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Urea-based fertilization strategies to reduce yield-scaled N oxides and enhance bread-making quality in a rainfed Mediterranean wheat crop

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ABSTRACT

Urea fertilizer applications to calcareous soils can result in significant nitrous oxide (N₂O) and nitric oxide (NO) emissions, predominantly via nitrification rather than denitrification. To address this, we explored several mitigation strategies based on improved urea management in a rainfed winter wheat (*Triticum aestivum* L.) crop during two consecutive cropping seasons with contrasting rainfall quantities and distribution. The strategies we investigated included the split application of urea at top dressing, the use of nitrification inhibitors (e.g. 2-(3,4-dimethyl-1H-pyrazol-1-yl) succinic acid isomeric mixture, DMPSA, and nitrapyrin), the urease inhibitor N-butyl thiophosphorictriamide (NBPT), or the double inhibitor DMPSA + NBPT. Emissions of N₂O, NO, methane (CH₄), as well as measurements of grain and straw yield and bread-making quality (protein content, reserve protein composition: glutenins and gliadins) were measured. Nitrogen (N) use efficiency (NUE) and N surplus were also calculated. Results were affected by rainfall, since the first cropping season experienced typical rainfall quantity and distribution, whilst the second cropping season was very dry, thus increasing significantly the yield-scaled emissions and N surplus, and markedly decreasing the NUE. In comparison to the single application of urea without inhibitors, all treatments generally decreased surface-scaled and yield-scaled emissions, with urea + DMPSA being the most effective and consistent mitigation option. Split urea and NBPT did not mitigate surface-scaled emissions in the dry cropping season, because of the marked peaks in N oxides after flowering, caused by inefficient crop N uptake. In the typical rainfall cropping season, the use of the double NBPT + DMPSA inhibitor led to the best balance between mitigation of yield-scaled N oxides emissions, N efficiency, crop yield and bread-making quality (i.e. increments in total protein, gliadins and glutenins). We did not observe any effect of nitrification inhibitors on grain yield (except in the dry cropping season) or the composition of gluten proteins. Our results suggest that the use of DMPSA with or without NBPT should be recommended to mitigate yield-scaled emissions of N oxides in rainfed semi-arid crops.

1. Introduction

Nitrogen (N) fertilization is essential to feed the increasing worldwide population through the enhancement of crop yields (Spiertz, 2010). On the other hand, N fertilizers can have a major impact on the environmental, e.g. through the release of gases such as ammonia (NH₃), nitric oxide (NO) or nitrous oxide (N₂O) to the atmosphere (Ussiri and Lal, 2013). Nitrous oxide is a powerful greenhouse gas (GHG) and an ozone-depleting substance (IPCC, 2014), while NO contributes to the formation of tropospheric ozone (O₃) and acid rain

(Pilegaard, 2013). The main biochemical processes involved in the emissions of both N oxides are nitrification (i.e. the oxidation of ammonium, NH₄⁺, to nitrate, NO₃⁻, by autotrophic or heterotrophic microorganisms under aerobic conditions) and denitrification (i.e. the reduction of NO₃⁻ to N₂O/N₂ by heterotrophic microbiota under anaerobic conditions) (Caranto and Lancaster, 2017; Hallin et al., 2017).

Urea fertilizer is the most commonly used nitrogen (N) fertilizer used worldwide due to its low cost, high N content and ease of management during transport and storage (Glibert et al., 2006). After

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application to the soil, it rapidly hydrolyses to release NH_3 and carbon dioxide (CO_2). The use of this NH_4^+ -N based fertilizer can result in lower N_2O emissions than NO_3^- -based fertilisers (such as ammonium nitrate) in agro-ecosystems where denitrification is a dominant soil process (e.g. grasslands in humid areas) (Smith et al., 2012; Harty et al., 2016; Roche et al., 2016). However, greater N_2O emissions can occur from urea (compared to NO_3^- -based fertilisers) under contrasting conditions, such as arable-crops in semi-arid areas (Zhang et al., 2016; Guardia et al., 2017; Volpi et al., 2017). This may be a result of the predominance of nitrification in semi-arid calcareous soils with low organic carbon (C) content (Aguilera et al., 2013) that causes NH_4^+ oxidation to be the main N_2O production pathway, even under irrigated conditions (Guardia et al., 2017, 2018). According to Tierling and Kuhlmann (2018), the accumulation of nitrite (NO_2^-) under non-denitrifying conditions plays a key role in the increase of N_2O production, and this is favored at high soil pH. Therefore, it is essential to find mitigation strategies for N oxides in semi-arid calcareous soils, where emissions from widespread urea fertilizer are significant.

One possible approach is the use of nitrification (NIs) and urease (UIs) inhibitors with urea. Dicyandiamide (DCD) has been one of the most widely used NIs worldwide, but its use is currently under discussion because traces of this inhibitor were found in New Zealand milk products (Chen et al., 2014). Plant interception and uptake, followed by grazing by dairy cows, were potential routes for this contamination of milk (Kim et al., 2012a; Marsden et al., 2015). There is therefore interest in other NIs, such as 2-(3,4-dimethyl-1H-pyrazol-1-yl) succinic acid isomeric mixture (DMPSA) or nitrapyrin (2-chloro-6-(trichloromethyl)-pyridine). Neither of these two NIs have been commercialized yet in Europe, although nitrapyrin was introduced as a NI to the US market in the 1960s (Goring, 1962). The efficacy of nitrapyrin has been demonstrated in other areas of the world under a range of management and environmental conditions (Thapa et al., 2016). DMPSA has been shown to reduce emissions of N oxides following calcium ammonium nitrate (Guardia et al., 2017, 2018) and ammonium sulphate (Huérffano et al., 2016) applications to soil, mainly under irrigated or humid rainfed conditions. However, the efficacy of DMPSA to reduce emissions of N oxides has not been tested with urea fertiliser in rainfed semi-arid conditions yet.

The use of NIs can result in negative trade-offs (pollution swapping), e.g. increased NH_3 volatilization (Qiao et al., 2015; Pan et al., 2016). Other possible mitigation strategies include the use of UIs such as N-butyl thiophosphorictriamide (NBPT), which delays the hydrolysis of urea thus reducing NH_3 emissions (Sanz-Cobena et al., 2008). Moreover, NBPT also has showed positive results in mitigating emissions of N oxides through the reduced availability of topsoil NH_4^+ and NO_3^- (Abalos et al., 2012). However, its N oxides mitigation efficacy may be more dependent on environmental conditions, thus leading to a lower average performance than NIs (Thapa et al., 2016). Therefore, it is important to evaluate the efficacy of NBPT in consecutive years in rainfed semi-arid areas, where high rainfall variability influences the effectiveness of N fertilizer mitigation strategies (Abalos et al., 2017). In this sense, the use of double NI-UI inhibitors may lead to mitigation of both N oxides and NH_3 emissions (Zaman and Nguyen, 2012). The potential for the double-inhibitor approach to improve the mitigation efficacy of N oxides (compared with NIs alone) has yet to be proved under rainfed semi-arid conditions.

Since NIs and UIs are known to improve the synchronization of the N applied with crop demand (Abalos et al., 2014a), other strategies such as splitting N application should be compared against inhibitors. Previous studies have shown the potential of smaller more frequent doses of fertiliser to enhance N recovery efficiency and decrease N losses (Bell et al., 2015; Wang et al., 2016; Xia et al., 2017). Some of these mitigation strategies can increase farm costs and decrease net margins for farmers (Sanz-Cobena et al., 2017), in comparison to the single application of urea without inhibitors. Consequently, possible improvements of farm benefits through increments in crop yields and

quality, or improvements in NUE, which can be affected by inhibitors or more split doses of fertilizer (Peltonen and Virtanen, 1994; López-Bellido et al., 2005; Grant et al., 2011; Abalos et al., 2014a) must be evaluated together with N oxides emissions.

A large number of previous studies have explored the use of NIs and UIs on gaseous emissions and crop yields, but a complete overview including crop quality (e.g. bread-making quality of wheat) is lacking. Previous studies have shown the potential of inhibitors and N application timing to increase plant N concentrations and/or influence N remobilization or protein composition of grain (Qiao et al., 2015; Thapa et al., 2016; Xue et al., 2016). In the case of bread-making wheat, an increase in grain protein content is closely linked to dough quality.

In this context, a field experiment was established to compare several strategies based on urea fertilization, including the use of NIs and/or UIs, and split dressings of urea. We hypothesized that all of these strategies would improve the balance between mitigation of yield-scaled N oxides emissions, NUE, crop yield and bread-making quality, compared to conventionally managed urea application. Although its contribution to GHG balance in semi-arid croplands is generally low (Aguilera et al., 2015) methane (CH_4) emissions, which also affect the GHG balance of agro-ecosystems (Snyder et al., 2009), were also measured.

2. Materials and methods

2.1. Site description

The field experiment was located in the National Center of Irrigation Technology, “CENTER” (latitude 40°25'1.31"N, longitude 3°29'45.07"W) in the Madrid region of Spain. According to the Soil Taxonomy of USDA the soil is a *Typic xerofluvent* (Soil Survey Staff, 2014) with a silt loam texture (10% clay, 59.5% silt, and 30.5% sand) in the upper horizon (0–20 cm). The main physico-chemical properties of the topsoil were: bulk density, 1.27 Mg m^{-3} ; water pH, 8.2; organic matter (Walkley-Black), 20.7 g kg^{-1} ; total N, 1.64 g kg^{-1} ; CaCO_3 , 8.16 g kg^{-1} ; extractable P (Olsen), 28.4 mg kg^{-1} ; total K, 3.14 g kg^{-1} . The site's mean annual average air temperature and annual rainfall during the last 10 years was 14.1 °C and 393 mm, respectively. The average rainfall from November to July (a typical winter cereal cropping period) was 296 mm (184 mm from February to July), while mean soil temperature (at 10 cm depth) for this period was 11.8 °C. Data for daily rainfall and daily air and soil temperatures were obtained from the meteorological station located at the field site.

2.2. Experimental design and management

A field experiment was carried out from October 2015 to October 2017, including two wheat cropping seasons: year 1 (2015/2016) and year 2 (2016/2017). The same plots were used in the two years. A complete randomized block design with three replicates was used, with each plot covering an area of 64 m^2 (8 m × 8 m). The application of fertilizers was adjusted to provide the equivalent of 120 $\text{kg total N ha}^{-1}$ for all treatments during the cropping period. The different fertilizer treatments were: 1) Urea applied in one dose (U); 2) Urea + NBPT (U + NBPT); 3) Urea + DMPSA (U + DMPSA); 4) Urea + NBPT + DMPSA (U + DI); 5) Urea + Nitrapyrin (U + NIT) 6) Urea split (SU) in two applications (60 kg N ha^{-1} + 60 kg N ha^{-1}); and 7) Control with no N fertilization (control). The proportion of DMPSA in the fertilizers was 0.8% of the NH_4^+ -N, whereas NBPT was applied at 0.13% of ureic N. NBPT and DMPSA based products were provided by EuroChem Agro in a granular form, and were homogeneously applied to the soil by hand. Nitrapyrin was applied at a rate of 0.35% of the applied N (w/w). The mixture U + NIT was obtained by dissolving U and nitrapyrin in water. The solution was then sprayed with a manual applicator. All fertilizers were applied to the soil surface at tillering stage (26th February 2016 and 23rd February 2017). In the case of SU

treatment, the second N fertilizer dose was applied at the beginning of stem elongation (31st March 2016 and 29th March 2017). Soil phosphorus (Olsen method) and potassium (K) concentrations were analyzed prior to the beginning of the experiment. No additional P or K fertilizer was applied since the soil content for both were sufficient for wheat production. The field was sown with winter wheat (*Triticum aestivum* L. 'Ingenio') on 27th October 2015 and *Triticum aestivum* L. 'Marcopolo' on 11th November 2016 at 210 kg ha⁻¹. A cultivator pass was performed after sowing and also to incorporate the wheat residues after harvesting. The field was kept free of weeds, pests and diseases, following local practices (including herbicides and pesticides). Because of the low rainfall during the spring of year 2, three 20 mm irrigation events (27th April, 3rd and 8th May 2017) were performed using sprinklers.

2.3. Gas sampling and analyses

During the first 30 days following each fertilizer application, sampling for gaseous emissions occurred 2–3 times per week since this was considered the most critical period for high fluxes. Afterwards, the frequency of sampling was decreased progressively (although the high frequency sampling period was extended in year 2 due to climatic conditions). The GHG (N₂O and CH₄) fluxes were measured using the closed chamber technique, as described in detail by Guardia et al. (2017). One chamber per plot was used for this analysis. 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 and avoid the soil disturbance associated with the insertion of the chambers in the soil (Hutchinson and Livingston, 2001). The plants were cut when their height surpassed that of the chamber. The rings were only removed during management events and GHG measurements were always taken with wheat plants inside the chamber. 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 (Reeves and Wang, 2015). Gas samples were taken via a septum in the chamber lid and placed in 20 ml pre-evacuated vials at 0, 30 and 60 min to test the linearity of headspace gas accumulation. The concentrations of N₂O and CH₄ were quantified by gas chromatography, using a HP-6890 gas chromatograph (GC) equipped with a headspace autoanalyzer (HT3), both from Agilent Technologies (Barcelona, Spain). HP Plot-Q capillary columns transported the gas samples to a ⁶³Ni electron-capture detector (ECD) to analyze the N₂O concentrations and to a flame-ionization detector (FID). The increases in N₂O and CH₄ concentrations within the chamber headspace were generally linear during the 1 h sampling period (> 90% of cases, particularly when the highest fluxes or emission peaks were reported, R² > 0.90). In the case of nonlinear fluxes, linear regressions were performed, since it has been described as the recommended option for three sampling points (Venterea et al., 2012).

NO fluxes were measured using a gas flow-through system on the same days as the N₂O measurements (during summer period). One chamber per plot was used for this analysis (volume 22 L, diameter 35 cm and height 23 cm). In this case, the interior of the chamber was covered with Teflon® to minimize the reactions of NO_x with the walls and the chamber had inlet and outlet holes (Guardia et al., 2017). The nitric oxide was analysed using a chemiluminescence detector (AC31M-LCD, Environment S.A., Poissy, France). Air (filtered through a charcoal and aluminium/KMnO₄ column to remove O₃ and NO_x) was passed through the headspace of the chamber, and the gas samples were pumped from the chambers at a constant flow rate to the detection instruments via Teflon® tubing. The ambient air concentration was measured between each gas sampling. As proposed by Kim et al. (1994), the NO flux was calculated from a mass balance equation, considering the flow rate of the air through the chamber and the increase in NO concentration with respect to the control (chamber placed over the

chemiluminescence equipment) when the steady state concentration was reached.

2.4. Soil and crop sampling and analyses

Soil samples were taken concurrently with gas samples in order to determine the moisture content, NH₄⁺-N and NO₃⁻-N concentrations, and relate them to the gaseous emissions. Three soil cores (0–10 cm) were randomly sampled in each microplot and then mixed and homogenized in the laboratory. The soil NH₄⁺-N and NO₃⁻-N concentrations were analyzed using 8 g of soil extracted with 50 mL of KCl (1 M) and measured by automated colorimetric determination using a flow injection analyzer (FIAS 400 Perkin Elmer) with a UV-V spectrophotometer detector. Water-filled pore space (WFPS) was calculated by dividing the volumetric water content by the total soil porosity, assuming a particle density of 2.65 g cm⁻³. The gravimetric water content was determined by oven-drying soil samples at 105 °C with a MA30 Sartorius® moisture analyzer.

The wheat was harvested on 21st June 2016 and 21st June 2017 with a research plot combine (Wintersteiger Inc.). Previous to this, the plants of one row were harvested to determine the total N content of grain and straw, which were measured using a TruMac CN Leco elemental analyzer. Grain proteins (gliadins and glutenins) were sequentially extracted using the modified Osborne method (Bean et al., 1998; Bean and Lookhart, 2000). Samples were analysed using a Beckman® 2100 P/ACE system controlled by a System Gold Software version 810. Proteins were detected by UV absorbance at 214 nm with a photo diode array (PDA) detector. As described by Ronda et al. (2008), in order to reduce the lack of reproducibility usually obtained in electrophoretic analysis, the lys-tyr-lys tripeptide (Sigma- Aldrich, Inc, USA) was used as internal standard.

2.5. Calculations and statistical methods

Cumulative gas emissions during the experimental period were calculated by linear interpolation between sampling dates. The global-warming potential (GWP) of N₂O and CH₄ emissions was calculated in units of CO₂ equivalents (CO₂-eq) over a 100-year time horizon. A radiative forcing potential relative to CO₂ of 265 was used for N₂O and 28 for CH₄ (IPCC, 2014). Greenhouse gas intensity (GHGI) and yield-scaled NO emissions (YSNO) were calculated as the ratios of GWP to grain yield and NO-N emissions to grain yield, respectively (Shang et al., 2011). The N₂O and NO emission factors (EFs) were calculated as the ratio of the cumulative emissions (subtracting those of the control) to the total synthetic N applied. The N₂O and NO mitigation percentages were calculated using the EFs. The NUE was calculated as the ratio of the total N in aboveground biomass in each fertilized treatment (subtracting the value of the control) to the total N applied through synthetic fertilizer. The N surplus of fertilized treatments was calculated as the N application minus the aboveground N uptake (van Groenigen et al., 2010).

Analyses of variance were performed using *Statgraphics Plus 5.1*. Data distribution normality and variance uniformity were previously assessed using the Shapiro-Wilk test and Levene's statistic, respectively, and log-transformed when necessary. Means were separated by Least Significant Difference (LSD) test at *P* < 0.05. For non-normally distributed data, the Kruskal–Wallis test was used on non-transformed data to evaluate differences at *P* < 0.05. Simple Linear Regression analyses were performed to determine the relationships between N₂O-N, NO-N, and CH₄-C fluxes with soil NH₄⁺-N, NO₃⁻-N, WFPS and soil temperature, as well as among some yield/quality variables.

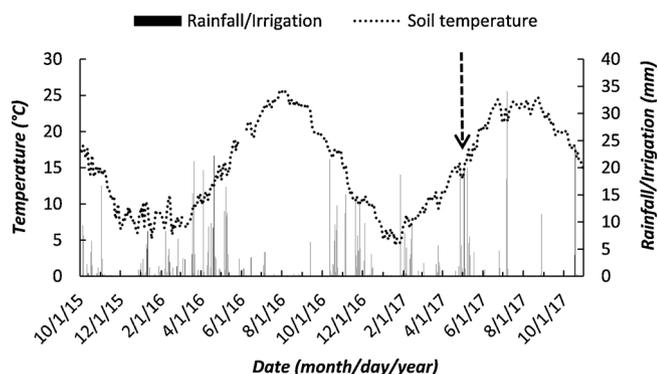


Fig. 1. Daily soil temperatures (at 10 cm depth) and rainfall distribution throughout both wheat cropping cycles. The 20 mm irrigation events (indicated with a dotted arrow) were performed on 27th April, 3rd May and 8th May of 2017.

3. Results

3.1. Environmental conditions and soil mineral N

Average soil temperatures at 10 cm depth and rainfall distribution throughout both years are shown in Fig. 1. Total precipitation over the wheat cropping cycle (October–July) was 319 mm and 272 mm for year 1 and 2, respectively. In the February–July period, the accumulated rainfall was 243 mm and 99 mm for year 1 and 2, respectively. Soil WFPS in the February–July period (Fig. 2a, b) ranged from 11% to 60% (in year 1) and from 3% to 50% (in year 2).

Mineral N concentrations in the topsoil increased markedly after fertilization (data not shown). During year 1, treatments containing NIs (i.e. U + DMPSA, U + NIT and U + DI) generally increased average soil NH_4^+ concentrations in comparison to U, particularly from the stem elongation stage (Fig. 3a). In agreement, average NO_3^- concentrations

decreased in these treatments with respect to U, particularly during tillering and stem elongation (Fig. 3c). The U + NBPT treatment generally reduced the average NH_4^+ and NO_3^- concentrations (Figs. 3a, c), while SU reduced mineral N concentrations until the second fertilizer dressing, after which NH_4^+ and NO_3^- contents increased. In year 2, the treatments containing NIs generally resulted in greater soil NH_4^+ concentrations (particularly after stem elongation) and lower NO_3^- concentrations (Figs. 3b, d). The main differences with respect to year 1 occurred for U + NBPT, which only decreased mineral N contents (in comparison to U) during the first period (tillering). Mineral N concentrations after flowering for year 2 were significantly higher than those of year 1. Specifically, the soil NO_3^- content after harvesting reached $43 \text{ mg N kg soil}^{-1}$, much higher than that of the previous year ($< 5 \text{ mg N kg soil}^{-1}$) (data not shown).

3.2. Gaseous emissions

3.2.1. N oxides emissions

Emissions of N oxides from mid-February to the end of May, including fertilization events in each year, are shown in Figs. 4 and 5. Nitrous oxide emissions ranged from -0.12 to $0.94 \text{ mg N m}^{-2} \text{ d}^{-1}$ (year 1) and from -0.14 to $1.07 \text{ mg N m}^{-2} \text{ d}^{-1}$ (year 2) (Fig. 4). In year 1, N_2O peaked on 25th March (30 days after first fertilization), reaching $0.35 \text{ mg m}^{-2} \text{ d}^{-1}$ on average for the fertilized treatments (Fig. 4a, c). In year 2, the main increases were observed 15 days (10th March, reaching $0.35 \text{ mg m}^{-2} \text{ d}^{-1}$ on average for the fertilized treatments), 67 days (1st May, reaching $0.38 \text{ mg m}^{-2} \text{ d}^{-1}$ on average for the fertilized treatments) and 76 days (10th May, reaching $0.57 \text{ mg m}^{-2} \text{ d}^{-1}$ on average for the fertilized treatments) after fertilization (Fig. 4b, d). The U treatment resulted in the highest N_2O EFs in both years were (0.12% and 0.20% in years 1 and 2, respectively). During the first year, U resulted in significantly higher cumulative N_2O losses than inhibitor-based treatments or SU (Table 1). The N_2O cumulative fluxes from SU were greater than those of U + DMPSA, but similar to those of U + NIT, U + DI or U + NBPT. In the second year, U + NBPT and SU did not decrease N_2O cumulative losses in comparison to U (Table 1). The U + DMPSA treatment was the most effective mitigation treatment of all inhibitor-based strategies, with significantly lower emissions than U + NIT (showing U + DI intermediate results, Table 1). At the end of this second year and after a rainfall event in mid-October, an increase in N_2O emissions was noticed (20th October, reaching on average $0.37 \text{ mg N m}^{-2} \text{ d}^{-1}$). This peak was concurrent with an increase in soil moisture (soil WFPS was $51.8 \pm 0.4\%$). No significant differences between treatments were observed in this peak (data not shown). On average, cumulative N_2O fluxes were increased by 36% in this second year (average 369.3 g ha^{-1}) compared to those in year 1 (average 270.8 g ha^{-1}). Regarding the relationship of N_2O fluxes with soil properties, in year 2, daily N_2O fluxes were positively correlated with WFPS ($P = 0.008$, $n = 35$, $r = 0.45$) and negatively with soil NH_4^+ ($P = 0.007$, $n = 35$, $r = -0.56$). These significant correlations were not observed in year 1.

Nitric oxide emissions ranged from -0.36 to $7.76 \text{ mg N m}^{-2} \text{ d}^{-1}$ (year 1) and from -1.31 to $21.2 \text{ mg N m}^{-2} \text{ d}^{-1}$ (year 2) (Fig. 5). Nitric oxide peaks were generally concurrent with those of N_2O . The NO EFs ranged from 0.2% to 1.4% (year 1) and from 0.0 to 1.6% (year 2), with highest values corresponding to U. As in the case of N_2O , U resulted in the highest NO cumulative emissions in year 1 (Table 1), while U + DMPSA led to the lowest emissions of N fertilized treatments, being significantly lower than those of U + NBPT, U + NIT and SU. In the second year, U + DMPSA was also the N fertilizer treatment that caused the lowest cumulative emissions, which were even lower than those of control. The U + DI and U + NIT also decreased cumulative NO fluxes (by 94% and 77%, respectively, $P < 0.05$) with respect to U, but SU and U + NBPT did not decrease NO emission in comparison to U, as a result of high emissions after irrigation events (Fig. 5b, d). A strong and positive correlation between N_2O and NO fluxes was found ($P = 0.000$,

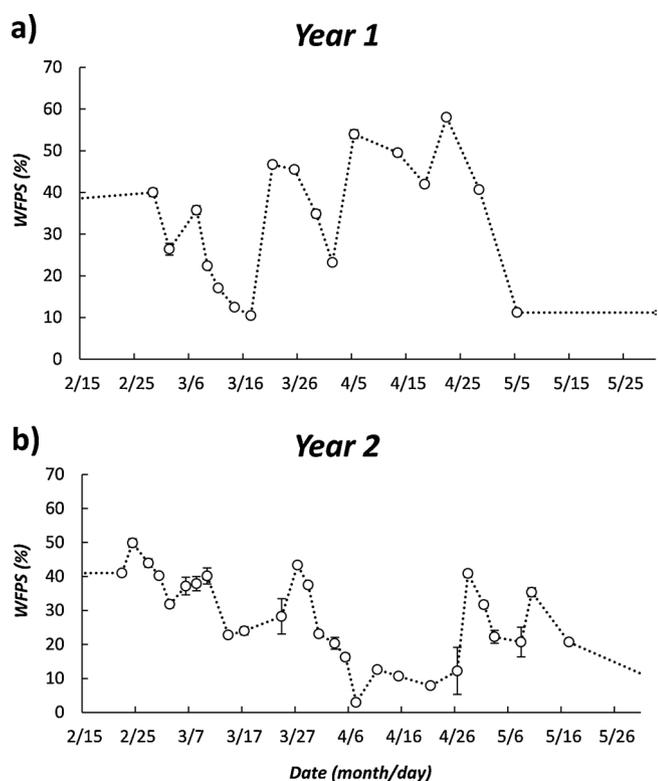


Fig. 2. Pattern of soil WFPS (0–10 cm) in years 1 (a) and 2 (b). Vertical bars indicate standard errors.

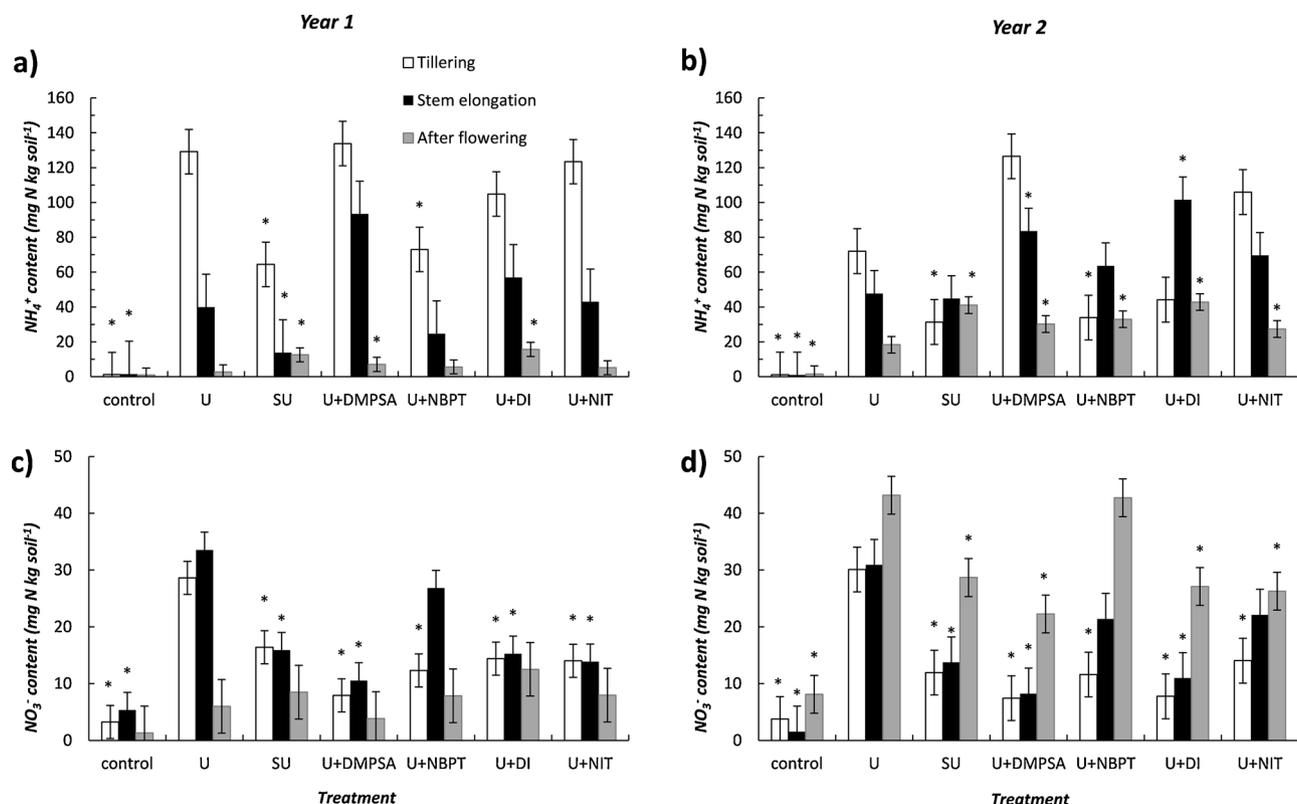


Fig. 3. Average NH_4^+ (a, b) and NO_3^- (c, d) concentrations in the topsoil (0–10 cm) during tillering, stem elongation and from flowering to the end of the experimental period in the different treatments: U (Urea), U+NIT (Urea+Nitrapyrin), U+NBPT (Urea+NBPT), U+DMPSA (Urea+DMPSA), U+DI (Urea+DMPSA+NBPT), split urea (SU) and control. Data are provided separately for year 1 (a, c) and 2 (b, d). * indicates treatments are significantly different from U in the corresponding period by applying the LSD test at $P < 0.05$. Vertical bars indicate standard errors ($n=3$).

$n = 42$, $r = 0.72$). Regarding soil properties, cumulative NO fluxes correlated with mean soil NH_4^+ contents ($P = 0.011$, $n = 42$, $r = 0.65$), while daily NO fluxes correlated with NO_3^- concentrations throughout year 2 ($P = 0.022$, $n = 35$, $r = 0.66$).

3.2.2. Methane oxidation

Methane fluxes ranged from -1.70 to $2.60 \text{ mg m}^{-2} \text{ d}^{-1}$ (year 1), and from -1.62 to $1.69 \text{ mg m}^{-2} \text{ d}^{-1}$ (year 2). The soil acted as a CH_4 sink on most sampling dates, although emission peaks of CH_4 were observed during both years about one month after N fertilization (data not shown). No significant differences in cumulative CH_4 oxidation were reported between treatments in either of the two years (Table 1). If GWP is considered for both GHGs, CH_4 uptake by soil was only 4–13 % of N_2O emissions; therefore, the net result ($\text{CH}_4 + \text{N}_2\text{O}$) is the emission (on average for fertilized treatments) of $70.3 \pm 3.6 \text{ kg CO}_2\text{-eq ha}^{-1}$ and $97.8 \pm 4.7 \text{ kg CO}_2\text{-eq ha}^{-1}$ in years 1 and 2, respectively.

3.3. Crop yield

Average grain yields were 2850 (Table 2) and 845 kg ha^{-1} (Table S1) while wheat straw yields were 6095 (Table 2) and 6610 kg ha^{-1} (Table S1) in years 1 and 2, respectively. Even though there were no significant differences between fertilized treatments for grain or straw yield during the first year, yields from the control plots were significantly lower than the fertilized treatments. During year 1, average grain yield was slightly above the average regional value (MAPAMA, 2016). Conversely, in the following wheat cropping season, grain yields were much lower than in year 1 ($< 1500 \text{ kg ha}^{-1}$). Only SU, U+DMPSA, control and U+NIT exceeded $1000 \text{ kg grain ha}^{-1}$ and showed a positive response with respect to U. During year 2, straw production was similar for all treatments, including the control treatment, with the exception of SU which had the highest straw yield.

3.4. Bread-making quality

Average grain N contents (Table 2) were 2.8% and 3.2% in year 1 (variety 'Ingenio') and 2 (variety 'Marpolito'), respectively ($P < 0.001$). The use of different wheat varieties in each year means that the differences in crop yields and quality between years were not only influenced by the meteorological conditions, but also by the different genetic characteristics of both cultivars. In the first year, grain from the control had the lowest protein content. U+DI significantly increased the grain protein content in comparison to U+DMPSA, U, SU and U+NIT, with U+NBPT having an intermediate value. In the second year, the results were similar, although U and U+NBPT had similar grain protein content to U+DI. Further, the control did not result in a lower grain protein content than the SU, U+NIT or U+DMPSA treatments. In this cropping season (year 2), grain yield and grain N content were negatively correlated ($P = 0.000$, $n = 28$, $r = -0.86$). The U+DI treatment had the highest total gliadin and glutenin concentrations in both years (Table 2), and was significantly higher than that of some of the other fertilized treatments. However, differences between treatments regarding gluten proteins composition were generally small. On average, gliadins accounted for 64.6% and 68.3% of total gluten proteins in years 1 and 2, respectively (data not shown). The corresponding gliadin to glutenin ratios were 1.9 and 2.3 (data not shown). Grain N content was positively correlated with both gliadin ($P = 0.000$, $n = 56$, $r = 0.74$) and glutenin contents ($P = 0.000$, $n = 56$, $r = 0.55$) in both years.

3.5. N efficiency and yield-scaled emissions

In the first year, U+DI led to the numerically highest NUE value, while SU decreased N efficiency compared to U+NBPT and U+DI (Table 3). Regarding the N surplus, the treatments involving NBPT (U

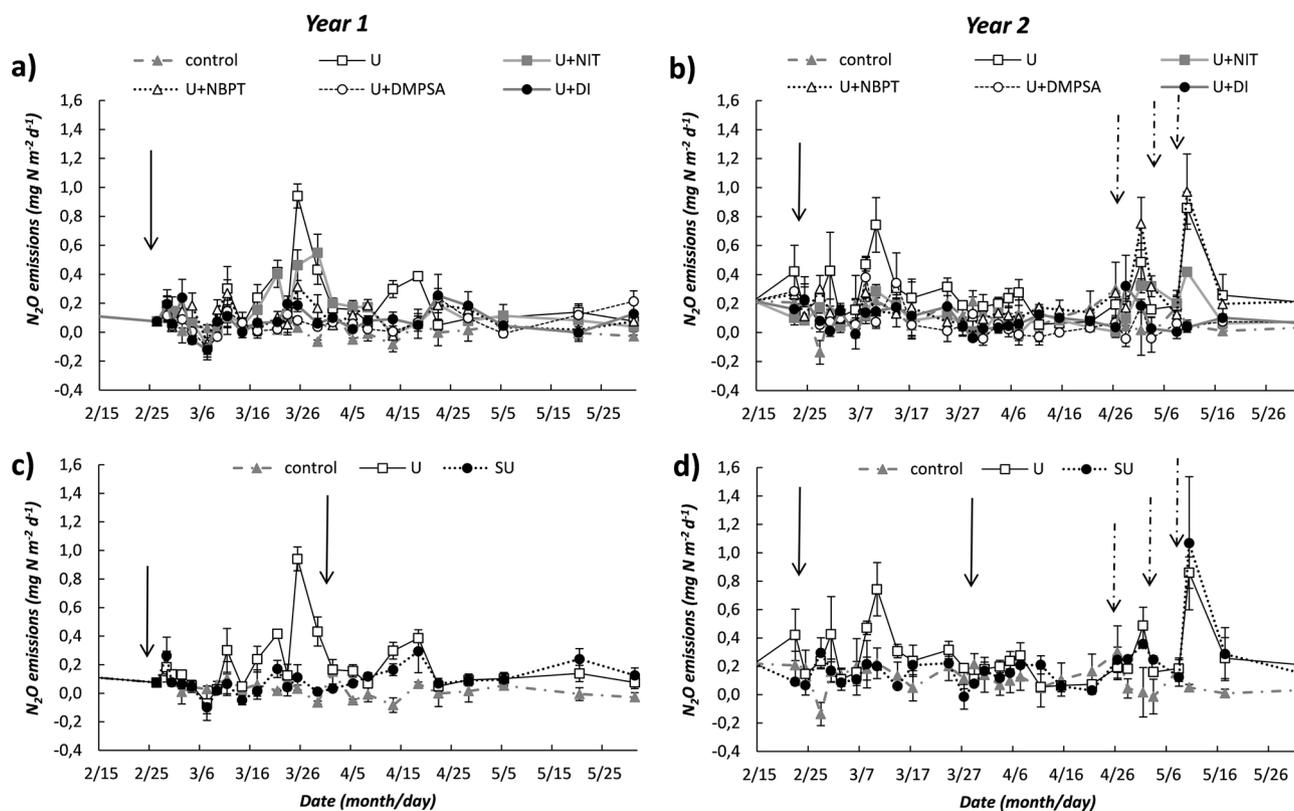


Fig. 4. N_2O emissions in the February–May period in year 1 (a, c) and 2 (b, d) in the different treatments: U (Urea), U + NIT (Urea + Nitrapyrin), U + NBPT (Urea + NBPT), U + DMPSA (Urea + DMPSA), U + DI (Urea + DMPSA + NBPT), split urea (SU) and control. Data are provided separately for inhibitor-based treatments (a, b) and split urea (SU, c, d); and for year 1 (a, c) and 2 (b, d). The plain and dotted arrows indicate N fertilization and irrigation events, respectively. Vertical bars indicate standard errors ($n = 3$). Outside of the represented period, emissions of N_2O were generally negligible (-0.1 to $0.1 \text{ mg N m}^{-2} \text{ d}^{-1}$), and fluxes are not shown to improve readability of the figures.

+NBPT and particularly U + DI) had the lowest surpluses while the highest value was reported for SU ($P < 0.05$) in year 1. In the second year, SU and U resulted in the highest and lowest NUEs (and therefore the lowest and highest surplus), respectively, with the rest of the treatments having intermediate results. The average NUEs were 62.2% and 29.8% in years 1 and 2, respectively ($P < 0.001$). In agreement, the average N surplus in the second year ($-14.9 \text{ kg N ha}^{-1}$) was higher than that in year 1 ($-23.8 \text{ kg N ha}^{-1}$).

In year 1, GHGI and YSNO emissions followed a similar pattern as N_2O and NO emissions, respectively (Table 3). In the second year, U and U + NBPT led to the highest GHGIs, while SU significantly decreased this index (thus counterbalancing its high N_2O losses). NI-based treatments resulted in the lowest GHGIs, but the double inhibitor increased the amount of $\text{CO}_2\text{-eq}$ emitted per kilogram of grain yield, compared to U + DMPSA. YSNO emissions were decreased in all fertilized treatments in comparison to U (except in U + NBPT), with U + DMPSA being the most effective N-fertilized option. Average GHGI and YSNO were increased in year 2 ($183.1 \text{ g CO}_2\text{-eq kg grain}^{-1}$ and $2.45 \text{ g N kg grain}^{-1}$, respectively) with respect to year 1 ($23.7 \text{ g CO}_2\text{-eq kg grain}^{-1}$ and $0.26 \text{ g N kg grain}^{-1}$, respectively) by factors of 7.6 and 9.1, respectively ($P < 0.001$).

4. Discussion

4.1. Effect of NIs on N oxides emissions

The new inhibitor DMPSA was consistently efficient in mitigating N_2O losses in both year 1 (68% mitigation) and 2 (100% mitigation) (Table 1). High mitigation efficacies with the use of this NI, in comparison to those reported by the meta-analysis of Gilsanz et al. (2016), provides evidence of the importance of nitrification as a dominant

processes generating N_2O in these calcareous and low organic C content soils (Cui et al., 2012; Guardia et al., 2018) and the effectiveness of DMPSA. In the typical rainfall cropping season (year 1), the higher mitigation efficacy of U + DMPSA for NO (86%) than for N_2O (68%) also supports the relevance of nitrification, which has been suggested as the main source of NO (Medinets et al., 2015). The correlation between NH_4^+ depletion and N_2O emissions (see section 3.2.1) and by the higher NO fluxes compared to those of N_2O in both years (Akiyama et al., 2004) are also in agreement with the importance of nitrification. Indeed, average NO emissions exceeded those of N_2O by factors of 2.8 and 3.0 in years 1 and 2, respectively. Field studies measuring both N_2O and NO emissions after DMPSA application are currently limited to irrigated conditions and the use of calcium ammonium nitrate (Guardia et al., 2017), therefore, this study demonstrates that DMPSA is also effective in mitigating both N oxides (NO and N_2O) from urea in rainfed crops.

In comparison to urea only, the addition of nitrapyrin to urea significantly reduced cumulative N_2O (by 30% and 77% in years 1 and 2, respectively) and NO emissions (by 67% and 67% in years 1 and 2, respectively). Even though the meta-analysis of Thapa et al. (2016) reported similar mitigation efficacies for DMPP (a pyrazole-based inhibitor) and nitrapyrin under a wide range of environmental conditions, in our study DMPSA always surpassed nitrapyrin (Table 1) regarding N oxides abatement. We hypothesize that the application of nitrapyrin as liquid solution may have enhanced the release of N oxides particularly after fertilization thus decreasing its efficacy, since soil moisture is a limiting factor for N oxides emissions, particularly in rainfed semi-arid crops (Pilegaard, 2013; Aguilera et al., 2013; García-Marco et al., 2014). This effect was noticed in the first year, especially for NO fluxes (Fig. 5a), which were similar or even higher than those of urea in the first weeks after N application. However, both NIs maintained their

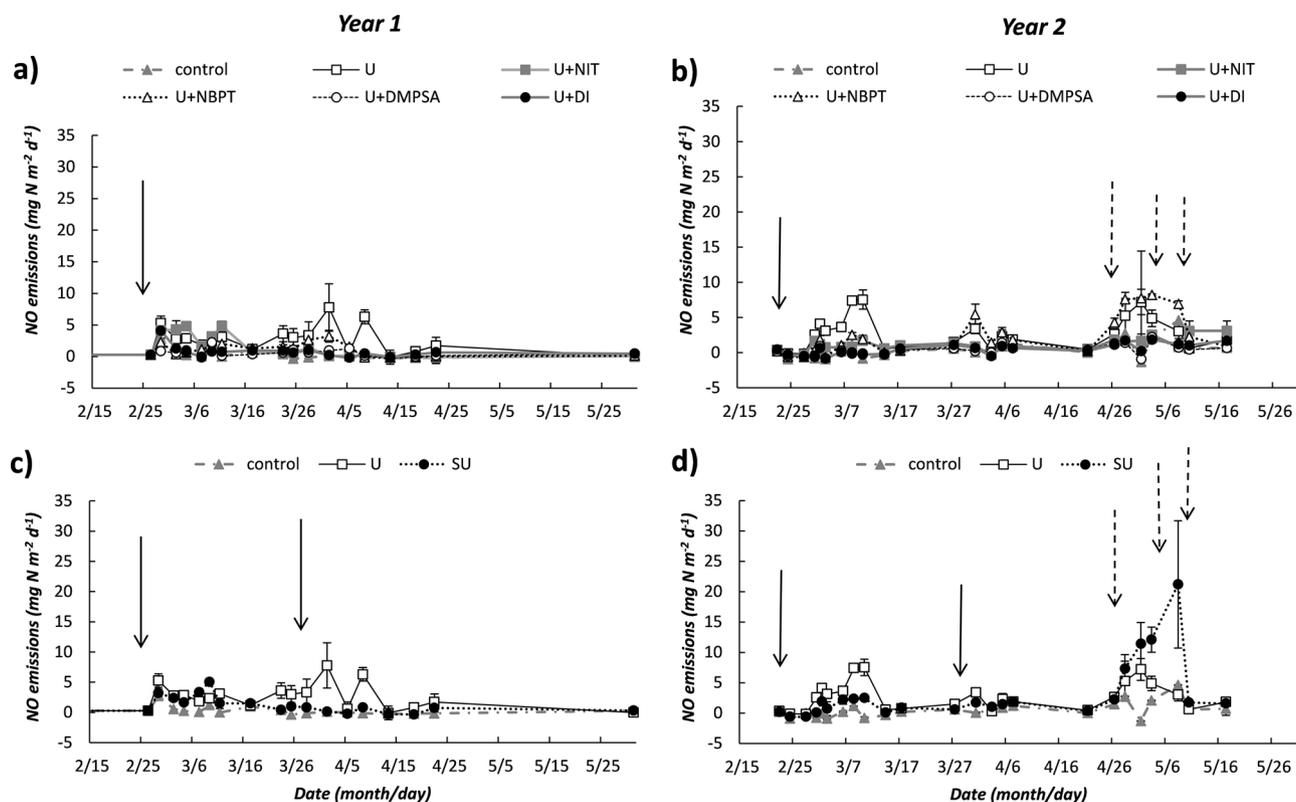


Fig. 5. NO emissions during the experimental period in the February–May period in year 1 (a, c) and 2 (b, d) in the different treatments: U (Urea), U + NIT (Urea + Nitrpyrin), U + NBPT (Urea + NBPT), U + DMPSA (Urea + DMPSA), U + DI (Urea + DMPSA + NBPT), split urea (SU) and control. Data are provided separately for inhibitor-based treatments (a, b) and split urea (SU, c, d); and for year 1 (a, c) and 2 (b, d). The plain and dotted arrows indicate N fertilization and irrigation events, respectively. Vertical bars indicate standard errors ($n = 3$). Outside of the represented period, emissions of NO were negligible ($< 1 \text{ mg N m}^{-2} \text{ d}^{-1}$), and fluxes are not shown to improve readability of the figures.

mitigation efficacy consistently through cropping seasons for one to more than two months after N application, when the highest peaks of N oxides occurred (Figs. 4 and 5). This was supported by the increments in average soil NH_4^+ concentrations and decreases in average NO_3^- contents after tillering, in comparison to urea alone (Fig. 3). However, this was not observed for the N_2O peak in October 2017, 8 months after fertilization. Therefore, we did not observe any significant residual effect of any of the NIs applied, contrary to some previous findings (e.g. Alonso-Ayuso et al., 2016). The occurrence of N oxides pulses after the first rainfall events in autumn, arising from unusually high amounts of residual N (Fig. 3d) has been previously described by other authors (e.g. Sanchez-Martin et al., 2010; Leitner et al., 2017).

4.2. Effect of NBPT on N oxides emissions

Contrary to NIs, the effectiveness of the UI, NBPT, in mitigating N oxides emissions was greatly influenced by the meteorological conditions in each year. Indeed, the U + NBPT treatment significantly decreased N_2O and NO emissions during the first year (by 57% and 70%, respectively), compared to urea (Table 1). These values were lower than those obtained by Abalos et al. (2012) under similar conditions (86% for N_2O and 88% for NO), and also lower than those for DMPSA. However, during the second year the cumulative N emissions from U + NBPT were not significantly different from those from the urea treatment. In spite of the high NBPT effectiveness in reducing emissions prior to wheat flowering (Figs. 4 and 5), we observed marked N oxides pulses after flowering because of the high substrate (mineral N)

Table 1

Cumulative N_2O -N, NO-N and CH_4 -C fluxes for the different treatments: U (Urea), U + NIT (Urea + Nitrpyrin), U + NBPT (Urea + NBPT), U + DMPSA (Urea + DMPSA), U + DI (Urea + DMPSA + NBPT) and control. 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_2O 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	198.4 a	259.6 a	169.9 a	507.9 b	-140.2	-190.1
U	348.8 e	494.5 c	1886.8 d	1929.8 d	-235.5	-171.6
U + NBPT	263.5 bc	440.5 c	693.0 c	1857.2 d	-215.2	-23.8
U + NIT	303.8 d	341.4 b	738.7 c	981.2 c	-216.5	-75.4
U + DMPSA	245.9 b	281.0 a	408.2 b	363.3 a	-152.3	-63.4
U + DI	253.4 bc	306.6 ab	410.6 bc	487.6 b	-86.8	-235.1
SU	281.9 cd	425.5 c	821.3 c	2437.9 d	-99.5	-140.7
S.E.	9.9	25.1	191.0	191.2	53.8	70.5
P value	0.000	0.000	0.000	0.000	0.441	0.427

Table 2

Grain and straw yield, grain N and total gliadin and glutenin contents for the different treatments: U (Urea), U + NIT (Urea + Nitrapyrin), U + NBPT (Urea + NBPT), U + DMPSA (Urea + DMPSA), U + DI (Urea + DMPSA + NBPT) and control. 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	Grain yield (kg ha ⁻¹)		Straw yield (kg ha ⁻¹)		N content grain (%)		Total gliadin content (mV min mg ⁻¹ flour)		Total glutenin content (mV min mg ⁻¹ flour)	
	Year 1 ^a	Year 1 ^a	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
control	2199 a	3258 a	2.23 a	3.00 a	0.78 a	1.16 a	0.50 a	0.50 a		
U	2981 b	7339 b	2.86 b	3.39 c	1.03 ab	1.42 ab	0.56 ab	0.68 ab		
U + NBPT	3025 b	7048 b	2.97 bc	3.31 bc	1.00 ab	1.35 ab	0.55 ab	0.65 ab		
U + NIT	3077 b	6550 b	2.79 b	2.99 a	1.14 b	1.21 a	0.60 ab	0.55 a		
U + DMPSA	2959 b	5941 b	2.91 b	2.96 a	1.12 b	1.28 ab	0.62 b	0.58 a		
U + DI	2766 b	6107 b	3.18 c	3.40 c	1.17 b	1.58 b	0.67 b	0.81 b		
SU	2941 b	6155 b	2.85 b	3.11 ab	1.02 ab	1.34 ab	0.59 ab	0.56 a		
S.E.	154	540	0.09	0.08	0.11	0.11	0.07	0.07		
P value	0.029	0.000	0.000	0.001	0.236	0.217	0.281	0.056		

^a Grain and straw yields in 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 kg ha⁻¹).

availability resulting from the lower (drought-related) wheat N uptake (Fig. 3, see Section 3.1). When irrigation water was applied, this residual soil N resulted in marked increases in N oxides emission. Since these peaks occurred 67 and 76 days after N fertilization, the more temporary effect of NBPT had disappeared, in contrast to the NIs. Our results demonstrate that due to the climatic variability of rainfed semi-arid crops and the transient effect of NBPT, the potential of this strategy to mitigate N oxides emissions is uncertain and, in any case, lower than that of NIs. Therefore, the possible reduction of the efficacy of mitigation practices (e.g. inhibitors) under a global change scenario (i.e. increment of global temperatures, drought episodes) should be considered for the implementation of cost-effective practices by stakeholders and policymakers.

4.3. Effect of the double inhibitor (DMPSA + NBPT) on N oxides emissions

The mixture of UI and NI was developed to achieve a reduction in total N losses combining the beneficial effect of UIs on NH₃ volatilization abatement and that of NIs on the reduction of emissions of N oxides and N leaching (Kim et al., 2012b; Pan et al., 2016). In our study, U + DMPSA and U + DI generally resulted in similar N₂O and NO emissions (except lower NO emissions in year 2 from U + DMPSA). This result and the observed tendencies are in agreement with the study of Zhao et al. (2017), which reported that the effectiveness of DMPP alone significantly surpassed that of DMPP + NBPT in a calcareous soil. They speculated that this effect could be driven by the decomposition of NBPT and by other side reactions when both inhibitors are mixed. The low N₂O fluxes in this rainfed semi-arid crop masked this effect, so the significant reduction of N oxides together with the NH₃ mitigation potential of NBPT (Pan et al., 2016) should also be taken into account,

Table 3

N use efficiency (NUE), N surplus, greenhouse gas intensity (GHGI) and yield-scaled NO emissions (YSNO) for the different treatments: U (Urea), U + NIT (Urea + Nitrapyrin), U + NBPT (Urea + NBPT), U + DMPSA (Urea + DMPSA), U + DI (Urea + DMPSA + NBPT) and control. 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	NUE (%)		N surplus (kg N ha ⁻¹)		GHGI (g CO ₂ -eq kg grain ⁻¹)		YSNO (g N kg grain ⁻¹)	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
control	–	–	–	–	22.6 a	90.7 a	0.08 a	0.77 ab
U	61.1 ab	16.1 a	–22.6 bc	1.2 b	29.0 c	368.9 d	0.63 d	5.77 e
U + NBPT	65.5 bc	32.7 ab	–27.8 ab	–18.7 ab	21.4 a	263.9 cd	0.23 c	4.24 de
U + NIT	56.1 ab	22.9 ab	–16.6 bc	–5.2 ab	24.3 b	159.5 abc	0.24 c	1.74 bc
U + DMPSA	62.7 abc	30.3 ab	–24.4 bc	–15.8 ab	20.6 a	80.8 a	0.14 b	0.41 a
U + DI	77.3 c	25.0 ab	–42.0 a	–9.5 ab	23.4 ab	202.9 bc	0.22 c	1.37 bc
SU	50.4 a	51.5 b	–9.67 c	–41.3 a	24.7 b	115.3 ab	0.29 c	2.84 cd
S.E.	5.0	10.0	5.8	12.1	1.5	37.6	0.05	0.62
P value	0.000	0.157	0.000	0.146	0.000	0.000	0.000	0.000

since the amount of N loss is often greater than that of other gaseous forms (Zhou et al., 2016).

4.4. Effect of splitting urea applications on N oxides emissions

As for the U + NBPT treatment, our results indicated that SU only mitigated N oxides emissions during the first year. Therefore, its effectiveness is highly dependent on rainfall distribution after fertilization events. Our results suggest that in dry years with water scarcity during tillering and stem elongation, splitting the application of U may enhance the risk for N oxides pulses after subsequent rainfall/irrigation events, as a result of the inefficient N uptake by plants which leads to increased opportunities for microbial N transformations in soil (Abalos et al., 2014b). In addition, split applications under conditions of fast urea hydrolysis and subsequent nitrification (relative to N transport to roots) may cause splitting N to be inefficient as a surface-scaled N₂O mitigation strategy, as observed by Venterea et al. (2016) in a rainfed maize crop. Since splitting N can have a negative effect on N₂O losses reduction but also enhance crop yields (Wang et al., 2016), the assessment of yield-scaled emissions, grain yield and quality, surplus and N efficiency is needed to gain a complete view of the sustainability of these urea-based strategies.

4.5. Selecting the best mitigation strategies for rainfed winter wheat

The calculated N surpluses revealed that in year 1 (which was wetter than year 2) the crop obtained more N from the soil, via net mineralization. The scale of the calculated N surpluses for both years are within the range of “no effect” on yield scaled-emissions as suggested by Van Groenigen et al. (2010). However, both GHGI and YSNO

emissions were markedly increased in year 2, compared to year 1. The grain yields obtained in year 2 were visibly affected by severe drought conditions (Table 2). The setting-up of the irrigation system occurred after stem elongation stage, so the scarcity of soil moisture during the stages of tillering and stem elongation (Fig. 2), which are critical for N uptake (López-Bellido et al., 2005), resulted in a devastating effect on grain yield and a high content of soil residual N after these stages (see section 3.1 and Fig. 3), some of which was then lost to the environment. In this dry year, we observed a positive response of some N management strategies (SU, U + DMPSA, and U + NIT) on grain yields, compared to urea. We hypothesize that under optimum conditions (rainfall, N rate), the response of yield to fertilization management (e.g. inhibitors) is often masked (Rose et al., 2018). However, under limiting conditions (in this case, the severe drought), responses to N management can be detected. However, this depends on the extent of the limiting conditions (e.g. drought), which in year 2 resulted in very poor grain yields. Under extreme drought conditions, N fertilization was a useless and even counter-productive strategy, as shown by the similar grain yields in the control and the top-yielding treatments (SU and U + DMPSA).

Protein content was clearly influenced by the common negative relationship between grain N content and grain yield (Simmonds, 1995) that we also noticed. In both years, the use of NBPT (particularly U + DI) showed the potential to biofortify bread-making wheat through the enhancement of grain protein. Previous studies have shown that NH_4^+ -N based plant nutrition increases protein content (Fuertes-Mendizábal et al., 2013). We hypothesize that the slow-release effect of NBPT may prolong NH_4^+ availability, thus raising grain protein. In addition, the potential direct uptake of NBPT by the crop could have promoted N remobilization, as shown by Artola et al. (2011) and Cruchaga et al. (2013), leading to enhanced N content in the grain. Gluten proteins (gliadins and glutenins), which are related to bread-making quality (i.e. extensibility, tenacity and elasticity, Barak et al., 2013), followed a similar tendency as total grain proteins, reaching maximum values in U + DI treatment. The increments in grain protein were not observed for DMPSA, in agreement with Huérfano et al. (2016). All of the NIs (DMPSA, nitrapyrin or NBPT) showed a neutral effect on the composition of gluten proteins and therefore rheological properties. Contrary to other authors (e.g. Fuertes-Mendizábal et al., 2013; Xue et al., 2016), we did not observe any effect of splitting N fertilization on the composition of gluten proteins.

The cost of purchasing inhibitors (NIs and UIs) is one of the main barriers to their widespread use (Sanz-Cobena et al., 2017). Consequently, it is important to evaluate the effects of these products on N efficiency, crop yield and quality in order to obtain a complete view of their potential advantages, in addition to the documented public economic benefit of reducing the environmental impacts of N pollutants (Qiao et al., 2015; Yang et al., 2016). In the typical rainfall year, the U + DI treatment gave the best balance between N oxides mitigation, NUE, N surplus and protein content, while no effect of N fertilized treatments was observed regarding grain yield. The high NUE values and enhanced protein content in NBPT-based treatments (particularly U + DI) could be attributed to the abatement of NH_3 losses as widely reported by previous studies (Sanz-Cobena et al., 2014), which are quantitatively more relevant, from an agronomic perspective, than those of N oxides (Zhou et al., 2016). The U + DMPSA treatment resulted in the lowest GHGI (similar to that of U + NBPT and U + DI, Table 3) and YSNO emissions, suggesting it is a promising mitigation strategy. In the second year, U + DMPSA was again the most effective GHGI and YSNO mitigation strategy, while high yields in the SU treatment offset partially the high surface-scaled emissions. The enhancement of grain yields with the tillering-stem elongation fractionation of fertilizer dose, which was also observed by López-Bellido et al. (2005), only occurred in the driest year in our experiment. In agreement with these authors, SU also increased NUE nearly doubling that of other fertilizer treatments, although differences were only significant

when compared to urea alone.

5. Conclusions

Our results showed that all alternative treatments (inhibitors or splitting urea) decreased surface-scaled and yield-scaled GHG and NO emissions, compared to urea alone. In the first year (with a typical rainfall amount), the use of the double NBPT + DMPSA inhibitor led to the best balance between mitigation of yield-scaled N oxides emissions, N efficiency, and crop yield and bread-making quality (total N in grain and gluten proteins). In the following dry year, the grain yields did not respond positively to N fertilization, although NIs (nitrapyrin and DMPSA) increased grain yield in comparison to urea only. During this second year, the urea + NBPT treatment was not an effective mitigation strategy since the main N oxides peaks occurred when its effect had run out.

Splitting urea should be recommended rather than a single dressing application of urea, although its efficacy was lower in our study than that of the inhibitors. During dry years, splitting urea applications can improve grain yields but with the risk of increasing N oxides losses. The effectiveness of nitrapyrin in mitigating yield-scaled emissions was generally exceeded by that of DMPSA. The application method and rate of nitrapyrin application, which is still not used in Europe, should be improved to enhance its mitigation potential. The use of DMPSA with urea was the most effective yield-scaled emissions mitigation option, regardless of rainfall conditions. The use of NBPT (particularly in the double inhibitor treatment) showed the potential to biofortify wheat through the enhancement of grain protein, including those related to bread-making quality (glutenins and gliadins). Our results suggest that in spite of the inexistent effect of inhibitors on grain yield during typical rainfall years, the enhancement of NUE and/or grain quality, and also the increase of grain yield under drought conditions can help offset the price of these inhibitors for farmer.

Our results also showed that if water supply is not enough during tillering and stem elongation stages, the application of N fertilizers can be even counter-productive leading to higher yield-scaled emissions, N surpluses and low NUEs. These low N uptake efficiencies should be avoided to prevent severe gross margin penalties and pulses of N oxides emissions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.agee.2018.06.033>.

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