrate (CAN) instead of urea (100% of ureic N) could be an effective N oxide mitigation strategy (Volpi et al. 2017), because half of the N is applied through a nitric (N-NO₃⁻) form, thus supplying the substrate for denitrification. Moreover, the use of urea has been discouraged mainly in countries, such as Spain, where NH₃ emissions are above the committed targets (Sanz-Cobena et al. 2014), so substituting urea by CAN is expected to cause a win-win effect regarding N₂O and NH₃. Due to the lower relevance of denitrification under rainfed semi-arid conditions, the mixed application of NO₃⁻-N and ammonium (NH₄⁺-N) using CAN could also lead to a higher N availability and therefore be a more effective strategy for crop growth (Hagin et al. 1990). Whether the N oxide emissions from CAN under rainfed semi-arid conditions could be further reduced through fertilizer technologies or management of application timing has not been satisfactorily addressed yet.

The use of nitrification inhibitors (NIs) could improve the performance of CAN regarding N oxide mitigation or N efficiency enhancement, as suggested by several meta-analyses (Qiao et al. 2015; Thapa et al. 2016). Dicyandiamide (DCD) and 3, 4-dimethylpyrazole phosphate (DMPP) are two of the most used NIs worldwide (Yang et al. 2016). However, the use of DCD is under discussion due to plant uptake and traces in food (Pan et al. 2016). With respect to DMPP, the new inhibitor 2-(3,4-dimethyl-1H-pyrazol-1-yl) succinic acid isomeric mixture (DMPSA) allows the combination of DMP with CAN (Guardia et al. 2018a), whereas DMPP is not stable with basic-reaction fertilizers as CAN thus leading to DMP volatilization. Compared to DMPP, DMPSA showed similar inhibition efficacy under humid Mediterranean conditions when applied with ammonium sulphate (Huérffano et al. 2016). Both NIs are not taken up by plants at normal doses, and even at high rates only traces of DMPSA could be found in roots, but not in aboveground biomass (Rodrigues et al. 2018). The N oxide mitigation efficacy of DMPSA with CAN has also been demonstrated under irrigated (Guardia et al. 2017a, 2018a; Recio et al. 2018a) and rainfed conditions (Recio et al. 2018b). However, the effectiveness of NIs such as DMPP is highly dependent on rainfall amount and distribution in rainfed semi-arid crops (Abalos et al. 2017). Therefore, whether DMPSA with CAN is effective under rainfed semi-arid conditions in seasons with contrasting precipitation patterns (considering that CAN without NIs leads to low N oxide emissions, Zhang et al. 2016), still remains unanswered.

Enhanced efficiency fertilizers (including NIs) were developed to improve the synchronization between N supply and N acquisition by plants. Therefore, the comparison between the use of NIs and splitting the fertilizer without an inhibitor is also an issue of interest, since the management of N timing ('right time') is one of the pillars of an effective N fertilization regarding mitigation of N losses (Good and Beatty 2011).

Evaluating different fertilization strategies, such as the N source (including the use of NIs) or timing of application, at a farm scale requires not only the measurement of N emissions but also a consideration of the effects on crop yield and quality, which is affected by N fertilization management (Massoudifar et al. 2014). In the case of bread-making wheat (*Triticum aestivum* L.), the protein concentration and composition is influenced by the NH₄⁺ and NO₃⁻ supply and their availability in time (Fuertes-Mendizábal et al. 2013). Therefore, the delay in the oxidation of NH₄⁺ could affect some of

these parameters related to bread-making quality (gluten strength, elasticity, extensibility). To date, little information exists about the effect of NIs on bread-making quality in cereal crops under rainfed semi-arid conditions. Recio et al. (2018b) measured the SDS-sedimentation (SDSS) volume test, which is an indirect measurement of gluten strength, obtaining similar values with and without DMPA. The evaluation of bread-making quality through reserve protein composition analysis (i.e. gliadins and glutenins) is needed to assess thoroughly the economic and environmental performance of NIs.

The objective of this study was to evaluate the effect of CAN, split CAN and CAN+DMPA on the N oxide emissions, yield, bread-making quality and N use efficiency (NUE). Methane (CH₄) emissions from soils were also measured to calculate the carbon dioxide equivalent (CO₂-eq) emissions. We hypothesized that DMPA would be effective at mitigating N oxides when applied together with CAN under rainfed semi-arid conditions, without detriment to the yield or bread-making quality.

Materials and methods

Site description

We conducted a two-year field experiment at *Centro Nacional de Tecnología de Regadíos*, 'CENTER' (latitude 40°25'1.31"N, longitude 3°29'45.07"W) in the Madrid region (Spain). The soil was a *Typic xerofluvent* (USDA, Soil Survey Staff 2014) with a silty-loam texture: 8.8% clay, 55.9% silt (0.05–0.002 mm), and 35.4% sand (0.05–2 mm). The main properties of the upper horizon (0–20 cm) of the soil were: bulk density, 1.23 Mg m⁻³; pH (H₂O), 8.1; organic matter (Walkley-Black), 17.4 g kg⁻¹; CaCO₃, 13.1 g kg⁻¹. In the last 10 years, mean air temperature and cumulative annual rainfall were 14.1°C and 393 mm, respectively. A meteorological station located at the field site provided the meteorological data (e.g. rainfall, soil temperature), which is available at <http://eportal.mapama.gob.es/websiar/Inicio.aspx>

Experimental design and management

The experiment was carried out from October 2015 to October 2017, thus including two wheat cropping seasons: year 1 (2015/2016) and year 2 (2016/2017). The experiment was arranged in a complete randomized block design with three replications laid out in three blocks. Each plot covered an area of 64 m² (8 m × 8 m). The fertilizers were applied at a rate of 120 kg N ha⁻¹. The different fertilizer treatments were: 1) CAN; 2) CAN+DMPA; 3) CAN split (SCAN) in two applications (60 kg N ha⁻¹ + 60 kg N ha⁻¹); and 4) control with no N fertilization (control). The DMPA was applied at a rate of 0.8% of the NH₄⁺-N. All fertilizers were provided in granular form by EuroChem Agro and broadcasted over the soil surface at the tillering stage (i.e. end of February in both years). In the SCAN plots, the second N fertilization took place at the stem elongation stage (i.e. approximately 1 month after first dressing application). No phosphorus (P) or potassium (K) seeding fertilization was applied because the concentrations (Olsen P, 26.4 mg kg⁻¹; available K extracted in ammonium acetate pH = 7.0, 310 mg kg⁻¹) were high for both nutrients considering the soil and management conditions of this study. The field was seeded with winter wheat (*Triticum aestivum* L. var 'Ingenio') in 2015 and *Triticum aestivum* L. var 'Marcopolo' in 2016 at 210 kg ha⁻¹, between mid-October and mid-November. 'Ingenio' was a widespread cultivar used in the area, while in the second year a new bread wheat genotype with higher grain protein concentration ('Marcopolo') was seeded, since we expected that a greater response of N management in protein concentration and composition would be observed. After seeding and after harvesting, a cultivator labor (shallow ploughing 0–15 cm) was carried out.

Sampling and measurement of GHG and NO

Gas samples were taken 2–3 times per week during the first month after N application. Afterwards, the frequency of sampling was decreased progressively but it was never less than 15 days, increasing after rainfall events. Nitrous oxide and CH₄ fluxes were measured through closed opaque chambers

(placing one chamber per plot). The chambers (volume 22 L, diameter 35 cm and height 23 cm) were hermetically closed for 1 h, by fitting them into stainless steel rings, which had been inserted (at the beginning of the study) into the soil to a depth of 10 cm to minimize the lateral diffusion of gases. Gas samples were taken at the same time of day (10–12 am) in order to minimize any effects of diurnal variations in the emissions, and placed in 20 ml pre-evacuated vials at 0, 30 and 60 min. The concentrations of GHG fluxes were quantified with a gas chromatograph equipped with an electron-capture detector (ECD) and a flame-ionization detector (FID). More details about GHG sampling and analyses can be found in Guardia et al. (2018a).

On the same days as the GHG measurements, the NO fluxes were analyzed employing a gas flow-through system using also one chamber per plot. Nitric oxide was analysed using a chemiluminescence detector (AC31M-LCD, Environment S.A., Poissy, France), and the NO fluxes were calculated as explained in Abalos et al. (2014).

Plant and soil analyses

At grain maturity, 50 plants within each plot were randomly harvested to determine the total N concentration of grain and straw, which were analyzed with a TruMac CN Leco elemental analyser. Afterwards, a plot combine was used to harvest the wheat in mid-June during both years. The modified Osborne method (Bean et al. 1998; Bean and Lookhart 2000) was used to extract sequentially grain gliadins and glutenins. The analysis of the samples was done using a Beckman ® 2100 P/ACE system as described in Guardia et al. (2018b). Three soil cores (0–10 cm) per plot were taken 1–2 times per week to determine the moisture content (expressed as water-filled pore space, WFPS), NH_4^+ -N and NO_3^- -N concentrations. The soil NH_4^+ -N and NO_3^- -N concentrations were extracted and analysed by a colorimetric method using a flow injection analyzer as explained in Montoya et al. (2018). The soil WFPS was estimated as explained in Abalos et al. (2012), previously measuring the gravimetric water content by oven-drying the soil samples.

Calculations and statistical analysis

Linear interpolations between sampling dates were carried out to calculate the cumulative gas emissions. The global-warming potential (GWP) of N_2O and CH_4 emissions was expressed as CO_2 -eq using factors of 265 and 28 for N_2O and CH_4 , respectively (IPCC 2014). The greenhouse gas intensity (GHGI) and yield-scaled NO emissions (YSNO) were calculated as the ratios of CO_2 -eq (i.e., GWP) or NO emissions to grain yield. The N_2O and NO emission factors (EFs), which were used to calculate the N oxide mitigation percentages, were calculated as the difference between N_2O emissions from a fertilized treatment and the control treatment divided by the rate of synthetic N applied. The NUE (i.e. the proportion of synthetic N applied that was taken up in above-ground biomass of the crop) and N surplus (i.e. the difference between synthetic N applied and aboveground N uptake) were estimated as in explained by Van Groenigen et al. (2010).

All data were checked for assumptions of data distribution normality (Shapiro-Wilk) and variance uniformity (Levene), and log-transformed when necessary. Analyses of variance were carried out with block as a random factor and fertilizer as a fixed factor. The Least Significant Difference (LSD) test was used for multiple comparisons between means at $P < 0.05$. All statistical analyses were performed using *Statgraphics Plus 5.1*.

Results

Environmental conditions and soil mineral N

The total rainfall (Figure 1) over the wheat cropping cycle was 319 mm and 272 mm for years 1 and 2, respectively. In the February–July period, the precipitation accounted for 243 mm and 135 mm for

Figure 1. Daily soil temperatures (at a 10 cm depth) and rainfall distribution throughout both years.

years 1 and 2, respectively (Figure 1, S1). Average soil WFPS values during this period (Figure 2a,b) were 32.6% (11% to 60%) and 27.7% (3% to 50%) in years 1 and 2, respectively (Figure 2).

Marked peaks in both NH_4^+ and NO_3^- concentrations were observed after fertilization events. Generally, CAN+DMPA increased the mean NH_4^+ concentrations (Figure 3a,b) and decreased those of NO_3^- (Figure 3c,d) in comparison to CAN, although differences were only significant ($P < 0.05$) during stem elongation in year 2 (increase in NH_4^+ concentration, Figure 3b) and after harvesting in year 2 (decrease in NO_3^- concentration, Figure 3d). The effect of DMPA increasing soil NH_4^+ concentrations was maintained during approximately 1 month in year 1, being more long-lasting during year 2 (when a significant increase in soil NH_4^+ was observed on 17th May, i.e. 83 days after fertilization, data not shown). The use of DMPA increased the average NH_4^+ concentrations by 14–33% and decreased those of NO_3^- by 16–18%. These differences in average mineral N concentrations were only significant ($P < 0.05$) during the second year. A split application of CAN generally resulted in lower mineral N concentrations than CAN until flowering, and the opposite tendency was observed thereafter (except for NO_3^- concentrations in year 2, which maintained higher values in CAN after flowering). Mean NH_4^+ concentrations during the tillering stage were lower in year 2 than in year 1. Regarding NO_3^- concentrations, these were markedly higher after flowering in year 2 (Figure 3d) than in year 1 (Figure 3c). Specifically, the average NO_3^- concentration after harvesting was $37.2 \text{ mg N kg soil}^{-1}$ in year 2, much higher than that of the previous year ($<10 \text{ mg N kg soil}^{-1}$).

Gaseous fluxes

N oxide emissions

Average N_2O emission was $0.07 (-0.10 \text{ to } 0.29) \text{ mg N m}^{-2} \text{ d}^{-1}$ and $0.14 (-0.14 \text{ to } 0.62) \text{ mg N m}^{-2} \text{ d}^{-1}$ in years 1 and 2, respectively (Figure 4). The split application of CAN led to the highest N_2O emissions in both years, being statistically (year 1) and numerically (year 2) higher than those from CAN (Table 1). The use of DMPA led to similar N_2O losses as those of CAN during year 1. In year 2, CAN+DMPA tended to decrease cumulative N_2O emissions in comparison to CAN (by 32%) but this result was not statistically significant ($P > 0.05$). Average emissions during year 2 surpassed those of year 1 (by 37%) primarily due to the

Figure 2. Soil WFPS (0–10 cm) in years 1 (a) and 2 (b). Vertical bars indicate the standard errors ($n = 3$).

increases after rainfall events in May (Figure 4b) and a peak that was observed after the harvest (mid-October). Emissions at this peak reached on average 0.50 (0.36 – 0.62) $\text{mg N m}^{-2} \text{d}^{-1}$ and no significant differences between treatments were reported ($P > 0.05$).

Average NO emissions were 0.75 (-0.36 to 6.13) and 1.15 (-1.31 to 17.42) $\text{mg N m}^{-2} \text{d}^{-1}$ in the first and second year, respectively (Figure 5). During the first year, the greatest increases were observed after fertilization events, and cumulative NO emissions were statistically similar in all CAN-based treatments. The tendency was similar during the second year, but emissions were markedly enhanced after rainfall events in May (Figure 5b) in CAN and – particularly – SCAN treatments. As a consequence, cumulative NO fluxes were reduced in the order $\text{SCAN} > \text{CAN} > \text{CAN} + \text{DMPA} = \text{control}$.

Methane emissions

Average CH_4 fluxes were -0.15 (-1.62 to 2.06) $\text{mg C m}^{-2} \text{d}^{-1}$ and -0.13 (-1.86 to 1.32) $\text{mg C m}^{-2} \text{d}^{-1}$ in years 1 and 2, respectively (data not shown). Negative CH_4 fluxes (i.e. net CH_4 sink) were reported at most sampling dates, although net CH_4 emissions (>1 $\text{mg C-CH}_4 \text{ m}^{-2} \text{d}^{-1}$) were observed around 1 month after N fertilization during both years, and also after harvest (mid-October) in year 2. No significant differences in cumulative CH_4 oxidation were reported

Figure 3. Average NH_4^+ (a, b) and NO_3^- (c, d) concentrations in the topsoil (0–10 cm) from fertilization to the end of tillering (white columns) during the stem elongation stage (black columns) and from flowering to the end of the experimental period (grey columns) in the different treatments: CAN (calcium ammonium nitrate), CAN + the nitrification inhibitor 2-(3,4-dimethyl-1H-pyrazol-1-yl) succinic acid isomeric mixture NI (CAN + DMPSA), SCAN (split CAN) and control. Data are provided separately for campaigns 1 (a, c) and 2 (b, d). The * indicates the treatments are significantly different from CAN in the corresponding period. Vertical bars indicate standard errors ($n = 3$).

between treatments in any of the 2 years ($P > 0.05$) [Table 1](#)), although CAN+DMPSA and SCAN tended to increase CH_4 sink in comparison to CAN in both years. If both GHGs are expressed as $\text{CO}_2\text{-eq}$ emissions (see section 2.5), CH_4 sink compensated 6–14% of N_2O emissions.

Wheat yields

The average grain and straw yield in year 1 were 2689 and 5694 kg ha^{-1} , respectively. During year 1, the mean grain yield in CAN-based was close (i.e. 9% higher) than the average regional value (MAPAMA 2016), and the lowest grain and biomass productivities were obtained in the control plots. However, in year 2 (data not shown), only the control surpassed 1000 kg grain ha^{-1} , which was higher than that of the fertilized treatments ($P < 0.05$). In this year, straw yield was statistically similar for all treatments. The addition of DMPSA to CAN or splitting CAN showed no significant influence on grain yields in both years ($P > 0.05$).

Bread-making quality

In the first year, the grain protein concentration decreased as follows: SCAN = CAN > CAN + DMPSA > control ([Table 3](#)). No significant differences ($P > 0.05$) between treatments were observed in year 2, although a tendency similar to that of the previous year was observed

Figure 4. N_2O emissions in the February–May period in years 1 (a) and 2 (b) in the different treatments: control, CAN (calcium ammonium nitrate), CAN + the nitrification inhibitor 2-(3,4-dimethyl-1H-pyrazol-1-yl) succinic acid isomeric mixture NI (CAN + DMPSA) and SCAN (split CAN). The arrows indicate N fertilization events. Vertical bars indicate standard errors ($n = 3$). Outside of the represented period, emissions of N_2O were generally negligible (-0.1 to $0.1\ mg\ N\ m^{-2}\ d^{-1}$), and fluxes are not shown to improve the readability of the figures.

(data not shown). Average grain N concentrations (Table 3) were 2.8% and 3.2% in years 1 (var. 'Ingenio') and 2 (var. 'Marcopolo'), respectively ($P < 0.001$). The total gliadin concentration and total protein concentration behaved similarly in both years, with no significant differences between treatments in the second year ($P > 0.05$). The different treatments also did not affect the high molecular weight (HMW) glutenin concentration (not even the control). However, CAN was the treatment with the greatest low molecular weight (LMW) glutenin concentration in both years and was different ($P < 0.05$) from the unfertilized control (but not from SCAN or CAN+DMPSA).

Table 1. Cumulative N₂O-N, NO-N and CH₄-C fluxes for the different treatments: control, CAN (calcium ammonium nitrate), CAN + the nitrification inhibitor 2-(3,4-dimethyl-1H-pyrazol-1-yl) succinic acid isomeric mixture (CAN + DMPSA) and SCAN (split CAN). Different letters within columns indicate significant differences by applying the LSD test at $P < 0.05$. Standard Error (S.E., $n = 3$) is given for each effect.

Treatment	N ₂ O emissions (g N ha ⁻¹)		NO emissions (g N ha ⁻¹)		CH ₄ emissions (g C ha ⁻¹)	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
Control	195.2 a	260.0 a	172.1 a	508.8 a	-140.2	-214.9
CAN	239.7 b	356.3 bc	587.0 b	844.8 b	-157.2	-89.16
CAN+DMPSA	247.8 b	325.0 b	533.3 b	564.9 a	-185.9	-249.2
SCAN	285.5 c	384.8 d	553.3 b	2024.7 c	-239.0	-280.6
S.E.	9.7	10.7	42.9	79.3	41.9	75.2
<i>P</i> value	0.010	0.002	0.001	0.000	0.374	0.374

NUE, N surplus and yield-scaled emissions

The average NUE was 63% lower in year 2 (26.2%, ranging from 22.8% to 31.5% data not shown) than in year 1 (70.3%, ranging from 58.5% to 78.9%, Table 2). In agreement, average N surpluses were -33.6 and -11.0 kg N ha⁻¹ in years 1 (Table 2) and 2 (data not shown), respectively. The average GHGI and YSNO were increased in year 2 (data not shown, 206.2 g CO₂-eq kg grain⁻¹ and 2.63 g N kg grain⁻¹, respectively) with respect to year 1 (22.2 g CO₂-eq kg grain⁻¹ and 0.17 g N kg grain⁻¹, respectively, Table 2).

In a typical rainfall year (i.e., year 1), the NUE of CAN surpassed that of CAN+DMPSA (by 35%, showing SCAN intermediate results). The N surpluses were consistent with the NUE, with a lower N surplus for CAN than for CAN+DMPSA in year 1. SCAN was the treatment that resulted in the highest GHGI in year 1 (Table 2), whereas the YSNO losses were similar for all N-fertilized treatments in this year.

Discussion

Our results show that the variability in the amount and – particularly – in the distribution of rainfall after N application to soil is the key driver for N oxide emissions in semi-arid areas (e.g. Mediterranean), rather than the N fertilization management. We also show that N₂O emissions are lower than the IPCC default EF, being quantitatively surpassed by NO emissions. The rainfall regime during the first weeks following N fertilization affected nitrification rates (which is the key process in semi-arid areas), while the emissions peaks after rewetting events determined the efficacy of the mitigation strategies. The choice of best N fertilization strategies must consider this climatic variability, assuming that the mitigating effect may vary between years, as well as the yields (which are unpredictable) and the economic returns.

Effect of DMPSA on N oxide emissions

In year 2 (with severe drought conditions after N fertilization), DMPSA decreased the cumulative NO emissions ($P < 0.05$) and also tended to reduce those of N₂O (Table 1). However, no effect of DMPSA was observed in year 1 (with typical amount of rainfall after N fertilization). We hypothesize that the small losses of N oxides when applying CAN masked the effect of the inhibitor in year 1. Therefore, our results suggest that when climatic conditions (i.e. rainfall) lead to low N oxide emissions, the use of NIs may have a limited potential as fertilization has a small effect on emissions. In agreement with our results, Huérfano et al. (2016) found that the mitigating effect of DMPSA was only significant ($P < 0.05$) during the year with the highest N₂O fluxes, in which the emission factor (EF) of the treatment without an inhibitor was 0.36%. However, during the year with the lowest fluxes (EF of ammonium sulphate without DMPSA was lower than 0.1%) the DMPSA did not reduce the cumulative N₂O fluxes significantly ($P > 0.05$). In our experiment, the N₂O and NO emission

Figure 5. NO emissions in the February–May period in years 1 (a) and 2 (b) in the different treatments: control, CAN (calcium ammonium nitrate), CAN + the nitrification inhibitor 2-(3,4-dimethyl-1H-pyrazol-1-yl) succinic acid isomeric mixture NI (CAN + DMPSA) and SCAN (split CAN). The arrows indicate N fertilization events. Vertical bars indicate standard errors ($n = 3$). Outside of the represented period, emissions of N₂O were generally negligible ($<0.1 \text{ mg N m}^{-2} \text{ d}^{-1}$), and fluxes are not shown to improve the readability of the figures.

factors were lower than 0.35% except for that of NO in year 2 (the DMPSA was significantly effective in this case ($P < 0.05$), supporting these findings. Under similar conditions as those of year 1 (but with different N fertilization timing), Recio et al. (2018b) found that the use of DMPSA mitigated N₂O (by 75%) and NO (by 100%) emissions ($P < 0.05$), highlighting the relevance of rainfall conditions after N addition. In agreement with the results about gaseous emissions, significant differences ($P < 0.05$) in soil mineral N concentrations between CAN and CAN+DMPSA (i.e. increment in average NH₄⁺ concentrations during stem elongation and reduction of NO₃⁻ concentrations from flowering) were only reported in year 2.

Table 2. N use efficiency (NUE), N surplus, greenhouse gas intensity (GHGI) and yield-scaled NO emissions (YSNO) in year 1 for the different treatments: control, CAN (calcium ammonium nitrate), CAN + the nitrification inhibitor 2-(3,4-dimethyl-1H-pyrazol-1-yl) succinic acid isomeric mixture (CAN + DMPSA) and SCAN (split CAN). Different letters within columns indicate significant differences by applying the LSD test at $P < 0.05$. Standard Error (S.E., $n = 3$) is given for each effect. Data of year 2 have not been included in the Table, since due to the severe drought conditions the grain yields were, in all treatments, below the limit of profitability ($<1500 \text{ kg ha}^{-1}$), so this year can be considered irrelevant from an agronomic viewpoint.

Treatment	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})	NUE (%)	N surplus (kg N ha^{-1})	GHGI ($\text{g CO}_2\text{-eq kg grain}^{-1}$)	YSNO (g N kg grain^{-1})
Control	2190 a	3422 a	–	–	22.1 a	0.09 a
CAN	2989 b	6754 b	78.9 b	–43.9 a	19.8 a	0.20 b
CAN+DMPSA	2974 b	6130 b	58.5 a	–19.4 b	20.3 a	0.18 b
SCAN	2595 ab	6363 b	73.6 ab	–37.5 ab	26.4 b	0.21 b
S.E.	140	311	5.7	6.9	1.5	0.02
P value	0.030	0.001	0.068	0.097	0.006	0.005

In the second year, in which lower cumulative rainfall was recorded (Figure 1, S1), higher N oxide losses were found (Table 1). Although no significant correlations between N oxide emissions were found, the relevance of N_2O emissions due to soil rewetting after dry conditions has been previously highlighted (e.g., Bergstermann et al. 2011; Leitner et al. 2017), while emissions of NO are maximum at medium (40–50%) WFPS values (Pilegaard 2013). In agreement, García-Marco et al. (2014) reported that soil WFPS is the factor with the greatest influence on N_2O emissions. Our results showed that the scarcity of rainfall during wheat critical stages (i.e., tillering and stem elongation) caused a deficient plant N uptake, thus leading to significant amounts of residual soil N (particularly NO_3^-) after flowering (Figure 3), which was liable to being lost after the re-wetting of the soil (Leitner et al. 2017). The rainfall events in May (Figure 1) were inefficient for increasing the grain N uptake or yield (Tables 2 and 3) but caused a remarkable increase in N oxide emissions (particularly those of NO, Figure 5). In this case, DMPSA conserved its effect, thus showing low N oxide fluxes during these peaks. Our results confirm those of Guardia et al. (2017b) and Guardia et al. (2018b), who found that DMPP or DMPSA can be effective even 2 months after application. On the other hand, the effectiveness of DMPSA was not maintained during the peak that was reported after the first rainfall events in autumn (approximately 6 months after fertilization, see section 3.2.1). The interaction of N and water under rainfed conditions is therefore essential for achieving an optimum N nutrition leading to admissible NUEs and minimizing gaseous losses. This fact is crucial within the context of a global change and for regions with considerable rainfall variability and scarcity (e.g., Mediterranean regions). The variability and unpredictability of rainfall amount and distribution could cause the use of nitrification inhibitors to be recommendable to control N oxide fluxes in these regions. Our results also suggest that the high sampling frequency with a closed chamber technique method under rainfed semi-arid conditions should not be limited to the first month after fertilization, because significant emission peaks can occur after flowering and harvesting.

Effect of splitting CAN on N oxide emissions

Splitting N applications has been proposed as one of the ‘4R’ strategies (right fertilizer source, right rate, right place and right time) to reach the economic, social and environmental objectives at agro-ecosystem scale (Bruulsema et al. 2009). Indeed, splitting the N applied at dressing has an overall effect on reducing N losses (including those of N_2O) in wheat (Xia et al. 2017); and on increasing the yields or grain protein (López-Bellido et al. 2005). However, we generally observed an increment in N oxide emissions (particularly those of NO in year 2) in SCAN plots in comparison to CAN (Table 1). Since emissions from CAN were low, splitting the application of CAN did not cause a mitigation of these N losses through a better synchronization of N supply and plant demand. Moreover, our results suggest that late dressing applications can lead to residual soil N that is not efficiently taken-up or remobilized by plants and can result in emission peaks (Figures 4 and 5) after late rainfall events. Indeed, the contribution of the weeks following the second dressing application to annual N oxide

Table 3. Grain N content and amount of total gliadins, high molecular weight (HMW) and low molecular weight (LMW) glutenins in the different treatments: control, CAN (calcium ammonium nitrate), CAN + the nitrification inhibitor 2-(3,4-dimethyl-1H-pyrazol-1-yl) succinic acid isomeric mixture (CAN + DMPSA) and SCAN (split CAN). Different letters within columns indicate significant differences by applying the LSD test at $P < 0.05$. Standard Error (S.E., $n = 3$) is given for each effect.

Treatment	N content grain (%)		Total gliadin content (mV min mg ⁻¹ flour)		HMW glutenin content (mV min mg ⁻¹ flour)		LMW glutenin content (mV min mg ⁻¹ flour)	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
Control	2.22 a	3.02	0.75 a	1.17	0.09	0.09	0.32 a	0.41 a
CAN	3.12 c	3.22	1.05 bc	1.39	0.12	0.09	0.59 b	0.80 b
CAN+DMPSA	2.73 b	3.16	0.93 ab	1.29	0.10	0.09	0.49 ab	0.67 ab
SCAN	3.22 c	3.27	1.24 c	1.24	0.12	0.10	0.58 b	0.57 ab
S.E.	0.08	0.09	0.07	0.11	0.01	0.01	0.06	0.08
P value	0.000	0.071	0.010	0.576	0.305	0.685	0.029	0.042

emissions (27–29% for N_2O and 44–90% for NO) was higher than that of the first dressing application (7–13% for N_2O and 7–46% for NO). In our conditions, NO fluxes did not peak when soil WFPS values were steadily maintained in optimum values for nitrification (Pilegaard 2013). However, the scarce rainfall and warm temperatures decreased WFPS below 20% (Figure 2b), thus causing this ‘pulse effect’ after the soil rewetting. Our results suggest that splitting N rate in two or more applications may increase the chances for these rewetting pulses in rainfed semi-arid crops, in which drought periods can occur. The single application of CAN at dressing was better than the split tillering-stem elongation application, at least from an area-scaled emissions viewpoint. Further studies can be conducted assessing split applications during the tillering stage, even though a single application appears as a sufficiently efficient practice from environmental and practical (i.e., reduction of farm operations) perspectives.

Soil processes, N oxide emission factors and CH_4 sink

Despite the higher N oxides in the SCAN treatment, the EFs were generally low. For N_2O , the EFs were lower than 0.10% for all treatments. Our N_2O EFs were below the range found by Cayuela et al. (2017) in Mediterranean regions for synthetic fertilizers (even that of CAN), but within the range for inhibitors. Average NO EF was 0.32% (ranging from 0.30% in CAN+DMPSA to 0.35% in CAN) in year 1; and 0.53% (ranging from 0.05% in CAN+DMPSA to 1.30% in SCAN) in year 2. These values agreed with the EFs obtained in previous field studies (e.g., Abalos et al. 2012; Hickman et al. 2017; Recio et al. 2018b); and were below or within the range (0.71–1.61%, average 1.16%) reported in the global meta-analysis of Liu et al. (2017). Our results suggest the potential of N fertilizers containing NO_3^- -N to decrease N oxide emissions in comparison to urea or NH_4^+ -N-based N sources. In this sense, under similar experimental conditions, Guardia et al. (2018b) found that the N_2O EFs of urea ranged from 0.12% to 0.20%, being, therefore, higher than those of CAN. Regarding NO, a similar tendency is observed: 1.4–1.6% for urea (Guardia et al. 2018b) and 0.28–0.35% for CAN in this experiment. These results are in agreement with previous findings under irrigated Mediterranean conditions, e.g. Abalos et al. (2014), who reported significantly higher N_2O EFs from calcium nitrate than from urea or Guardia et al. (2017a) comparing urea and CAN in a maize crop. These results should not be generalized to denitrifying environments (e.g. humid grassland soils), where lower emissions from urea than from CAN have been found (Harty et al. 2016; Roche et al. 2016; Carswell et al. 2019).

Quantitatively, NO fluxes surpassed those of N_2O , which has been suggested as an indicator of the predominance of nitrification (Akiyama et al. 2004), as commonly accepted for Mediterranean regions (Aguilera et al. 2013). Nitrification was also crucial for the N oxide (particularly NO) emission pulses observed after the soil rewetting in year 2. During this emission peaking period, NO fluxes were also much higher than those of N_2O (Figures 4 and 5, S1). It has been demonstrated that the soil moisture status prior to rewetting episodes determines the magnitude of the emission bursts. In our study, the greater peaks observed in year 2 occurred after a period of 23 days of soil WFPS below 20% (even reaching values < 10%, Figure 2b). In the previous year, WFPS values below 20% were only observed in a 10-day period, never being lower than 10% (Figure 2a). Moreover, the rewetting event in year 1 took place during the crop stage when N uptake is expected to be more intense (i.e. stem elongation), thus reducing the potential for N losses. In agreement with our results, Bergstermann et al. (2011) found that the N_2O emission peak after soil rewetting was significantly higher when the soil WFPS was lower (i.e. 20% versus 75%) before rewetting. The delayed occurrence of the N_2 emission peak with respect to that of N_2O found by Bergstermann et al. (2011), suggests that an intense nitrification activity and/or a low N_2O reductase activity could have contributed significantly to these rewetting peaks. An interesting result was that NO emissions in the control plots were much higher when NO peaked after the rainfall episodes in May than during the rest of the experimental period (Figure S1), suggesting that the reactivation of soil microorganisms is *per se* an important driver of emission pulses (Liang et al. 2016). The critical contribution to annual cumulative losses of

these emission peaks after rewetting episodes could be particularly important in low organic C and basic/calcareous soils (e.g. Sanchez-Martin et al. 2010; Cui et al. 2012) thus conditioning the efficacy of mitigation practices.

As previously reported for non-flooded agro-ecosystems, a net CH₄ sink was reported in both years (Snyder et al. 2009). N fertilization did not affect the activity of methanotrophs, since no differences were observed between treatments, including the control (Table 1). The non-significant effect of DMPA on CH₄ oxidation ($P > 0.05$) is consistent with the meta-analysis of Qiao et al. (2015) for NIs. Interestingly, some CH₄ peaks were observed at the same dates as some N₂O increments (e.g. after harvest in year 2) causing a weak but significant correlation between both GHGs ($P = 0.02$, $n = 62$, $r = 0.29$).

Agronomic response and bread-making quality

Wheat yields seemed to be strongly influenced by the differences in the amount and distribution of rainfall between both years. The extremely low grain yields suggested critical water scarcity conditions during tillering, stem elongation and flowering, and also a deficient grain filling stage, which may have been caused by the severe drought conditions. We must clarify that the significant differences in crop yields and quality between years can be attributable not only to the rainfall conditions, but also to the different wheat varieties. However, it seems obvious that in the second year, when a severe drought occurred during critical wheat stages (Figure 1), fertilizing with N was counterproductive. Surprisingly, the control was the treatment that resulted in the lowest yield-scaled emissions in this dry year, despite the abatement of LMW glutenin concentrations *cf.* CAN. Due to the extremely low yields, year 2 can be considered irrelevant from an agronomic viewpoint. For this reason, the yield-based and NUE data from this year have not been considered for the discussion. Since only 1 year of valid data was obtained due to the erratic rainfall distribution in Mediterranean areas, further research is needed to obtain robust conclusions on the effect of CAN-based strategies on yields and NUE.

Our results showed that splitting CAN or using DMPA did not affect the grain and straw yields. Our results agreed with those of Huérfano et al. (2016) for wheat and Guardia et al. (2017a) for maize, who obtained similar grain yields with and without DMPA. However, contrary to López-Bellido et al. (2005), we did not find any positive response to splitting CAN at dressing on grain yield. We hypothesized that the low N losses from CAN application in this semi-arid Mediterranean soil as N₂O (Cayuela et al. 2017; Abalos et al. 2017), NH₃ (because the N was applied as CAN, Sanz-Cobena et al. 2014; Pan et al. 2016) or leaching (limited by rainfed conditions) caused a very limited effect of NI or a split application. The DMPA treatment even tended to decrease the gliadin and LMW glutenin concentration and decreased ($P < 0.05$) the grain protein and NUE in the typical rainfall year (var 'Ingenio', Tables 2 and 3). The depletion of NUE was clearly opposed to the results of the meta-analysis of Abalos et al. (2014b). Supporting our findings, the meta-analysis of Yang et al. (2016) reported that the effect of DCD on plant N uptake was positive, whereas that of DMPP (a pyrazole-based NI) was insignificant, with some observations leading to a negative response (CI: -1.6% to 5.8%). Our results may suggest that when inhibitors are applied together with optimum N rates (as 120 kg N ha⁻¹ for wheat) the positive effect of inhibitors on agronomic efficiency may not be as notable as when adjusted or deficit rates are used (Rose et al. 2018).

Gliadin concentration is a key bread-making quality indicator which is closely related to dough extensibility, while glutenins are mainly associated with gluten strength (Girard et al. 2018). Previous studies have shown that protein concentration and therefore protein composition are clearly influenced by N management. Our results revealed a negligible effect of splitting CAN or the use of DMPA on reserve protein composition. These findings were not in agreement with those of Fuertes-Mendizábal et al. (2010), who found an increment of HMW glutenin subunits ($P < 0.05$) with the split application of N, but supported those of Garrido-Lestache et al. (2005) in durum wheat or Guardia et al. (2018b) in bread wheat. The former study found no significant effect of splitting urea or DMPA on gliadin and glutenin concentrations using the same bread wheat varieties under similar conditions ($P > 0.05$). Comparing data from both studies, similar glutenin and HMW concentrations

were found between urea (Guardia et al. 2018b) and CAN (this study). However, the latter treatment increased by 36% the concentration of LMW glutenins (0.70 and 0.51 mV min mg⁻¹ flour for CAN and urea, respectively). LMW glutenins are significantly related to dough resistance and extensibility (Wieser 2007) and are key quality components in bread and – particularly – durum wheat, so further research is needed comparing urea and CAN in both wheat species.

Conclusions

While splitting CAN applications at dressing is not an acceptable option from a N oxide mitigation viewpoint, the effectiveness of DMPA with CAN depended on the amount and distribution of rainfall. During the dry year 2, higher NO peaks occurred after the rewetting of the soil and the effectiveness of DMPA was significant ($P < 0.05$) under these conditions, thus improving the performance of calcium ammonium nitrate. Even though in a typical rainfall year, the use of DMPA did not improve the performance of CAN, this NI should be recommended to avoid high emission peaks in years with low N uptake efficiency and high N losses.

Our results also demonstrated that under extreme drought conditions with soil moisture being the limiting factor for N uptake, N fertilization is counterproductive in rainfed cereal crops: the response on crop yields is not observed, but high N oxide emissions can occur after late soil rewetting events.

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