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Increasing N use efficiency while decreasing gaseous N losses in a non-tilled wheat (*Triticum aestivum* L.) crop using a double inhibitor

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ABSTRACT

No tillage management is being encouraged nowadays to prevent soil erosion and contribute to carbon (C) sequestration. However, N fertilizer should be managed properly to optimize N use efficiency and to avoid the side-effects of losses of gaseous nitrogen (N), particularly those of ammonia (NH_3), which are expected to increase due to the presence of residues in the topsoil. In this context, a non-tilled (two years after conversion from tillage) field experiment was set up in central Spain, in which urea treated with a combination of urease (N-butyl thiophosphorictriamide, NBPT) and nitrification (2-(3,4-dimethyl-1H-pyrazol-1-yl) succinic acid isomeric mixture, DMPSA) inhibitors was compared with the use of urea without inhibitors. Fluxes of NH_3 (using the integrated horizontal flux method with three replicates), nitrous oxide (N_2O), methane (CH_4) and soil respiration were measured throughout the wheat (*Triticum aestivum* L.) cropping cycle under rainfed conditions, together with ancillary soil measurements (e.g., soil moisture and mineral N), yield and crop uptake of N. Gaseous N losses from urea without inhibitors accounted for 10.35% and 0.66% (for NH_3 and N_2O, respectively) of total N applied. The use of the double inhibitor resulted in a significant mitigation of both losses (by 50.5% and 91.6% for NH_3 and N_2O, respectively). The double inhibitor increased N use efficiency expressed as crop recovery efficiency and partial nutrient balance, and decreased N surplus, in comparison with urea without inhibitors. In spite of a similar grain yield, the unfertilized control had the lowest aboveground N uptake rate, due to the higher concentrations of grain and straw N for the fertilized treatments. Under the conditions of our study, NH_3 volatilization in a short-term no-till managed field can be minimized using a double (urease *plus* nitrification) inhibitor. This strategy had no negative side effects on N surplus, CH_4 uptake or N_2O mitigation and led to a significant improvement of N use efficiency.

1. Introduction

The mitigation of the negative impacts of soil erosion is a key driver of the increasing importance of conservation agriculture practices, e.g., no tillage, particularly in semi-arid croplands (Kassam et al., 2012; Tan et al., 2020). The enhancement or conservation of soil organic carbon (C) stocks with the adoption of no tillage (Aguilera et al., 2013; Chenu et al., 2019) is currently a pillar of several initiatives aiming to increase the sustainability of crop production, e.g., the 4 per 1000 initiative, which was launched by the French government at the twenty-first session of the Conference of the Parties (COP 21) (Soussana et al., 2019; Rumpel et al., 2020). Moreover, several benefits of no tillage on soil physical (Li et al., 2019) or biological (Nunes et al., 2020) quality have been highlighted in recent meta-analyses, e.g., improvements in water stable aggregates, hydraulic conductivity, available water capacity, microbial biomass, soil respiration or permanganate oxidizable C, among other soil health indicators. However, the objective of increasing crop sustainability through no tillage could be compromised if its adoption leads to an increase of losses of gaseous nitrogen (N) to the atmosphere e.g., the greenhouse gas (GHG) nitrous oxide (N_2O) or -particularly- the reactive gas ammonia (NH_3). Along these lines, the meta-analysis of Huang et al. (2018) reported an overall increase in N_2O

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emissions in comparison with conventional tillage, with increases dependent on environmental and management (e.g., fertilizer placement or experiment duration) conditions. Regarding NH_3 emission, the presence of crop residues on the soil surface with no tillage management may reduce the contact between ammonium ($N\text{-}NH_4^+$) released from urea hydrolysis and soil colloids and, therefore, act as a barrier for urea incorporation, thus potentially increasing N volatilization. This effect was reported by e.g., Pinheiro et al. (2018) in subtropical humid conditions using semi-open static chambers, and summarized across studies by the recent meta-analysis of Ma et al. (2021). Since the reduction of NH_3 losses is a priority in countries that currently exceed the emissions permitted under government regulations (Sanz-Cobena et al., 2014a, 2014b), some N management strategies are needed to maintain the benefits of no tillage and minimize these negative side-effects.

The potential risk for the increase of NH_3 volatilization from no tillage management in comparison with conventional tillage could be further increased at basal fertilization (i.e., when the soil cover depends mostly on the management of residues from the previous crop due to the incipient growth of the current crop) rather than at dressing fertilization (e.g., when cereals are at tillering stage thus covering a significant proportion of soil surface and masking the differences between tillage systems). Although applying N at seeding has been discouraged due to the potential increase of N losses (Abalos et al., 2017), particularly to groundwater (Xia et al., 2017), this practice may be necessary to prevent N immobilization (Cao et al., 2020) when the residues from the previous crop have a high C:N ratio (e.g., those of cereal crops). In these conditions, the use of urea is a widespread and cost-effective option due to its N-richness, low price, manageable storage and the lower GHG emissions associated with its industrial production, in comparison with other N sources such as ammonium nitrate (Guardia et al., 2019a), but with the negative trade-off of increasing NH_3 emissions (Pan et al., 2016). The incorporation of urea into the soil (mechanical or through irrigation) has been highlighted as an effective practice to mitigate NH_3 emissions (Sanz-Cobena et al., 2014a, 2014b; Pan et al., 2016), but the options in rainfed no-tilled agro-ecosystems are mainly limited to the use of urease inhibitors such as N-butyl thiophosphorictriamide (NBPT). The meta-analysis of Ti et al. (2019) estimated that the use of urease inhibitors mitigated NH_3 volatilization by 53.1%, overall. However, this reduction was not significant for wheat. Rainfed semi-arid cropping systems are associated with a high variability of rainfall amount and distribution, thus affecting the amount of NH_3 volatilized and, therefore, the cost-effectiveness of urease inhibitors as a NH_3 mitigation strategy. Additional measurements under these conditions are therefore needed, with the Integrated Horizontal Flux (IHF) providing a meteorology-sensitive method (Pacholski et al., 2008); considered as a reference technique. Surface requirements and the distance between plots often limit the number of replicates when using IHF (Recio et al., 2018) and field-studies using three replicates are scarce, thus limiting the robustness of the results. To our knowledge, only Recio et al. (2020) has measured NH_3 volatilization using the IHF method comparing urea with and without a double inhibitor, under irrigated conditions and using two replicates. No studies measuring NH_3 volatilization with micrometeorological techniques in non-tilled agro-ecosystems have been carried out so far.

Due to the variable effectiveness of NBPT in the mitigation of N_2O emissions (Guardia et al., 2018a), the use of a double nitrification and urease inhibitor is expected to be the most advisable solution to significantly mitigate both NH_3 and N_2O losses (Ding et al., 2011; Zaman and Nguyen, 2012; Recio et al., 2020). Most trials using double inhibitors have studied the combination of NBPT with the nitrification inhibitor diciandyamide (DCD), while no studies have looked at the effect of combining 2-(3,4-dimethyl-1H-pyrazol-1-yl) succinic acid isomeric mixture (DMPSA) with NBPT under rainfed conditions whilst simultaneously measuring N_2O and NH_3 emissions. The use of a double inhibitor was confirmed as the most-effective option among other enhanced-efficiency fertilizers for increasing N recovery in the crop (Sha et al., 2020), thus reducing the risks for high N surpluses and N losses to the atmosphere or waterbodies. However, even though several global studies have found a significant effect of enhanced-efficiency fertilizers on N use efficiency (NUE) and yields, this is often masked under field conditions where recommended N rates are often used (Rose et al., 2018). No significant increases in crop N uptake have been reported with the double inhibitor with respect to urea alone in some previous studies (e.g., Guardia et al., 2018a; Fu et al., 2020; Recio et al., 2020; Montoya et al., 2021). Nevertheless, the significant improvements found by e.g., Zaman and Nguyen (2012), Zaman et al. (2013), or Zhao et al. (2017), as well as the positive trends regarding oil yield (Montoya et al., 2021) or protein content and composition (Guardia et al., 2018a) under rainfed Mediterranean conditions suggest that this effect should be still explored.

In our field experiment, we measured NH_3 volatilization in a non-tilled wheat (*Triticum aestivum* L.) crop using the IHF method with three replicates in order to evaluate the mitigation potential of a double nitrification and urease inhibitor at seeding and dressing fertilization. In addition, the variability and the statistical significance of using three replicates for the NH_3 fluxes compared with two were studied. The side-effects on soil mineral N, N_2O emission, methane (CH_4) uptake, carbon dioxide (CO_2) fluxes, yield and NUE indicators were also evaluated. We hypothesized that under the conditions of our study, the use of the double inhibitor would mitigate NH_3 and N_2O losses (Recio et al., 2020), whilst increasing aboveground N uptake (Guardia et al., 2018a; Sha et al., 2020).

2. Materials and methods

2.1. Site description

The experiment was carried out during one annual cropping campaign (2019–2020) at the “Centro Nacional de Tecnología de Regadíos CENTER”, situated near Madrid (central Spain, latitude 40°25N, longitude 3°29′W). The experiment was split into three periods: from seeding fertilization to dressing fertilization (Period I), from dressing fertilization to harvest (Period II) and from harvest to the end of the experimental period (Period III). The experiment ended when the next crop was seeded, thus covering the whole wheat cycle and post-harvest period. The site has a typical Mediterranean climate with most rainfall occurring during spring and autumn and a dry and hot period during summer. During the last 10 years, the mean air temperatures during the months corresponding to Periods I, II and III were 5.5, 15.3 and 23.2 °C, respectively. Average cumulative rainfall during these periods was 109, 151 and 33 mm, respectively. The soil is a silt loam (Bouyoucos) *Typic xerofluvent*, (Soil Survey Staff, 2014) whose main physicochemical properties in the topsoil (0–15 cm) are: clay, 85.4 g kg^{-1}; silt, 520.3 g kg^{-1}; sand, 394.3 g kg^{-1}; pH (soil: water 1:2.5), 8.2; electrical conductivity (soil: water 1:5), 0.56 dS m^{-1}; total carbonates (calcimeter), 132.1 g kg^{-1}; total organic matter (Walkley-Black), 22.8 g kg^{-1}; bulk density (core samplers, Grossman and Reinsch, 2002), 1.4 g cm^{-3}. Data for the 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

Three different treatments: urea (U); urea with the double inhibitor NBPT+DMPSA (U+DI) and a control with no N fertilization, were arranged in a randomized block design with three replicates (Fig. S1). The plots had a surface area of 0.14 ha (36 m × 40 m) with a separation of 50 m. In the previous campaigns (2018–2019 and 2017–2018), the field was seeded with barley (*Hordeum vulgare* L.) and rape (*Brassica napus* L.), respectively. The field has been traditionally managed with conventional tillage, but the no-tillage management was first established for the barley crop campaign (2018–2019). Wheat (*Triticum aestivum* L. var. ‘Marcopolo’ was sown at the end of October over the residues of the previous crop (second campaign under no-tillage) at 200 kg seed ha^{-1}. Previously, the herbicides 'Cosmic' ® (glyphosate 36% w/v) and 'Trago' ® (derivate of 2,4-D) were applied at 2.5 and 0.5 L ha^{-1}, respectively. The fertilizers were applied at a rate of 40 kg N ha^{-1} (seeding fertilization, 26th November 2019) and 120 kg N ha^{-1} (dressing fertilization at tillering stage, 18th February 2020), giving a total rate of 160 kg N ha^{-1}. For the U+DI plots, the percentage of DMPSA in the fertilizer was 0.8% of ureic-N, while the urease inhibitor NBPT was 0.13% of the ureic-N. This fertilizer was supplied by EuroChem Agro. To guarantee an even application of fertilizers, we split the plots in 16 quadrants and applied the same amount of fertilizer to each. The field was kept free of pests following local practices, and two ancillary irrigation events (on 26th February and 9th March, 25 mm in total) were applied due to the dry and warm conditions following dressing fertilization.

2.3. NH_3 volatilization measurements and the effect of the number of replicates

Ammonia fluxes were measured using the micrometeorological mass-balance integrated horizontal flux (IHF) method as described in detail by Recio et al. (2018). Briefly, each central mast was equipped with five passive flux samplers (Leuning et al., 1985; Viguria et al., 2015) consisting of traps of oxalic acid, placed at different heights (0.25, 0.65, 1.25, 2.05 and 3.05 m above crop canopy). Two additional background masts with three passive flux samplers (0.25, 1.25, 3.05 m height) were placed outside of the fertilized plot (more than 50 m apart, Fig. S1) in the dominant wind direction for the area, in order to determine NH_3 background concentrations. Passive flux samplers were changed and/or collected 1, 3, 6, 9 14, 17, 23, 31 and 43 days after basal fertilization (Period I); and 1, 3, 6, 9, 13, 16, 21, 29 and 35 days after dressing fertilization (Period II). The IHF method permits the calculation of cumulative NH_3 emissions over the entire period as the sum of NH_3 volatilized during each measurement period. The net vertical flux (F, μg m^{-2} s^{-1}) from the treated surface is then calculated as explained in Bai et al. (2017) and Recio et al. (2018). Briefly, the horizontal fluxes are integrated over the height intervals and divided by the distance between the downwind and upwind boundaries (fetch), as follows:

$$F_{vert} = \frac{1}{x}\left[\int_{z_{i-1}}^{z_i}\left(\overline{u\cdot C}\right)_{plot}\cdot dz - \int_{z_{i-1}}^{z_i}\left(\overline{u\cdot C}\right)_{bg}\cdot dz\right] \tag{1}$$

Were F_{vert} is the vertical flux, x is the mean fetch length, z_{i-1} and z_i denote the height interval and the vector product $u{\cdot}C$ is the mean horizontal flux (u is the mean horizontal wind speed and C is the NH_3 concentration (μg m^{-3}) in the air which is trapped in the different passive flux samplers). Because of the design of the passive flux samplers, the influence of the wind speed is negligible and so the value of the mean horizontal flux ($u{\cdot}C$) is equated (by using a mass balance equation) to the mass of NH_3 collected per unit of time (sampling period) and the effective cross-sectional area of the sampler, which is determined in wind tunnel calibrations. Ammonia flux at the upwind mast position (plot) is subtracted from the downwind mast position (background, *bg*). Due to the rectangular shape of the plots, fetch length was calculated based on the mean wind directions (data from the meteorological station located at the experimental site) for one-hour periods.

Most studies in which NH_3 volatilization was measured using IHF methodology, were performed with two replicates (e.g., Sanz-Cobena et al., 2011; Recio et al., 2018, 2020; Montoya et al., 2021). In this trial, the variability obtained with three replicates (i.e., standard deviations) was analyzed. The statistical differences between the total cumulative fluxes for U and U+DI using the nine possible combinations of two replicates were analyzed using the Student-*t*-test at $P < 0.05$.

2.4. GHG sampling and analyses

Greenhouse gases were sampled using opaque static chambers (closed during one hour and placed over stainless steel rings). Two chambers (36 cm diameter, 19 cm height) per plot were randomly distributed and fertilized individually at the same rate as the corresponding main plots. The sampling frequency was 2–3 times per week during the first month after fertilization (Periods I and II). Afterwards, the sampling frequency gradually decreased, except when rainfall/irrigation events took place. During Period III, gas samples were only taken monthly, except after rainfall events. Three samples (0, 30 min and 60 min after closure) were taken at each sampling event, and the fluxes were calculated by linear regressions as recommended for three sampling points (Venterea et al., 2012). The plants fit under the chambers throughout the wheat cycle, so it was not necessary to clip them. More details about the chambers and sampling procedure can be found in Guardia et al. (2018a). Greenhouse gas concentrations were measured using a gas chromatograph HP-6890 GC (Agilent Technologies, Barcelona, Spain) with ECD and FID detectors, as described in Guardia et al. (2018a). Cumulative emissions were calculated by linear interpolation between consecutive sampling dates.

2.5. Soil and crop sampling and analyses

Soil samples (four soil cores in the 0–10 cm layer) were taken on all gas sampling occasions, even though soil mineral N was only analyzed for approximately half of them (with increased frequency after both fertilization events). The soil samples were extracted (soil:KCl 1 M 1:6.25) and then N-NH_4^+ and nitrate (N-NO_3) were analyzed by colorimetric detection using a flow injection analyzer. For all soil sampling occasions, the water-filled pore space (WFPS) was calculated using the bulk density and soil moisture by oven-drying at 105 °C. At harvest, wheat biomass was collected for four 0.25 m × 0.25 m squares in each plot and transported to the laboratory to separate grain and straw, which were weighed to estimate grain and straw yields and the harvest index. Once the plot samples were homogeneously mixed, a representative subsample of grain and straw was chosen to analyze total N in grain and biomass using the Dumas method, as explained in Guardia et al. (2018a). Soil dissolved organic C (DOC) and water-soluble N were determined by extracting homogeneously mixed soil with deionized water (1:6.25), and then analyzed with a total organic C and N analyzer (multi N/C 3100 Analityk Jena) by the Dumas method. The equipment is provided with IR and chemiluminiscence detectors to analyze the gaseous compounds derived from C and N, respectively. The data of these two soil variables (DOC and water-soluble N) were only used for the multivariate analyses.

2.6. NUE calculations and statistical analyses

Crop recovery efficiency (CRE) was calculated using the following equation:

$$CRE = \frac{U_N - U_0}{F} \tag{2}$$

Were 'U_N' and 'U_0' are the total plant N taken up into the removed plant components (grain + straw) for the fertilized (U and U+DI) and control plots, respectively; and 'F′ was the total N applied with the fertilizer (160 kg N ha^{-1}). The partial nutrient balance (PNB, denoted as 'NUE' by the EU Nitrogen Expert Panel and the study of Quemada et al., 2020) index was calculated as the ratio between 'U_N' and 'F′. The N surplus was estimated by the difference between 'F′ and 'U_N' (Quemada et al., 2020).

The statistical analyses, performed with Statgraphics software, consisted of analyses of variance taking into account the experimental design, i.e., "Block" and "Fertilizer" effects were included in the linear model. Previously, the variance homogeneity (Levene) and data normal distribution (Shapiro-Wilk) were checked, and the data were log-transformed when these assumptions were not met. Orthogonal contrast analyses were performed for comparing the different treatments (U versus U+DI and fertilized treatments versus control) at $P < 0.05$ significance level. The P values of each contrast were obtained using Student's t distribution with degrees of freedom equal to those of the model residual. Linear correlation analyses between soil parameters, temperatures and gaseous fluxes were also conducted (previously checking the normal distribution of the data). Two types of analyses were performed: i) using the average values at each sampling event (n = number of sampling days); and ii) using the average or cumulative values throughout the experiment (n = number of experimental units).

3. Results

3.1. *Environmental conditions, soil moisture and mineral N*

Mean air temperatures during periods I, II and III were 7.8, 13.8 and 21.5 °C, respectively (Fig. 1a). The cumulative rainfall in periods I, II and III was 107, 206 and 106 mm, respectively (Fig. 1a). The average maximum daily wind speed during the first week after fertilization was 6.8 and 3.9 m s^{-1} in periods I and II, respectively (data not shown), while mean air temperatures were 11.0 and 7.9 °C, respectively. Soil WFPS ranged from 17% to 72% (Fig. 1b). The first month after seeding fertilization, all WFPS values were between 50% and 70%. However, during the first month after dressing fertilization, several values between 30% and 50% were also observed. After harvest (Period III), intermediate values (41–64%) were reported after rainfall events.

The double inhibitor tended to increase N-NH_4^+ concentrations in the topsoil in the first and second Period (Fig. 2a). The effect of the NBPT was observed during the first week after basal fertilization (Fig. 1c), decreasing average N-NH_4^+ contents on 27th November. The effect of DMPSA enhancing N-NH_4^+ contents was observed 9 days after fertilization. A similar behavior was observed after dressing fertilization (second period), in which the use of the double inhibitor decreased N-NH_4^+ concentrations until 9 days after fertilization, with an increase thereafter, particularly on 24th March. After harvest (Period III), all N-NH_4^+ concentrations were below 15 mg N kg $soil^{-1}$ (Fig. 1c) and tended to be higher in the control (Fig. 2a).

During the first Period, an effect of the double inhibitor on soil N-NO_3^- availability was observed on some days (e.g., after mid-December, Fig. 1d), but no significant differences were observed in the average values for the whole period after seeding fertilization (Fig. 2b). Conversely, the significant reductions of N-NO_3^- in U+DI with respect to U after dressing fertilization led to lower ($P < 0.05$) average N-NO_3^- concentrations in the second Period and during the whole cropping cycle. The lowest soil mineral N contents were observed in the control plots (Fig. 2). After harvest (Period III), soil N-NO_3^- availability increased notably after the rainfall events in all treatments, including the control (Fig. 1d and 2b).

3.2. *Gaseous emissions*

3.2.1. *Ammonia volatilization and effect of the number of replicates*

After seeding fertilization, NH_3 fluxes peaked at 1 and, particularly, 3 days after fertilization, reaching 113.4 and 42.3 g N ha^{-1} day^{-1} for U and U+DI, respectively (Fig. 3a). Afterwards, daily fluxes decreased to values below 8 g N ha^{-1} day^{-1}. Cumulative emissions were significantly reduced in U+DI treatment in comparison with U (Table 1), accounting for 23.8% and 15.4% of N applied for U and U+DI, respectively. After dressing fertilization, maximum fluxes were reported 6 days after fertilization, reaching a flux of 34.7 and 10.3 g N ha^{-1} day^{-1} for U and U+DI, respectively (Fig. 3b). Afterwards, daily fluxes decreased and were below 2.5 g N ha^{-1} from 16 days after fertilization in both treatments. Cumulative volatilization was also significantly higher for U than for U+DI (5.9% and 1.7%, respectively, of the total N applied at dressing).

The hypothesis test for all the possible combinations of two replicates (Table 2) showed that five of the nine combinations would not reject the null hypothesis at the end of the experimental period ($P > 0.05$, three of them with $P > 0.10$) as opposed to that for three replicates (Table 1). With respect to the variability of the three replicates for the different periods, in the case of U, which was the treatment with the highest NH_3 fluxes, the average values ± standard deviations in Periods I, II and total were 9.5 ± 2.9, 7.1 ± 0.9 and 16.6 ± 3.6 kg N ha^{-1}, respectively.

3.2.2. *Nitrous oxide emissions*

After seeding fertilization, N_2O fluxes increased in the N-fertilized treatments, particularly in U, reaching 1.4 mg N m^{-2} d^{-1} 14 days after fertilization (Fig. 1e). One month after fertilization, fluxes in U decreased to similar values as the unfertilized control. A similar behavior was observed after N application at dressing, even though in this case the daily fluxes for U reached 2.8 mg N m^{-2} d^{-1}. During Periods I and II, the N_2O fluxes in the U+DI treatment were low and similar to those from the control plots, with a maximum value of 0.3 mg N m^{-2} d^{-1}. After harvest (Period III), short-lived episodes of N_2O peaks (Fig. 1e) were detected, particularly after a rainfall event in mid-August (25 mm, Fig. 1a), reaching 0.9 mg N m^{-2} d^{-1} in U treatment. This post-harvest period accounted for 15% (U) to 45% (control) of total cumulative emissions (Table 1). The double inhibitor decreased annual cumulative N_2O emissions by 91.6% with respect to U ($P < 0.05$; Table 1), and its mitigating effect was significant in Periods I and II, while a similar but non-significant reduction ($P < 0.06$) was also observed in Period III. Cumulative N_2O emissions were significantly correlated with average N-NO_3^- concentrations (Table S1, $P < 0.001$, n = 18, r = 0.90), while daily N_2O fluxes were positively correlated with soil WFPS (Table S2, $P < 0.01$, n = 34, r = 0.46) and respiration fluxes ($P < 0.05$, n = 34, r = 0.37).

3.2.3. *Methane and respiration fluxes*

Fertilized and control plots were net CH_4 sinks (Table 1), although some positive fluxes were observed occasionally in the three experimental periods, most notably those on 5th May (0.8 mg C m^{-2} d^{-1}), 14th August (1.0 C m^{-2} d^{-1}, 3 days after the soil rewetting) and 13th October (1.1 C m^{-2} d^{-1}) (Fig. S2a). Methane uptake was negatively correlated with dissolved organic C content ($P < 0.001$, n = 14, r = −0.82). Cumulative CH_4 consumption rates were higher in U+DI than in U ($0.05 < P < 0.10$, Table 1). Daily respiration fluxes (Fig. S2b) ranged from 0.1 to 1.1 g C m^{-2} d^{-1} and from 0.8 to 7.0 g C m^{-2} d^{-1} in Periods I and II, respectively. No significant differences in CO_2 emissions were observed between treatments in any of these two periods or in the annual cumulative fluxes (Table 1). After harvest, the mid-August rainfall also caused a short-term rise in CO_2 fluxes, particularly in the fertilized plots.

3.3. *Yield components and N use efficiency*

The unfertilized control had statistically similar straw and grain yields (Table 3) as the fertilized treatments. However, the control treatment tended to have larger ($P = 0.118$) and smaller ($P = 0.053$) grain and straw yields, respectively, in comparison with the fertilized plots. Therefore, the control had a higher harvest index than U and U+DI (by 23.5% on average). However, the plots that did not receive any N from synthetic fertilizers had significantly lower N concentrations in both straw and grain. As a result, the N yield in aboveground biomass (straw + grain) was higher in U and U+DI than in the control plots ($P < 0.01$). When comparing the fertilized treatments with each other, no significant differences were found in grain and straw yields or the harvest index. The use of the double inhibitor, however, resulted in a numerically larger N content in straw (by 22%) and grain (by 5%). In comparison with U, the U+DI treatment significantly increased both PNB and CRE (by 14% and 36%, respectively), and decreased N surplus by 37% ($P < 0.05$).

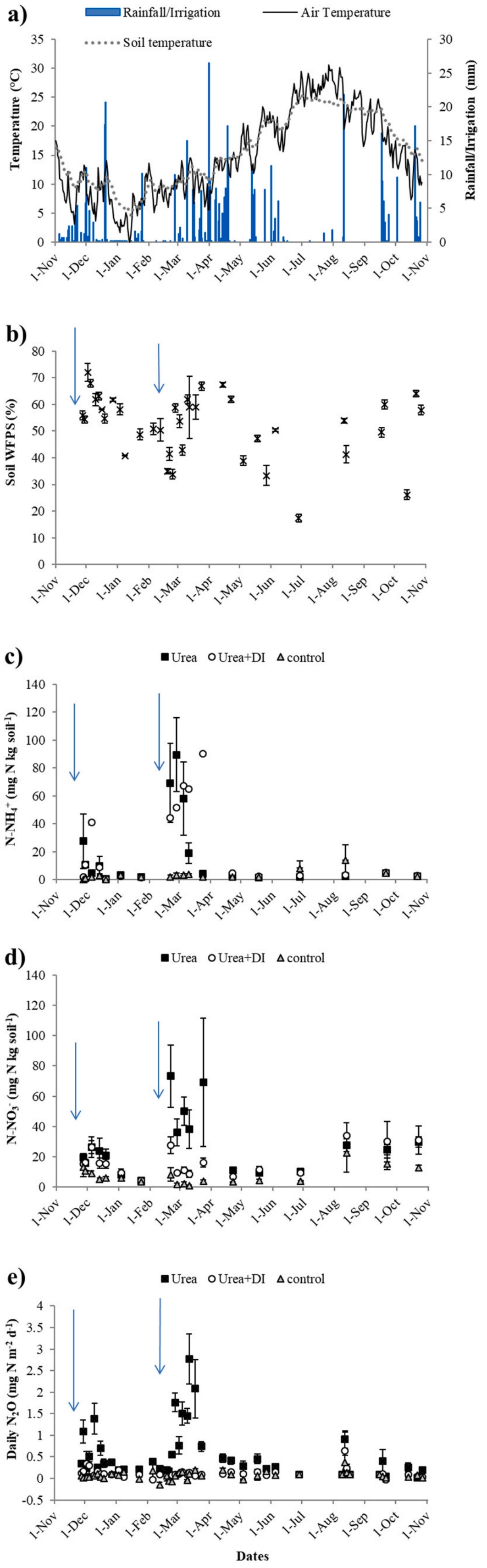

Fig. 1. (a) Air and soil temperatures, rainfall/irrigation, (b) soil WFPS, (c) soil NH_4^+ and (d) NO_3^- concentrations, and (e) N_2O emissions during the experimental period. Treatments were urea (U), urea + NBPT + DMPSA (U+DI) and control. Vertical bars indicate standard errors, and the arrows denote the fertilization events.

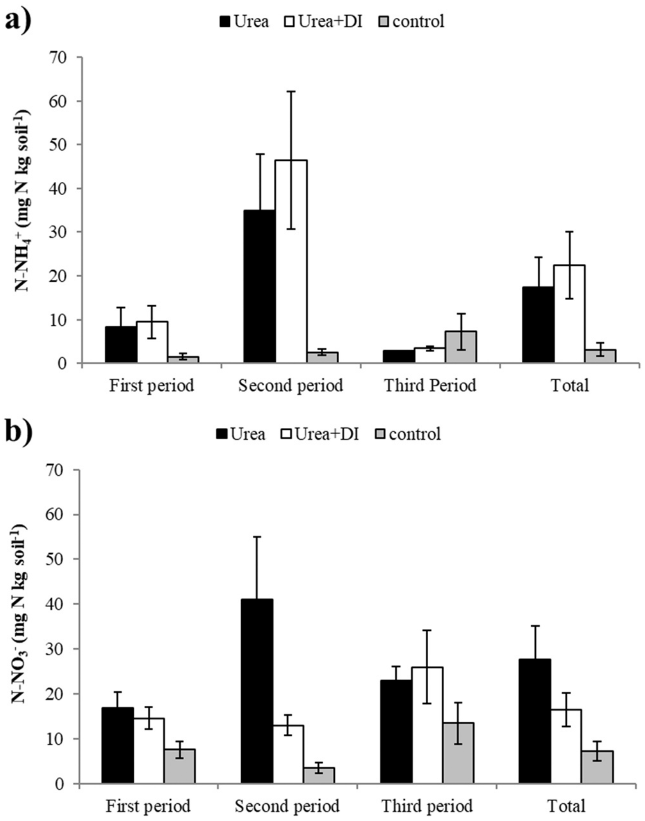

Fig. 2. Average NH_4^+ and NO_3^- concentrations for the urea (U), urea + NBPT + DMPSA (U+DI) and control treatments during three periods and for the whole experimental period. The three periods were: from seeding fertilization (November 2019) to dressing fertilization (mid-February 2020, Period I), from dressing fertilization to harvest (mid-June, Period II) and from harvest to the end of the experimental period (Period III). Vertical bars indicate standard errors.

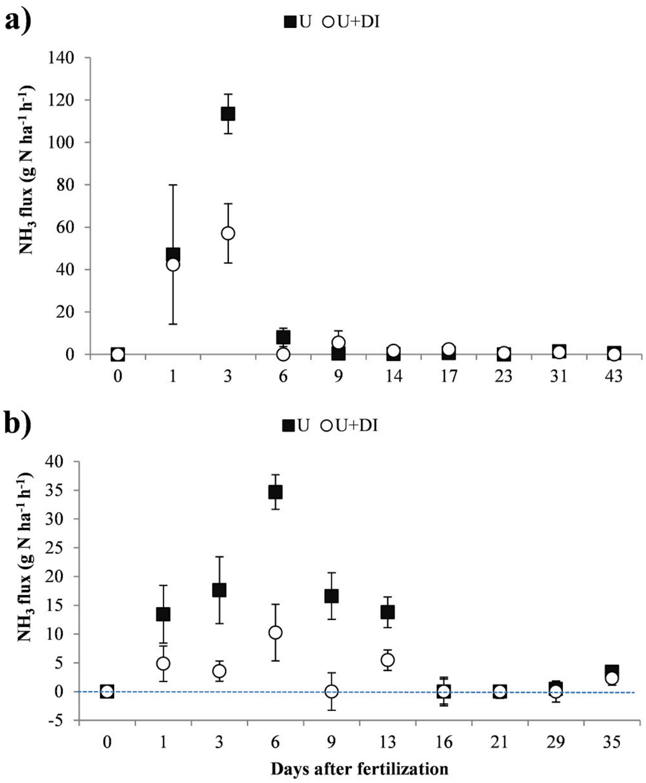

Fig. 3. Daily NH_3 fluxes after seeding (a) and dressing fertilization (b) in urea (U) and urea + NBPT + DMPSA (U+DI). Vertical bars indicate standard errors.

4. Discussion

4.1. Ammonia volatilization

Spain is one of the countries that exceed national NH_3 emission thresholds, so implementing NH_3 mitigation practices is a priority in the agricultural sector in this country (Sanz-Cobena et al., 2014a, 2014b). So far, little attention has been paid to the possible increase in N volatilization due to the expansion of non-tilled crops, as recently confirmed by Ma et al. (2021). The use of no tillage is increasing in Spain, the leading European country in the adoption of this practice (8% of the total surface of herbaceous crops, AEAC.SV, 2020), while this practice is also being extensively adopted in other countries worldwide (Shakoor et al., 2021). Moreover, urea remains the most used N-fertilizer in Spain (MAPA, 2018) and many other countries and so the contributions of urea to national NH_3 inventories should not be neglected. Our results show that the double inhibition from DMPSA + NBPT could be effective in reducing NH_3 emissions from urea in non-tilled agro-ecosystems.

The meteorological conditions during the days following fertilization might explain the higher cumulative NH_3 losses during the first Period than after dressing application (Table 1), despite the larger amount of synthetic N applied in Period II (120 kg N ha^{-1}) than in Period I (40 kg N ha^{-1}). Cumulative rainfall in the first 7 days after fertilization was 19.4 mm (2.6 mm during the first three days) and 0.2 mm in Periods I and II, respectively (Fig. 1a). Small amounts of precipitation are expected to stimulate NH_3 volatilization (Sanz-Cobena et al., 2011) since they act to dissolve urea without incorporating the fertilizer. In Period II, emissions were lower, but the peak lasted longer (Fig. 3), possibly because of the drier conditions, which led to lower urea dissolution rates mediated by surface moisture (dew). The higher N-NH_4^+ rates sampled after dressing (reaching up to 120 kg N ha^{-1}, Fig. 1c) were not in agreement with this hypothesis, although the large variability in NH_4^+ concentrations and the non-accounting of many potential entry or loss pathways to the mineral N pool add significant uncertainties to these soil recovery calculations. Volatilization is also strongly dependent on wind speed and air temperatures (Voglmeier et al., 2018). The conditions for NH_3 volatilization were more favorable after N application in the autumn (i.e., windier and warmer, see Section 3.1), thus possibly explaining the higher emissions. In addition, during the early stages of crop development, the “mulching effect” of previous crop stover and the higher urease concentrations produced by these residues, particularly under no-till management (Pinheiro et al., 2018) are more influential than after tillering, and could have also contributed to the higher emissions in the first Period (Fig. 3a, Table 1). The higher N uptake at the critical tillering stage (dressing fertilization) than that at seeding (basal fertilization) could have also contributed to reduce volatilization rates (Xia et al., 2017).

The use of the DMPSA + NBPT inhibitor mitigated NH_3 volatilization by 50.5% after both periods (35.4% and 71.0% for Periods I and II, respectively). In any case, the mitigation efficacies were slightly higher than those obtained by Ti et al. (2019) for urease inhibitors and wheat crops in a global meta-analysis. Our results suggest, therefore, that the combination of DMPSA with NBPT does not penalize the mitigation effect of the urease inhibitor alone.

4.2. Influence of the number of replicates on cumulative volatilization rates measured by the IHF method

The use of the IHF method for measuring NH_3 fluxes has generally been limited to one or two replicates due to operational and surface constraints (Recio et al., 2018). While differences between U and U+DI using three replicates were statistically significant (Table 1), five of the

Table 1

Cumulative N_2O, CH_4, CO_2 and NH_3 emissions for the different treatments: urea (U), urea + NBPT + DMPSA (U+DI), and control. Three periods were considered: from seeding fertilization (November 2019) to dressing fertilization (mid-February 2020, Period I), from dressing fertilization to harvest (mid-June, Period II) and from harvest to the end of the experimental period (Period III). The P value was calculated with Student's *t*-test and d.f. = 4. S.E. denotes the standard error of the mean.

Treatment	N_2O emission (kg N ha^{-1})				CH_4 emission (kg C ha^{-1})				CO_2 (Mg C ha^{-1})				NH_3 emission (kg N ha^{-1})		
	Period I	Period II	Period III	Total	Period I	Period II	Period III	Total	Period I	Period II	Period III	Total	Period I	Period II	Total
U	0.30	0.80	0.19	1.30	-0.14	-0.12	0.04	-0.22	0.55	3.59	0.91	5.05	9.50	7.06	16.56
U+DI	0.08	0.12	0.13	0.33	-0.24	-0.23	-0.15	-0.61	0.50	3.13	0.81	4.44	6.14	2.05	8.19
Control	0.05	0.09	0.11	0.25	-0.16	-0.19	0.06	-0.30	0.54	2.62	0.72	3.89	–	–	–
S.E.	0.02	0.03	0.02	0.05	0.06	0.14	0.11	0.12	0.13	0.43	0.09	0.55	1.22	0.49	1.50
Control versus fertilized	P = 0.007	P = 0.001	P = 0.056	P = 0.001	P = 0.736	P = 0.919	P = 0.462	P = 0.480	P = 0.899	P = 0.238	P = 0.990	P = 0.273	–	–	–
U+DI versus U	P = 0.002	P = 0.000	P = 0.053	P = 0.000	P = 0.311	P = 0.612	P = 0.297	P = 0.081	P = 0.795	P = 0.494	P = 0.721	P = 0.477	P = 0.049	P = 0.002	P = 0.017

Table 2

Difference between estimated means, standard error (SE), degrees of freedom (df), confidence interval (lower and upper confidence level, CL) and hypothesis test (t ratio and *P* value) for the 9 possible combinations of replicates in urea (U) and urea + NBPT + DMPSA (U+DI) for the cumulative volatilization fluxes in Period I + Period II.

Contrast	Estimate	SE	df	Lower CL	Upper CL	t ratio	*P* value
U+DI - U	-6.169	1.855	2	-14.152	1.814	-3.325	0.080
U+DI - U	-7.116	1.748	2	-14.636	0.404	-4.071	0.055
U+DI - U	-6.438	1.969	2	-14.911	2.036	-3.269	0.082
U+DI - U	-8.007	3.629	2	-23.620	7.607	-2.206	0.158
U+DI - U	-8.954	3.575	2	-24.336	6.428	-2.504	0.129
U+DI - U	-8.276	3.688	2	-24.146	7.595	-2.244	0.154
U+DI - U	-9.734	1.959	2	-18.163	-1.305	-4.969	0.038
U+DI - U	-10.681	1.857	2	-18.672	-2.689	-5.750	0.029
U+DI - U	-10.003	2.067	2	-18.898	-1.107	-4.838	0.040

nine combinations of replicates would confirm the null hypothesis (Table 2) for the conditions of our study. Our results suggest that the bias when using two replicates is negligible when NH_3 fluxes are low (e.g., in Period II, standard deviation for U accounted for 13.2% of the average value). The effect of the number of replicates is, however, noteworthy when fluxes are higher (e.g., in Period I the standard deviation for U was 2.9 kg N ha^{-1}, equivalent to 30.5% of the average value). Under the conditions of our study, the estimates of NH_3 volatilization from urea would have differed from the three-replicate average value by − 10.9% to + 10.6% (−16.9% to +12.9% in the first period) if only two replicates had been used (i.e., the combinations leading to the lowest and highest volatilization rates). However, it should be pointed out that our conclusions about the number of replicates are based on a small dataset and are site-specific. Therefore, further research is needed to analyze in depth the effect of varying the number of replicates on the experimental bias, variability of data and the mitigation potential of recommended strategies.

4.3. *Nitrous oxide emissions*

The use of a combined nitrification and urease inhibitor was chosen in this experiment with the goal of achieving an effective mitigation of both NH_3 and N_2O emissions. The presence of NBPT delays the hydrolysis of urea for 10–15 days, thus favoring the incorporation of the fertilizer into the soil. During this period, the availability of the substrate for nitrification (NH_4^+) is expected to decrease (Fan et al., 2018), as was indeed observed following both fertilization events (Fig. 1c, Section 3.1). The DMPSA inhibitor, which acts as a copper (Cu) chelator (Corrochano-Monsalve et al., 2021b), delays the oxidation of NH_4^+ to hydroxylamine (Ruser and Schulz, 2015), which is the first step of nitrification, a process that leads to the emissions of N oxides as by-products (Butterbach-Bahl et al., 2013). Therefore, its addition is expected to increase topsoil NH_4^+ concentrations once the effect of NBPT has ended (Fig. 1c), and the combined use of both products (NBPT and DMPSA) may lead to a temporary (e.g. a few weeks following N application) reduction of soil NO_3^- content (Fig. 1c). Our soil measurements of mineral N were, therefore, consistent with these dynamics, particularly following dressing fertilization. The reduction of NO_3^- availability indirectly limits the emission of N_2O from denitrification, a stepwise reduction process that is dominant at WFPS > 60% (Pilegaard, 2013) and/or in soil anaerobic microsites, especially when crop residues are present (Li et al., 2016; Rummel et al., 2020). Favorable conditions for this process (and its coupling with nitrification) could have occurred under our conditions after both fertilization events (Fig. 1b).

The double inhibitor also tended to mitigate the pulse after the rewetting episode in summer (Fig. 1e), even though the effect of the nitrification inhibitor DMPSA is not expected to remain after nearly seven months following dressing fertilization. Guardia et al. (2018a) did

Table 3
Grain and straw yields, N content in grain and straw, aboveground N uptake, harvest index, crop recovery efficiency (CRE), partial nutrient balance (PNB) and N surplus for the different treatments: urea (U), urea + NBPT + DMPSA (U+DI), and control. The P value was calculated with Student's *t*-test and d.f. = 4. S.E. denotes the standard error of the mean.

Treatment	Grain yield (kg ha^{-1})	N content in grain (%)	Straw yield (kg ha^{-1})	N content in straw (%)	Aboveground N uptake (kg N ha^{-1})	Harvest index	CRE (%)	PNB	N surplus (kg N ha^{-1})
U	2706	2.9	5233	0.82	119.1	0.34	27.3	0.74	40.9
U+DI	2770	3.0	5256	0.99	134.7	0.34	37.0	0.84	25.3
Control	3109	1.9	4291	0.41	75.5	0.42	–	–	–
S.E.	152.5	0.04	435.2	0.05	5.2	0.00	2.0	0.02	3.3
Control versus fertilized	P = 0.118	P = 0.000	P = 0.053	P = 0.002	P = 0.001	P = 0.000	–	–	–
U+DI versus U	P = 0.780	P = 0.075	P = 0.957	P = 0.081	P = 0.124	P = 0.520	P = 0.045	P = 0.045	P = 0.045

not find any effect of the double inhibitor on a rewetting peak after summer. However, Montoya et al. (2021) observed a significant mitigation of N_2O emissions with the double inhibitor (in comparison with urea) for a rewetting pulse that occurred around 3 months after dressing fertilization. All of these results under rainfed Mediterranean conditions suggest that the double inhibitor can be effective regardless of the effect during the N_2O peaks after rewetting episodes. Our results confirm the relevance of these peaks in semi-arid areas and the need for considering the post-harvest period to obtain reliable emission factors (Cayuela et al., 2017; Shang et al., 2020).

The emission factor from urea alone (0.66%) was slightly higher than the new revised factor from IPCC (2019) for semi-arid areas (0.5%), which is the same as that proposed by the meta-analysis of Cayuela et al. (2017) under rainfed Mediterranean conditions. The emission factor from U+DI (< 0.1%) was within the range obtained by Cayuela et al. (2017) for inhibitors in Mediterranean areas.

4.4. *Methane and soil respiration fluxes*

The U+DI treatment increased cumulative CH_4 oxidation with respect to urea alone at a > 90% confidence level (Table 1). Studies, such as those of Rime and Niklaus (2017) or the meta-analysis of Qiao et al. (2015) have not found a significant effect of nitrification inhibitors or an influence of environmental or management conditions on CH_4 fluxes. Current available information regarding the effect double nitrification-urease inhibitors is scarce, although no effect on CH_4 sink has been reported by e.g., Guardia et al. (2018a) or Montoya et al. (2021). Our results were mainly driven by the enhanced uptake potential observed in Period I (Table 1), some days during Period II (10th March – 22nd April) and immediately after the August rewetting event (Fig. S2a). These periods generally coincided with higher topsoil NH_4^+ concentrations in U+DI with respect to U plots (Fig. 1c), and also with lower concentrations of NO_3^- (Fig. 1d) in the plots that received the double inhibitor. Temporary changes in the availability of mineral N species could have influenced the higher CH_4 uptake in U+DI plots due to: i) the toxic effect of high NO_3^- concentrations on methanotrophs (Chen et al., 2019); ii) methanotrophs being favored under conditions of hypoxia and NH_4^+ availability (Walkiewicz et al., 2018); and iii) the effect of DMPSA on the availability of Cu (Corrochano-Monsalve et al., 2021b) or iron, which are co-factors of methanogenic and methanotrophic enzymes (Glass and Orphan, 2012; Semrau et al., 2018).

Carbon dioxide fluxes represent the respiration from soil microorganisms and crop roots. The highest fluxes coincided with spring warm temperatures, intense crop development stages, and also with high N availability in soil after dressing applications (Fig. 1a, c, d, Fig. S2b), as also found by Wang et al. (2019). The possible effect of DMPSA on non-target microbial communities described by Corrochano-Monsalve et al. (2020b) should be further explored since the U+DI treatments had reduced daily respiration fluxes after both fertilization events (e.g., mid-December, late-February to mid-April, Fig. S2b). This effect was diluted afterwards (Table 1), possibly because of the contribution from root respiration to the CO_2 fluxes, which could even be increased with the double inhibitor due to the increased root biomass, as reported by e.g., Guardia et al. (2018b) for DMPSA in a maize crop. The numerically lower CO_2 fluxes in the control with respect to the fertilized treatments also suggest a lower root development and respiration. The soil rewetting episode in mid-August (Fig. 1a, b) led to a remarkable response in CH_4 fluxes (intense methanotrophic activity followed by a CH_4 emission peak, Fig. S2a) and soil respiration (Fig. S2b), supporting the findings of Liang et al. (2016), Barnard et al. (2020) and Manzoni et al. (2020).

4.5. *Considerations about the experimental conditions and their effect on gaseous emissions*

Our experiment was established only two years after conversion from conventional tillage to NT. Therefore, it could be argued that the effect of the different treatments can be affected by the duration of NT management. Although this factor has undeniable influence on N_2O emissions as pointed out by e.g., van Kessel et al. (2013) and Huang et al. (2018), we hypothesized that the conclusions on the mitigation efficacy of the DI could be considered valid for the medium- to long-term. Volatilization of NH_3 is a physical process which depends more on the environmental conditions and the presence of a plant mulch barrier (Pinheiro et al., 2018) than on the long-term changes of biochemical processes resulting from the adoption of no-till (Nunes et al., 2020). In the case of N_2O, Huang et al. (2018) did not find any significant effect of experimental duration on the influence of no-till on N_2O. Moreover, our results show a large and highly significant mitigation potential of the DI (Table 1), supporting the recent findings of Corrochano-Monsalve et al. (2021a) under humid conditions. It should be noted that the comparison between conventional tillage and NT was not evaluated in our experiment, even though the influence of tillage management on NH_3 volatilization has recently been described to be significant at a global scale (Ma et al., 2021). We recommend that the interaction between the use of the DI or urea-only and different tillage practices and their effect on CH_4 and CO_2 fluxes should be further explored, as well as the effect of the period under NT conditions.

4.6. *Crop yield and NUE*

The use of the double inhibitor improved the acquisition of N by the wheat plants, as demonstrated by the significant increases of CRE or PNB (and the reduction of N surplus) with respect to urea alone. An analysis of all field studies carried out in areas with less than 650 mm of annual rainfall reveals that only Zhao et al. (2017), who evaluated the combination of DMPP and NBPT, found a significant increase of N uptake with the use of the double inhibitor, contrary to e.g., Souza et al. (2019). Other authors reported non-significant positive tendencies, e.g., Maharjan and Venterea (2013) or Ding et al. (2015), while Guardia et al. (2018a) or Montoya et al. (2021) obtained positive results for protein composition or oil yield in wheat and rape, respectively.

The significant increases in aboveground N uptake values were a result of the increases in grain and –particularly- straw N concentrations (Table 3), since both grain and straw yields were similar for U and U+DI (and also the unfertilized control). Our results are consistent with the N response of wheat in Mediterranean cropping systems (Savin et al., 2019), for which there is a trade-off between grain N concentrations and grain yields (Table S1). We obtained higher grain N concentrations (2.9%) than the average value for Mediterranean (2.4%) and non-Mediterranean European (1.8%) wheat crops, although the influence of the cultivar type on potential protein content is also critical. The high protein contents are linked to an improvement of bread-making quality, shown by the positive correlation with SDS sedimentation test (an indicator of gluten strength) and the concentration of gluten proteins (gliadins and glutenins), which are related to e.g., dough extensibility and elasticity (Barak et al., 2013; Guardia et al., 2018a, 2019b). By contrast, we obtained lower than average (taking both fertilized treatments and comparing them with the average Mediterranean value, Savin et al., 2019) grain yield (2.7 versus 4.1 Mg ha^{-1} or 3.0 Mg ha^{-1} in the Madrid region, MAPA, 2018), aboveground N uptake (117 versus 131 kg N ha^{-1}) and N utilization efficiency (ratio between grain yield and N uptake, 23 versus 33 kg grain kg N^{-1}).

Low values of CRE (but not PNB) were obtained as a result of the high grain yields in the control treatment (Table 3) and the high N rate applied (160 kg N ha^{-1}), which may have largely exceeded crop demand, as supported by the significant residual soil N-NO_3^- concentrations in Period III (Fig. 1d and 2b). In our study, the soil (a “missing input” for the PNB approach) contributed greatly to plant N uptake; and indexes such as PNB may overestimate the NUE as the response of crop to synthetic N fertilizer. Approaches such as PNB, which do not take into account unfertilized control plots are, therefore, more appropriate for several years of continuous cropping and/or balances of complete rotations (Quemada et al., 2020). In any case, our results suggest i) a low potential of N mining in the short-term; and ii) that the inclusion of control plots can result in low CREs and high crop yields, if significant legacy N is available in the soil as a result of over-fertilizing previous crops. The use of control plots that have had several years without N application and an assessment of the variability of environmental conditions (rainfall, temperature) are recommended in future experiments to obtain fairer and more reliable values of some NUE indicators.

Despite the statistically similar grain/straw yields (Table 3) and the larger harvest index in the control with respect to the fertilized plots; these results were offset by the increases in plant N concentrations, which led to higher N yields in the U and –particularly- U+DI treatments. Although the grain yields were similar to the average value for rainfed conditions in the Madrid region (MAPA, 2018), some strategies could be explored to increase grain yields and economic benefit, such as the adjustment of N rate by considering the yield potential of the area (avoiding N overdose) or selecting a wheat cultivar with higher grain yield potential rather than a cultivar with high protein accumulation potential (wheat cultivars suitable for bread-making). Our results confirm the opportunities offered by N management (use of a double inhibitor) to increase the economic returns (through the increases in protein content or NUE in our case) and decrease environmental impacts with the mitigation of gaseous N emissions and N surplus (as an indicator of N likely lost to the environment, Sanz-Cobena et al., 2014a, 2014b). The cost-benefit-analysis of the use of the NBPT+DMPSA should be explored in further studies, considering the increase in the price of the fertilizer but also the potential increase of net income through e.g., enhanced protein concentration for bread-making products or animal feed, the potential reductions of synthetic N rates through CRE enhancement (Rose et al., 2018) or the potential policy incentives considering the reductions of environmental or health costs at a regional scale (Qiao et al., 2015).

5. Conclusions

Under the conditions of our study, the risk of increasing NH_3 volatilization when no tillage management is implemented (as demonstrated by previous studies) can be considerably reduced by the use of a double urease nitrification inhibitor, without negative side effects on N surplus, CH_4 uptake or N_2O mitigation and with a significant enhancement of NUE. The mitigation rates for both NH_3 and N_2O losses were comparable to those reported for urease and nitrification inhibitors alone, respectively. The variability of meteorological conditions (which influence the effectiveness of enhanced-efficiency fertilizers) in rainfed cropping systems in semi-arid areas highlights the need for addressing the mitigation potential in future experiments under different soil or climatic conditions. Our study also contributed to the knowledge base on GHG fluxes in the post-harvest period, when soil rewetting events significantly affect fluxes or uptake rates after long dry periods. In addition to the N_2O emission peaks from rewetting events, which can contribute significantly to the emission factors in semi-arid areas, we also observed clear effects on soil respiration (pulse) and CH_4 (a sink followed by net emission). Although these CO_2 and CH_4 fluxes are not that important when it comes to the global warming potential from agro-ecosystems (e.g., CH_4 only compensated 2% (in urea alone) to 21% (with the double inhibitor) of N_2O emissions under our conditions) exploring further these mechanisms will improve our knowledge on C balances and soil dynamics.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2021.107546.

References

Abalos, D., Sanz-Cobena, A., Andreu, G., Vallejo, A., 2017. Rainfall amount and distribution regulate DMPP effects on nitrous oxide emissions under semiarid Mediterranean conditions. Agric. Ecosyst. Environ. 238, 36–45.

AEAC.SV, 2020. Asociación Española de Agricultura de Conservación-Suelos Vivos (in Spanish). Available online at ⟨http://www.agriculturadeconservacion.org/index.php/agricultura-de-conservacion/la-ac-en-espana⟩.

Aguilera, E., Lassaletta, L., Gattinger, A., Gimeno, B.S., 2013. Managing soil carbon for climate change mitigation and adaptation in Mediterranean cropping systems: a meta-analysis. Agric. Ecosyst. Environ. 168, 25–36.

Bai, M., Sun, J., Denmead, O.T., Chen, D., 2017. Comparing emissions from a cattle pen as measured by two micrometeorological techniques. Environ. Pollut. 230, 584–588.

Barak, S., Mudgil, D., Khatkar, B.S., 2013. Relationship of gliadin and glutenin proteins with dough rheology, flour pasting and bread making performance of wheat varieties. LWT-Food Sci. Technol. 51, 211–217.

Barnard, R.L., Blazewicz, S.J., Firestone, M.K., 2020. Rewetting of soil: revisiting the origin of soil CO_2 emissions. Soil Biol. Biochem. 147, 107819.

Butterbach-Bahl, K., Baggs, E.M., Dannenmann, M., Kiese, R., Zechmeister-Boltenstern, S., 2013. Nitrous oxide emissions from soils: how well do we understand the processes and their controls? Philos. Trans. R. Soc. Lond. B Biol. Sci. 368, 20130122.

Cao, Y., Zhao, F., Zhang, Z., Zhu, T., Xiao, H., 2020. Biotic and abiotic nitrogen immobilization in soil incorporated with crop residue. Soil Tillage Res. 202, 104664.

Cayuela, M.L., Aguilera, E., Sanz-Cobena, A., Adams, D.C., Abalos, D., Barton, L., Ryals, R., Silver, W.L., Alfaro, M.A., Pappa, V.A., Smith, P., Garnier, J., Billen, G., Bouwman, L., A Bondeau, A., Lassaletta, L., 2017. Direct nitrous oxide emissions in Mediterranean climate cropping systems: emission factors based on a meta-analysis of available measurement data. Agric. Ecosyst. Environ. 238, 25–35.

Chenu, C., Angers, D.A., Barré, P., Derrien, D., Arrouays, D., Balesdent, J., 2019. Increasing organic stocks in agricultural soils: knowledge gaps and potential innovations. Soil Tillage Res. 188, 41–52.

Chen, S., Hao, T., Goulding, K., Misselbrook, T., Liu, X., 2019. Impact of 13-years of nitrogen addition on nitrous oxide and methane fluxes and ecosystem respiration in a temperate grassland. Environ. Pollut. 252, 675–681.

Corrochano-Monsalve, M., Bozal-Leorri, A., Sánchez, C., González-Murua, C., Estavillo, J.M., 2021a. Joint application of urease and nitrification inhibitors to diminish gaseous nitrogen losses under different tillage systems. J. Clean. Prod. 289, 125701.

Corrochano-Monsalve, M., González-Murua, C., Bozal-Leorri, A., Lezama, L., Artetxe, B., 2021b. Mechanism of action of nitrification inhibitors based on dimethylpyrazole: a matter of chelation. Sci. Total Environ. 752, 141885.

Corrochano-Monsalve, M., González-Murua, C., Estavillo, J.M., Estonba, A., Zarraonaindia, I., 2020b. Unraveling DMPSA nitrification inhibitor impact on soil bacterial consortia under different tillage systems. Agric. Ecosyst. Environ. 301, 107029.

Ding, W.X., Chen, Z.M., Yu, H.Y., Luo, J.F., Yoo, G.Y., Xiang, J., Zhang, H.J., Yuan, J.J., 2015. Nitrous oxide emission and nitrogen use efficiency in response to nitrophosphate, N-(n-butyl) thiophosphoric triamide and dicyandiamide of a wheat cultivated soil under sub-humid monsoon conditions. Biogeosciences 12 (3), 803–815.

Ding, W.X., Hongyan, Y.Y., Cai, Z.C., 2011. Impact of urease and nitrification inhibitors on nitrous oxide emissions from fluvo-aquic soil in the North China Plain. Biol. Fert. Soils 47 (1), 91–99.

Fan, X., Yin, C., Yan, G., Cui, P., Shen, Q., Wang, Q., Chen, H., Zhang, N., Ye, M., Zhao, Y., Li, T., Liang, Y., 2018. The contrasting effects of N-(n-butyl) thiophosphoric triamide (NBPT) on N_2O emissions in arable soils differing in pH are underlain by complex microbial mechanisms. Sci. Total Environ. 642, 155–167.

Fu, Q., Abadie, M., Blaud, A., Carswell, A., Misselbrook, T.H., Clark, I.M., Hirsch, P.R., 2020. Effects of urease and nitrification inhibitors on soil N, nitrifier abundance and activity in a sandy loam soil. Biol. Fert. Soils 56 (2), 185–194.

Glass, J., Orphan, V.J., 2012. Trace metal requirements for microbial enzymes involved in the production and consumption of methane and nitrous oxide. Front. Microbiol. 3, 61.

Grossman, R.B., Reinsch, T.G., 2002. 2.1 Bulk density and linear extensibility. Methods of soil analysis: part 4. Phys. Methods 201–228.

Guardia, G., Aguilera, E., Vallejo, A., Sanz-Cobena, A., Alonso-Ayuso, M., Quemada, M., 2019a. Effective climate change mitigation through cover cropping and integrated fertilization: a global warming potential assessment from a 10-year field experiment. J. Clean. Prod. 241, 118307.

Guardia, G., González-Murua, C., Fuertes-Mendizábal, T., Vallejo, A., 2019b. 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. Arch. Agron. Soil Sci. 1–18.

Guardia, G., Sanz-Cobena, A., Sanchez-Martín, L., Fuertes-Mendizábal, T., González-Murua, C., Álvarez, J.M., Chadwick, D.R., Vallejo, A., 2018a. Urea-based fertilization strategies to reduce yield-scaled N oxides and enhance bread-making quality in a rainfed Mediterranean wheat crop. Agric. Ecosyst. Environ. 265, 421–431.

Guardia, G., Vallejo, A., Cardenas, L.M., Dixon, E.R., García-Marco, S., 2018b. Fate of 15N-labelled ammonium nitrate with or without the new nitrification inhibitor DMPSA in an irrigated maize crop. Soil Biol. Biochem. 116, 193–202.

Huang, Y., Ren, W., Wang, L., Hui, D., Grove, J.H., Yang, X., Tao, B., Goff, B., 2018. Greenhouse gas emissions and crop yield in no-tillage systems: a meta-analysis. Agric. Ecosyst. Environ. 268, 144–153.

IPCC, (Intergovernmental Panel on Climate Change), 2019. Refinement to the 2006 IPCC guidelines for national greenhouse gas inventories. In: Calvo Buendia, E., Tanabe, K., Kranjc, A., Baasansuren, J., Fukuda, M., Ngarize, S., Osako, A., Pyrozhenko, Y., Shermanau, P., Federici, S. (Eds.), Intergovernmental Panel on Climate Change (Switzerland).

Kassam, A., Friedrich, T., Derpsch, R., Lahmar, R., Mrabet, R., Basch, G., González-Sánchez, E.J., Serraj, R., 2012. Conservation agriculture in the dry Mediterranean climate. Field Crops Res. 132, 7–17.

Van Kessel, C., Venterea, R., Six, J., Adviento-Borbe, M.A., Linquist, B., van Groenigen, K.J., 2013. Climate, duration, and N placement determine N_2O emissions in reduced tillage systems: a meta-analysis. Glob. Change Biol. 19 (1), 33–44.

Leuning, R., Freney, J.R., Denmead, O.T., Simpson, J.R., 1985. A sampler for measuring atmospheric ammonia flux. Atmos. Environ. 19 (1967), 1117–1124.

Liang, L.L., Grantz, D.A., Jenerette, G.D., 2016. Multivariate regulation of soil CO_2 and N_2O pulse emissions from agricultural soils. Glob. Change Biol. 22, 1286–1298.

Li, Y., Li, Z., Cui, S., Jagadamma, S., Zhang, Q., 2019. Residue retention and minimum tillage improve physical environment of the soil in croplands: a global meta-analysis. Soil Tillage Res. 194, 104292.

Li, X., Sørensen, P., Olesen, J.E., Petersen, S.O., 2016. Evidence for denitrification as main source of N_2O emission from residue-amended soil. Soil Biol. Biochem. 92, 153–160.

Maharjan, B., Venterea, R.T., 2013. Nitrite intensity explains N management effects on N_2O emissions in maize. Soil Biol. Biochem. 66, 229–238.

Manzoni, S., Chakrawal, A., Fischer, T., Schimel, J.P., Porporato, A., Vico, G., 2020. Rainfall intensification increases the contribution of rewetting pulses to soil heterotrophic respiration. Biogeosciences 17 (15), 4007–4023.

MAPA, 2018. Anuario de Estadística, Ministerio de Agricultura Pesca y Alimentación. (in Spanish). Available online at: ⟨https://www.mapa.gob.es/es/estadistica/temas/publicaciones/anuario-de-estadistica/2019/default.aspx?parte=3⟩.

Ma, R., Zou, J., Han, Z., Yu, K., Wu, S., Li, Z., Liu, S., Niu, S., Horwath, W.R., Zhu-Barker, X., 2021. Global soil-derived ammonia emissions from agricultural nitrogen fertilizer application: a refinement based on regional and crop-specific emission factors. Glob. Change Biol. 27 (4), 855–867.

Montoya, M., Vallejo, A., Corrochano-Monsalve, M., Aguilera, E., Sanz-Cobena, A., Ginés, C., González-Murua, C., Álvarez, J.M., Guardia, G., 2021. Mitigation of yield-scaled nitrous oxide emissions and global warming potential in an oilseed rape crop through N source management. J. Environ. Manag. 288, 112304.

Nunes, M.R., Karlen, D.L., Veum, K.S., Moorman, T.B., Cambardella, C.A., 2020. Biological soil health indicators respond to tillage intensity: a US meta-analysis. Geoderma 369, 114335.

Pacholski, A., Cai, G.X., Fan, X.H., Ding, H., Chen, D., Nieder, R., Roelcke, M., 2008. Comparison of different methods for the measurement of ammonia volatilization after urea application in Henan Province, China. J. Plant Nutr. Soil Sci. 171 (3), 361–369.

Pan, B., Lam, S.K., Mosier, A., Luo, Y., Chen, D., 2016. Ammonia volatilization from synthetic fertilizers and its mitigation strategies: a global synthesis. Agric. Ecosyst. Environ. 232, 283–289.

Pilegaard, K., 2013. Processes regulating nitric oxide emissions from soils. Philos. Trans. R. Soc. Lond. B Biol. Sci. 368 (1621), 20130126.

Pinheiro, P.L., Recous, S., Dietrich, G., Weiler, D.A., Giovelli, R.L., Mezzalira, A.P., Giacomini, S.J., 2018. Straw removal reduces the mulch physical barrier and ammonia volatilization after urea application in sugarcane. Atmos. Environ. 194, 179–187.

Qiao, C., Liu, L., Hu, S., Compton, J.E., Greaver, T.L., Li, Q., 2015. How inhibiting nitrification affects nitrogen cycle and reduces environmental impacts of anthropogenic nitrogen input. Glob. Change Biol. 21, 1249–1257.

Quemada, M., Lassaletta, L., Jensen, L.S., Godinot, O., Brentrup, F., Buckley, C., Foray, S., Hvid, S.K., Oenema, J., Richards, K.G., Oenema, O., 2020. Exploring nitrogen indicators of farm performance among farm types across several European case studies. Agric. Syst. 177, 102689.

Recio, J., Montoya, M., Ginés, C., Sanz-Cobena, A., Vallejo, A., Alvarez, J.M., 2020. Joint mitigation of NH_3 and N_2O emissions by using two synthetic inhibitors in an irrigated cropping soil. Geoderma 373, 114423.

Recio, J., Vallejo, A., Le-Noe, J., Garnier, J., García-Marco, S., Álvarez, J.M., Sanz-Cobena, A., 2018. The effect of nitrification inhibitors on NH_3 and N_2O emissions in highly N fertilized irrigated Mediterranean cropping systems. Sci. Total Environ. 636, 427–436.

Rime, T., Niklaus, P.A., 2017. Spatio-temporal dynamics of soil CH_4 uptake after application of N fertilizer with and without the nitrification inhibitor 3, 4-dimethylpyrazole phosphate (DMPP). Soil Biol. Biochem. 104, 218–225.

Rose, T.J., Wood, R.H., Rose, M.T., Van Zwieten, L., 2018. A re-evaluation of the agronomic effectiveness of the nitrification inhibitors DCD and DMPP and the urease inhibitor NBPT. Agric. Ecosyst. Environ. 252, 69–73.

Rummel, P.S., Pfeiffer, B., Pausch, J., Well, R., Schneider, D., Dittert, K., 2020. Maize root and shoot litter quality controls short-term CO_2 and N_2O emissions and bacterial community structure of arable soil. Biogeosciences 17 (4), 1181–1198.

Rumpel, C., Amiraslani, F., Chenu, C., Cardenas, M.G., Kaonga, M., Koutika, L.S., Ladha, J., Madari, B., Shirato, Y., Smith, P., Soudi, B., Soussana, J.F., Whitehead, D., Wollenberg, E., 2020. The 4p1000 initiative: opportunities, limitations and challenges for implementing soil organic carbon sequestration as a sustainable development strategy. Ambio 49 (1), 350–360.

Ruser, R., Schulz, R., 2015. The effect of nitrification inhibitors on the nitrous oxide (N_2O) release from agricultural soils—a review. J. Plant Nutr. Soil Sci. 178 (2), 171–188.

Sanz-Cobena, A., Lassaletta, L., Estellés, F., Del Prado, A., Guardia, G., Abalos, D., Aguilera, E., Pardo, G., Vallejo, A., Sutton, M.A., Garnier, J., Billen, G., 2014a. Yield-scaled mitigation of ammonia emission from N fertilization: the Spanish case. Environ. Res. Lett. 9 (12), 125005.

Sanz-Cobena, A., Lassaletta, L., Estellés, F., Del Prado, A., Guardia, G., Abalos, D., Aguilera, E., Pardo, G., Vallejo, A., Sutton, M.A., Garnier, J., Billen, G., 2014b. Yield-scaled mitigation of ammonia emission from N fertilization: the Spanish case. Environ. Res. Lett. 9 (12), 125005.

Sanz-Cobena, A., Misselbrook, T., Camp, V., Vallejo, A., 2011. Effect of water addition and the urease inhibitor NBPT on the abatement of ammonia emission from surface applied urea. Atmos. Environ. 45 (8), 1517–1524.

Savin, R., Sadras, V.O., Slafer, G.A., 2019. Benchmarking nitrogen utilisation efficiency in wheat for Mediterranean and non-Mediterranean European regions. Field Crops Res. 241, 107573.

Semrau, J.D., DiSpirito, A.A., Gu, W., Yoon, S., 2018. Metals and methanotrophy. Appl. Environ. Microbiol. 84 (6).

Shakoor, A., Shahbaz, M., Farooq, T.H., Sahar, N.E., Shahzad, S.M., Altaf, M.M., Ashraf, M., 2021. A global meta-analysis of greenhouse gases emission and crop yield under no-tillage as compared to conventional tillage. Sci. Total Environ. 750, 142299.

Shang, Z., Abdalla, M., Kuhnert, M., Albanito, F., Zhou, F., Xia, L., Smith, P., 2020. Measurement of N_2O emissions over the whole year is necessary for estimating reliable emission factors. Environ. Pollut. 259, 113864.

Sha, Z., Ma, X., Wang, J., Lv, T., Li, Q., Misselbrook, T., Liu, X., 2020. Effect of N stabilizers on fertilizer-N fate in the soil-crop system: a meta-analysis. Agric. Ecosyst. Environ. 290, 106763.

Soil Survey Staff, 2014. Keys to Soil Taxonomy, Washington, DC, USA: USDA, Natural Resources Conservation Service, Washington.

Soussana, J.F., Lutfalla, S., Ehrhardt, F., Rosenstock, T., Lamanna, C., Havlík, P., Richards, M., Wollenberg, E.L., Chotte, J.L., Torquebiau, E., Ciais, P., Smith, P., Lal, R., 2019. Matching policy and science: rationale for the '4 per 1000-soils for food security and climate'initiative. Soil . Res. 188, 3–15.

Souza, E.F., Rosen, C.J., Venterea, R.T., 2019. Contrasting effects of inhibitors and biostimulants on agronomic performance and reactive nitrogen losses during irrigated potato production. Field Crops Res. 240, 143–153.

Tan, Z., Leung, L.R., Li, H.Y., Tesfa, T., Zhu, Q., Huang, M., 2020. A substantial role of soil erosion in the land carbon sink and its future changes. Glob. Change Biol. 26 (4), 2642–2655.

Ti, C., Xia, L., Chang, S.X., Yan, X., 2019. Potential for mitigating global agricultural ammonia emission: a meta-analysis. Environ. Pollut. 245, 141–148.

Venterea, R.T., Parkin, T.B., Cardenas, L., Petersen, S.O., Pedersen, A.R., 2012. Data analysis considerations. In: de Klein, C.A.M., Harvey, M.J., editors. Nitrous oxide chamber methodology guidelines. Global Research Alliance on Agricultural Greenhouse Gases.

Viguria, M., Sanz-Cobeña, A., López, D.M., Arriaga, H., Merino, P., 2015. Ammonia and greenhouse gases emission from impermeable covered storage and land application of cattle slurry to bare soil. Agric. Ecosyst. Environ. 199, 261–271.

Voglmeier, K., Jocher, M., Häni, C., Ammann, C., 2018. Ammonia emission measurements of an intensively grazed pasture. Biogeosciences 15 (14), 4593–4608.

Walkiewicz, A., Brzezińska, M., Bieganowski, A., 2018. Methanotrophs are favored under hypoxia in ammonium-fertilized soils. Biol. Fert. Soils 54 (7), 861–870.

Wang, R., Hu, Y., Wang, Y., Ali, S., Liu, Q., Guo, S., 2019. Nitrogen application increases soil respiration but decreases temperature sensitivity: combined effects of crop and soil properties in a semiarid agroecosystem. Geoderma 353, 320–330.

Xia, L., Lam, S.K., Chen, D., Wang, J., Tang, Q., Yan, X., 2017. Can knowledge-based N management produce more staple grain with lower greenhouse gas emission and reactive nitrogen pollution? A meta-analysis. Glob. Change Biol. 23 (5), 1917–1925.

Zaman, M., Nguyen, M.L., 2012. How application timings of urease and nitrification inhibitors affect N losses from urine patches in pastoral system. Agric. Ecosyst. Environ. 156, 37–48.

Zaman, M., Zaman, S., Adhinarayanan, C., Nguyen, M.L., Nawaz, S., Dawar, K.M., 2013. Effects of urease and nitrification inhibitors on the efficient use of urea for pastoral systems. Soil Sci. Plant Nutr. 59 (4), 649–659.

Zhao, Z., Wu, D., Bol, R., Shi, Y., Guo, Y., Meng, F., Wu, W., 2017. Nitrification inhibitor's effect on mitigating N_2O emissions was weakened by urease inhibitor in calcareous soils. Atmos. Environ. 166, 142–150.