J. Plant Nutr. Soil Sci. 2020, *000,* 1–12 **DOI:** 10.1002/jpln.201900561

Utility of stabilized nitrogen fertilizers to reduce nitrate leaching under optimal management practices

Noemí Mateo-Marín[1]*, **Dolores Quílez**[1], and **Ramón Isla**[1]

[1] Unidad de Suelos y Riegos (asociada a EEAD-CSIC), Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA). Avda. Montañana 930, 50059, Zaragoza, Spain

Abstract

Background: The inadequate application of nitrogen (N) to crops has increased the reactive N in the atmosphere and in the surface and ground waters. Stabilized N-fertilizers with nitrification (NI) and urease (UI) inhibitors have been proposed to reduce these environmental problems without affecting or even increasing crop productivity.
Aim: The objective of this study was to evaluate, in a maize–maize–wheat rotation, if the use of the NI 3,4-dimethylpyrazole phosphate (DMPP) and the UIs N-(n-butyl) thiophosphoric triamide (NBPT) and monocarbamide dihydrogen sulfate (MCDHS) reduces N leaching without compromising yield under optimal management of N and water.
Methods: The experiment was conducted in 24 drainage lysimeters with two soil types with contrasting water holding capacity under Mediterranean irrigated conditions. The fertilizer treatments were urea, urea with DMPP, urea with NBPT, and urea with MCDHS. For the maize crop, conventional fertilizer application was split into 6- and 13-leaf stages, whereas stabilized fertilizers were applied as a single application at the 6-leaf stage. All fertilizer treatments were applied at late tillering in the wheat crop.
Results: The soil mineral N was measured at the beginning and the end of each crop season, but no differences were found among fertilizer treatments. Differences in the volume of water drained or the cumulative mass of nitrate depending on the fertilizer were not significant (three-year treatment average of 200 L m^{-2} and 22 kg N ha^{-1} in the Deep soil, and 334 L m^{-2} and 40 kg N ha^{-1} in the Shallow type, respectively). No consistent significant differences were found in agronomic parameters (chlorophyll measurements, yield, and total N uptake) between the fertilizer treatments.
Conclusion: Based on the results, the use of stabilized N-fertilizer could be recommended to reduce the number of N applications in maize without compromising grain yield but with no advantages to reduce nitrate-leaching losses if N rates are managed properly under efficient irrigation management practices.

Key words: 3,4-dimethylpyrazole phosphate / monocarbamide dihydrogen sulphate / N-(n-butyl) thiophosphoric triamide / nitrate leaching / nitrification inhibitor / urease inhibitor

Accepted June 28, 2020

1 Introduction

Food production depends on the addition of nitrogen (N) fertilizers to obtain profitable crop yields (*Timilsena* et al., 2015), especially under irrigated conditions (*Berenguer* et al., 2009). Nevertheless, excessive N application causes environmental problems such as contamination of surface and ground waters by nitrate (*Peña-Haro* et al., 2010) or atmospheric contamination through the release of nitrogen oxides and ammonia (*Huérfano* et al., 2015; *Timilsena* et al., 2015). In semiarid Mediterranean conditions, high nitrate (NO_3^-) concentrations are found in irrigation return flows (*Barros* et al., 2012) and have been related to mismanagement of N application, inadequate irrigation practices, or inefficient irrigation systems that lead to water pollution (*Cavero* et al., 2012).

Enhancing nitrogen use efficiency (NUE) seems to be a good approach for addressing the triple challenge of environmental degradation, climate change, and food security (*Zhang* et al., 2015) and for reaching the Sustainable Development Goals of the 2030 Agenda (*United Nations*, 2015). This increase of NUE can be accomplished by improving the synchronization between the N supply and crop demand, and by reducing N losses using stabilized N-fertilizers that include nitrification and urease inhibitors (*Abalos* et al., 2014). Nitrification inhibitors (NIs) are compounds that delay the bacterial oxidation of ammonium (NH_4^+) to nitrite in the soil for a certain period by depressing the activity of *Nitrosomonas* bacteria (*Zerulla* et al., 2001). Urease inhibitors (UIs) inactivate the urease enzyme, consequently, the enzymatic hydrolysis of urea is

---

* Correspondence: N. Mateo-Marín; e-mail: nmateo@cita-aragon.es

slowed down or even stopped (*Snyder* et al., 2009), delaying the conversion of urea to ammonium and thus to nitrate. Different studies in maize (*Díez-López* et al., 2008; *Díez* et al., 2010), wheat (*Carrasco* and *Villar*, 2001), and other crops (*Serna* et al., 2000; *Egea* and *Alarcón*, 2004) have described potential reductions in nitrate losses by leaching using different NIs under irrigated conditions. Nitrate leaching reduction by NIs has been estimated at approximately 17%, with an increment in yield production of 3%, according to the meta-analysis of *Quemada* et al. (2013). UIs have also shown to be effective in reducing NO_3^- leaching (*Abalos* et al., 2014), yet *Rawluk* et al. (2001) indicate the risk of rapid movement of urea deeper into the soil profile due to its high solubility.

Rose et al. (2018), in a re-evaluation of the effectiveness of nitrification inhibitors, found that they achieve higher yields over conventional fertilizers at sub-optimal N rates, and the key question that arose is whether N loss can be reduced by applying inhibitors without loss of yield while being economically viable. Moreover, the general utility of these stabilized N-fertilizers in increasing NUE have been also questioned (*Yang* et al., 2016; *Rose* et al., 2018) due to their interactions with other climatic, edaphic, and management factors. To obtain such information, further studies across a range of crops and environments are needed.

Two of the most commonly used nitrification and urea inhibitors are 3,4-dimethylpyrazole phosphate (DMPP) and N-(n-butyl) thiophosphoric triamide (NBPT) (*Abalos* et al., 2014). More recently, a Spain-based fertilizer company released the technology DURAMON® based on the addition of the molecule monocarbamide dihydrogen sulfate (MCDHS; international patent WO 2007/132032 A1) to urea fertilizers with the potential to stabilize the urea-N through the inhibition of the urease enzyme. No information is available in the scientific literature about the effectiveness of this product to improve NUE compared to the above-mentioned and more-studied inhibitors.

For conventional fertilizers, the most extended and recommended practice in irrigated maize is to split N into two side-dress applications to increase its efficiency. Stabilized N-fertilizers might reduce the number of N applications, which would decrease fuel needs and operation time (*Huérfano* et al., 2015). Nevertheless, most of the studies addressing the effectiveness of inhibitors consider neither this issue nor the importance of irrigation management practices to increase NUE and reduce N losses. Besides, there is an absence of studies developed during a complete crop rotation established with N fertilizers managed at near-optimal management rates with rational irrigation management. Accordingly, the objective of this research is to assess, under semiarid irrigated conditions in a three-year rotation (maize–maize–wheat), the effect of three stabilized N-fertilizers (urea with DMPP, NBPT, and MCDHS) on crop productivity, nitrogen use efficiency, and nitrate losses by leaching in two soil types with contrasting water holding capacity. The hypothesis was that, in the case of maize, a single application of stabilized urea could reduce nitrate leaching compared to the conventional two side-dress urea applications, maintaining crop productivity.

2 Material and methods

2.1 Site and experimental design

This study was conducted from 2015 to 2017 in the CITA experimental field 'Soto Lezcano' in the middle Ebro river basin (Zaragoza, Spain), where the climate is semiarid Mediterranean-continental (mean annual maximum and minimum air temperatures of 21.4 and 8.3°C, respectively; yearly average precipitation of 319 mm; yearly average reference evapotranspiration of 1,239 mm; period 2004–2018).

An experimental facility with 24 concrete-made drainage lysimeters (size 2.0 m × 2.5 m, and 1.5-m depth) was used for the research. Lysimeters were filled in 2013 with soil from two different fields, twelve lysimeters with each soil type, to represent two contrasting soil types that frequently appear in the Ebro valley area. The soils are denominated in the study as "Deep" (restricted to 1.25-m soil depth) and "Shallow" (restricted to 0.50-m soil depth). The soil in the lysimeters was over a layer of gravel of 1 m for Shallow soil and 0.25 m for Deep soil. The physicochemical characteristics of the two soils are presented in Tab. 1. The main differences between both soils are the soil depth and the soil stoniness, which confer contrasting soil water holding capacity (223.3 mm in Deep soil and 63.2 mm in Shallow soil).

A crop rotation of maize–maize–wheat (*Zea mays* L. hybrid 'Pioneer P1758' and bread-making wheat *Triticum aestivum* L. cv. 'Rimbaud') was followed according to the management description in Tab. 2. The areas surrounding the lysimeters were also sowed to the same crop to avoid border effects. Previously to this experiment, the lysimeters were cropped with sunflower (2014) and barley (2015) with no differences in fertilization among lysimeters.

The experiment for each soil type had a completely randomized block design with three replicates for each treatment. The side-dressing fertilizer treatments evaluated along the three-year rotation were: (1) standard urea (Urea), (2) urea with the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP), (3) urea with the urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT), and (4) urea with the urease inhibitor monocarbamide dihydrogen sulfate (MCDHS). In maize crops, Urea treatment was split into two applications at the V6 and V13 stages (*Ritchie* et al., 1986) as is usual among local farmers, whereas in treatments with stabilized urea, fertilizers were applied in a single application at the V6 stage, as is normally recommended to farmers by the companies that commercialize these products. In the wheat crop, N was applied at late tillering in a single N dose for all treatments since it is the habitual practice among local farmers (Tab. 2). Directly after N application, a short irrigation event was applied to wash the fertilizer into the soil to reduce N losses by ammonia volatilization.

The proportion of inhibitor substance relative to nitrogen was established by the fertilizer companies as 0.8, 0.13, and 1.5% for DMPP, NBPT, and MCDHS, respectively. Only one of the three stabilized fertilizers (NBPT) used in the experiment is a commercial product (UTEC®), and the other two (DMPP and

Table 1: Main characteristics of Deep and Shallow soils.

Soil characteristics	**Deep soil**			**Shallow soil**	
	0–30 cm	**30–60 cm**	**60–125 cm**	**0–25 cm**	**25–50 cm**
Soil texture	Clay-loam	Clay-loam	Loam	Clay-loam	Clay-loam
Sand (%)	29	31	33	24	30
Silt (%)	52	51	48	40	36
Clay (%)	19	18	19	36	34
Stoniness (%vol.)	3.1	0.9	7.0	11.4	15.2
Available water (mm)	54.5	54.5	114.3	32.1	31.1
P (Olsen) (mg kg^{-1})	30.7	7.8	12.4	14.5	17.5
K (NH_4Ac) (mg kg^{-1})	499	236	72	225	202
Organic matter (%)	1.46	0.94	0.79	2.04	1.24
Soil pH (1:2.5 H_2O)	8.27	8.65	8.04	7.71	7.65

Table 2: General crop management description in field trials.

	Maize 1	**Maize 2**	**Wheat**
Sowing date	04/05/2015	14/04/2016	10/11/2016
Harvest date	05/10/2015	13/09/2016	03/07/2017
Seed rate (plants ha^{-1} or kg seed ha^{-1})	88083	87000	286
Date N pre-planting	30/04/2015	13/04/2016	–
Date N side-dress 1	15/06/2015	06/06/2016	27/02/2017
Date N side-dress 2	20/07/2015	05/07/2016	–
Total N applied (kg N ha^{-1})			
Deep soil	211	173	150
Shallow soil	236	211	150
Irrigation + Rain (mm)[a]	985	945	609
Crop E.T. (mm)[b]	918	866	578

[a] From sowing to harvest;
[b] obtained from soil water balance.

MCDHS) were prepared *ad hoc* for the study by the manufacturing companies.

At pre-sowing, 50–100–150 kg ha^{-1} ($N–P_2O_5–K_2O$) was applied to maize crops and 0–229–154 kg ha^{-1} ($N–P_2O_5–K_2O$) to wheat. The total N rates, shown in Tab. 2, were calculated each year taking into account the soil mineral nitrogen (SMN) at pre-planting in the upper part of the soil profile (0–25 cm in Shallow soil and 0–30 cm in Deep soil) and considering previous studies in the area showing that maize requires 250 kg N ha^{-1} (*Isla* et al., 2006) of available N (SMN at pre-planting + N from fertilizer). Wheat received a constant rate of 150 kg N ha^{-1} in both soil types.

The weekly irrigation requirements were calculated from the Penman–Monteith reference evapotranspiration and the crop coefficients of maize and wheat according to *Martínez-Cob* (2008) and FAO procedures (*Allen* et al., 1998), respectively. The salinity of the irrigation water (average of 1.5 dS m^{-1} over the three seasons) was above the threshold maize salt tolerance (*Ayers* and *Westcot*, 1985), and a 20% surplus of irrigation water was added over the irrigation requirements to avoid salt accumulation in the soil and the associated yield reduction due to salt stress. Crops were sprinkler irrigated and the total water received in each lysimeter was measured every irrigation with a rain gauge. The total water applied to each crop is presented in Tab. 2.

Weeds and pests were controlled according to standard practices of the area to guarantee adequate growth of maize and wheat and no special problems were observed during the study.

2.2 Soil mineral nitrogen

Soil sampling was performed at the beginning of each season and after harvest to evaluate the SMN. Shallow soil was sampled in two depth intervals (0–25 and 25–50 cm) and Deep soil in three depth intervals (0–30, 30–60, and 60–120 cm). At each lysimeter and for each soil depth, two-soil core samples were taken using an auger (5-cm diameter) and combined for further analyses. A subsample was used to calculate soil water content by gravimetry (drying at 105°C until constant weight). Another subsample of 10 g of fresh soil was extracted with 30 mL of 2 N KCl, shaken for 30 min, and filtered through a cellulose filter. The nitrate and ammonium concentrations in the extracts were analyzed by colorimetry using a segmented flow analyzer (AutoAnalyzer 3, Bran+Luebbe, Norderstedt, Germany).

2.3 Drainage water

Drainage from each lysimeter was collected weekly in 50-L graduated tanks set in an underground gallery, and the volume was measured. A 30-mL subsample was collected from each tank to analyze nitrate and ammonium concentrations using a segmented flow analyzer. The mass of nitrate leached was calculated for each sampling date as the product of drainage volume by nitrate concentration. The ammonium concentration was analyzed only during the first year (2015) because it was extremely low (average of 0.10 mg N L^{-1}; $n = 310$) compared to that of nitrate (18.1 mg N L^{-1}).

2.4 Crop nitrogen status and yield

The nutritional status of maize and wheat plants was evaluated using a portable chlorophyll meter (SPAD-502®, Minolta Camera Co., Ltd., Osaka, Japan) at different growth stages. In maize, SPAD readings were taken on the youngest fully developed leaf at the sixth leaf (V6), tenth leaf (V10), and on the ear leaf at the thirteenth leaf (V13), tasseling (VT), and milky grain (R3) stages according to *Ritchie* et al. (1986) scale. In wheat, SPAD readings were taken on the previous to the last unfolded leaf at anthesis half-way (GS-65), caryopsis water ripe (GS-71), and medium milk (GS-75) stages according to the *Zadoks* et al. (1974) scale.

At maize maturity (October 02, 2015, and September 13, 2016), all ears in each lysimeter were hand-harvested to determine grain yield (reported on the basis of 140 g kg^{-1} moisture content) and number of grains per square meter. The rest of the aerial parts (stem + leaves) were harvested and a subsample was dried to determine the total dry aboveground biomass.

At wheat maturity (July 03, 2017), a 0.73-m^2 subsample was randomly hand-harvested from each plot to determine biomass yield and number of grains per square meter. The rest of the plot was mechanically harvested by an experimental combine to determine grain yield (reported on the basis of 120 g kg^{-1} moisture content).

N content was analyzed from dry (at 65°C) and finely ground grain and plant samples of maize and wheat by dry combustion (TruSpec CN, LECO, St. Joseph, MI, USA). NUE was calculated as the ratio between total N extracted in the aboveground biomass and the N applied by fertilization.

2.5 Statistical analysis

The effect of fertilizer treatments on the different variables was analyzed separately for Deep and Shallow soils and for the three experimental years: Maize 1 (from sowing Maize 1 to sowing Maize 2), Maize 2 (from sowing Maize 2 to sowing Wheat) and Wheat (from sowing to end of September). Some variables were also analyzed for maize crop (as the sum of both maize seasons) and for the whole rotation (from sowing Maize 1 to end of September 2017).

Data were subjected to analysis of variance, and differences among fertilizer treatment means were established with Tukey's test. In the case of repeated measurements over time (nitrate mass in drainage), a repeated measure analysis was performed with the MIXED procedure considering a first-order autoregressive structure covariance model AR(1). Linear regression was used to relate yield with yield components. In all tests, the default level of significance considered was 0.05. Statistical analyses were performed using SAS® software (University Edition, SAS Institute Inc., Cary, NC, USA).

3 Results

3.1 Soil mineral nitrogen

No differences in SMN among fertilizer treatments were found in the two types of soil during the development of the experiment (Tab. 3). Overall, the different treatments presented a similar coefficient of variation (18% in Deep soil and 20% in Shallow soil on average), indicating a reasonable variability in SMN among replicated plots. A high increase in SMN was observed between the harvest of Maize 1 and the sowing of Maize 2 in both soil types. Thus, averaging across treatments, the SMN increased from 37 to 155 kg N ha^{-1} in the Deep soil and 24 to 52 kg N ha^{-1} in the Shallow soil.

3.2 Nitrate losses by drainage

The volume of drainage was not affected by fertilizer treatments in any of the three years in the two soil types (Tab. 4). Averaging over the years and soil types, the volume of water drained during the wheat crop was approximately 36% of that drained during maize crops. A high proportion of drainage (74%) happened during the period from seeding to harvest, *i.e.*, during the crop cycle (Fig. 1). Overall, using the measured volumes of drainage, rain, and irrigation, the leaching fraction for the whole rotation was 0.07 for the Deep soil and 0.13 for the Shallow soil.

The weekly mass of nitrate leached (Fig. 2), analyzed using a repeated measure procedure, did not show significant differences among treatments for any of the three crops in the two soil types. No differences among treatments in the mass of nitrate leached were found for the crop period, intercrop period, or 30-day post-fertilization period (data not shown).

Table 3: Average (n = 3) of soil mineral nitrogen content (kg N ha^{-1}) in the different treatments for the whole soil profile (0–120 cm in Deep soil and 0–50 cm in Shallow soil) at different times during the maize–maize–wheat rotation. No significant differences were found among treatments in the two soil types.

	Maize 1		**Maize 2**		**Wheat**
	Pre-plant (21/04/15)	**Harvest (19/10/15)**	**Pre-plant (08/04/16)**	**Harvest (20/09/16)**	**Harvest (10/07/17)**
Deep soil					
Urea	64.7	36.2	165.8	38.9	66.0
DMPP	68.0	36.1	161.3	40.9	62.2
NBPT	54.6	37.4	137.2	37.4	61.4
MCDHS	59.6	36.5	156.6	40.0	42.2
Shallow soil					
Urea	18.9	28.0	65.0	26.3	42.8
DMPP	10.6	21.5	51.7	28.1	31.0
NBPT	15.6	28.7	49.1	24.3	58.3
MCDHS	18.1	21.1	43.0	27.8	41.4

Table 4: Average (n = 3) of cumulative drainage (mm) in the different fertilizer treatments (Urea, DMPP, NBPT, and MCDHS) for the three crops (Maize 1, Maize 2, and Wheat) and the two soil types (Deep and Shallow). For maize, the period includes the crop period (sowing to harvest) and the intercrop period (harvest to the following crop sowing). For wheat, the period goes from sowing to the end of September. No significant differences were found among treatments in the two soil types.

	Maize 1	**Maize 2**	**Wheat**
Deep soil			
Urea	68	98	48
DMPP	80	81	40
NBPT	71	54	25
MCDHS	82	94	60
Shallow soil			
Urea	163	119	90
DMPP	157	120	108
NBPT	151	121	90
MCDHS	154	127	97

Considering the whole three-year rotation, higher cumulative losses of nitrate were observed in the Shallow soil (40.4 kg N ha^{-1}) compared to the Deep soil (22.0 kg N ha^{-1}). However, no significant differences were observed in the cumulative mass of nitrate leached among treatments in any of the two soil types (Tab. 5). In both soils, most of the nitrate was leached during the crop period (82% and 84% for Deep and Shallow soil, respectively) and the period within a month after the fertilization date accounted for 33% (Deep soil) and 44% (Shallow soil) of the total N leached.

3.3 Nutritional status of maize and wheat

No significant differences among treatments were observed in SPAD meter readings for the first maize crop (2015) for the five sampling dates in any of the two soil types (Fig. 2). However, in the second maize crop (2016) there were significant differences among treatments on some sampling dates. In Deep soil, MCDHS and DMPP showed lower SPAD values than NBPT and Urea at later growth stages, although only the MCDHS (on average 11% lower than NBPT and Urea) was significantly different at the VT stage. In Shallow soil, the SPAD values of the MCDHS treatment were 14% and 16% lower than those of the NBPT treatment at the VT and R3 stages, respectively. In wheat crop, no significant differences were found in SPAD values among treatments at any time for the two soil types (Fig. 3).

3.4 Total aboveground biomass and grain yield

The maize yield averaged 19.0 and 15.4 Mg ha^{-1} in 2015 (Maize 1) and 2016 (Maize 2), respectively (Tab. 6). Grain yield in Deep soil was significantly higher than that in Shallow soil (on average 18% higher). Variations in maize grain yield among plots across years and soil types were significantly related to kernel weight (R^2 = 0.74) and number of grains per square meter (R^2 = 0.70). The grain yield of wheat averaged 7.5 Mg ha^{-1} and was 39% higher in Deep soil than in Shallow soil.

Differences in yield performance were observed among treatments in Shallow soil but not in Deep soil (Tab. 6). No significant differences in maize grain yield among treatments were observed in Deep soil, but differences ($p < 0.1$) were observed in Shallow soil in both seasons. In Shallow soil, maize yield showed significant differences among treatments for the pooled data of the two seasons. Thus, MCDHS treatment had a 15% lower grain yield than NBPT. Similarly, total aboveground biomass was 10% lower in MCDHS compared to NBPT. In the case of wheat, Urea showed a 10% higher yield than the treatment with DMPP (Shallow soil), although no significant differences among treatments were observed in aboveground biomass.

3.5 Plant nitrogen concentration and nitrogen use efficiency

The grain N content of maize ranged between 1.23% and 1.38% depending on the year, soil type, and treatment (Tab. 7). No significant differences in maize grain N content among treatments were observed in the two years for the two soil types. Some minor, although statistically significant, differences were found in the wheat grain N content since it was

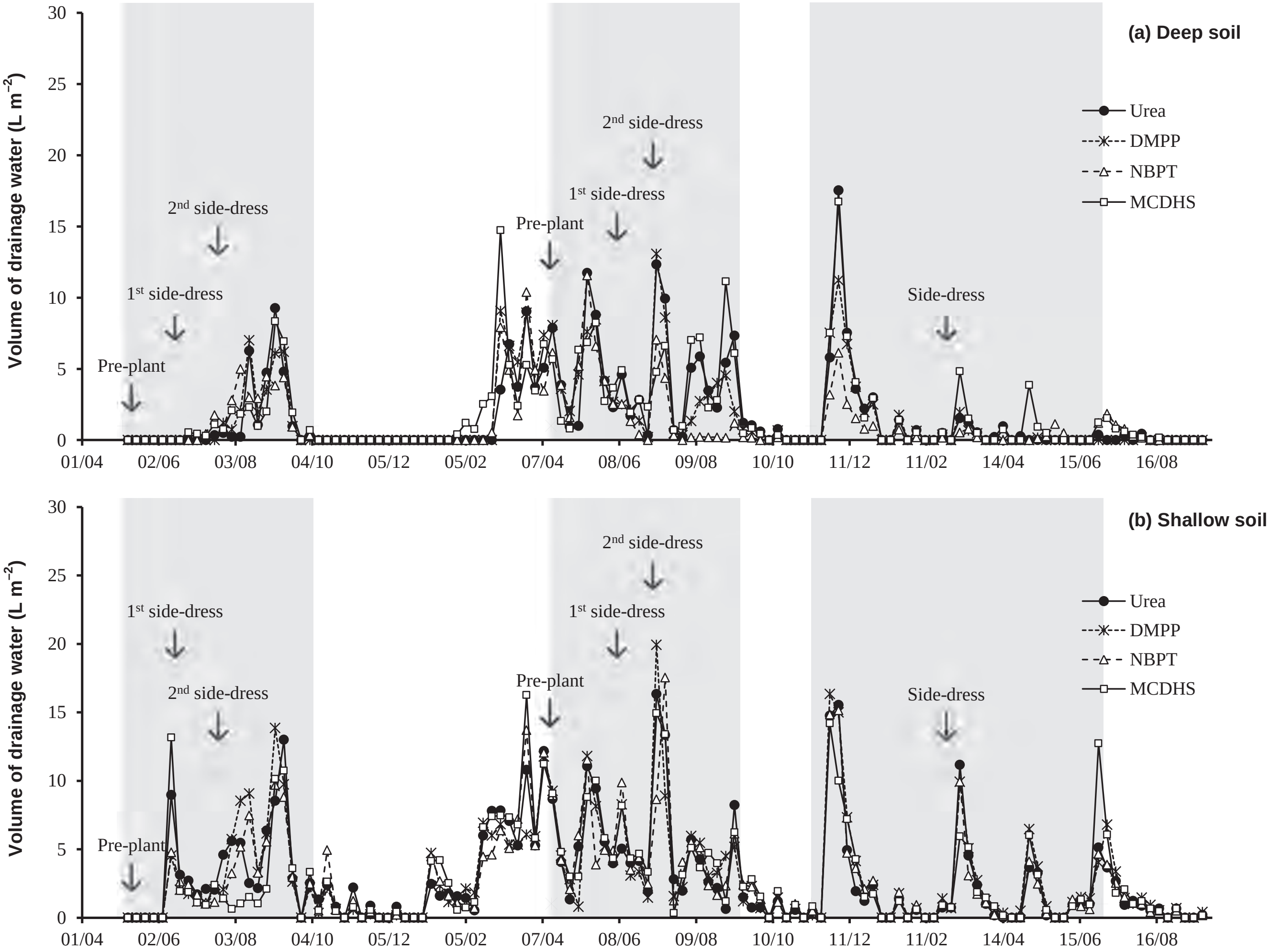

Figure 1: Averages of weekly volume of drainage water (L m^{-2} $week^{-1}$, *n* = 3) for the different fertilizer treatments (Urea, DMPP, NBPT, and MCDHS). The dynamic is presented for the Deep (a) and Shallow soil (b). The shadow area shows the period between seeding and harvest for each crop (Maize 1, Maize 2, Wheat).

Table 5: Average (*n* = 3) of the cumulative mass of nitrate (kg N ha^{-1}) leached in the different fertilizer treatments (Urea, DMPP, NBPT, and MCDHS). The results are presented separately by soil type (Deep and Shallow) and periods[a]. No significant differences were found among treatments in the two soil types.

	Maize 1	Maize 2	Wheat	Maize 1+2	Whole rotation
Deep soil					
Urea	6.8	8.3	3.3	15.1	18.4
DMPP	7.2	7.5	3.7	14.7	18.4
NBPT	15.3	7.8	1.7	23.0	24.7
MCDHS	11.4	11.5	3.6	23.0	26.5
Shallow soil					
Urea	19.8	19.2	4.5	39.0	43.5
DMPP	14.5	16.5	4.8	31.1	35.9
NBPT	25.7	19.6	5.0	45.3	50.2
MCDHS	14.3	13.7	4.1	28.0	32.0

[a] ‘Maize 1’, ‘Maize 2’ and ‘Wheat’ include the period from sowing to the following sowing. ‘Maize 1+2’ includes from Maize 1’s sowing to wheat’s sowing. ‘Whole rotation’ includes from Maize 1’s sowing to end September.

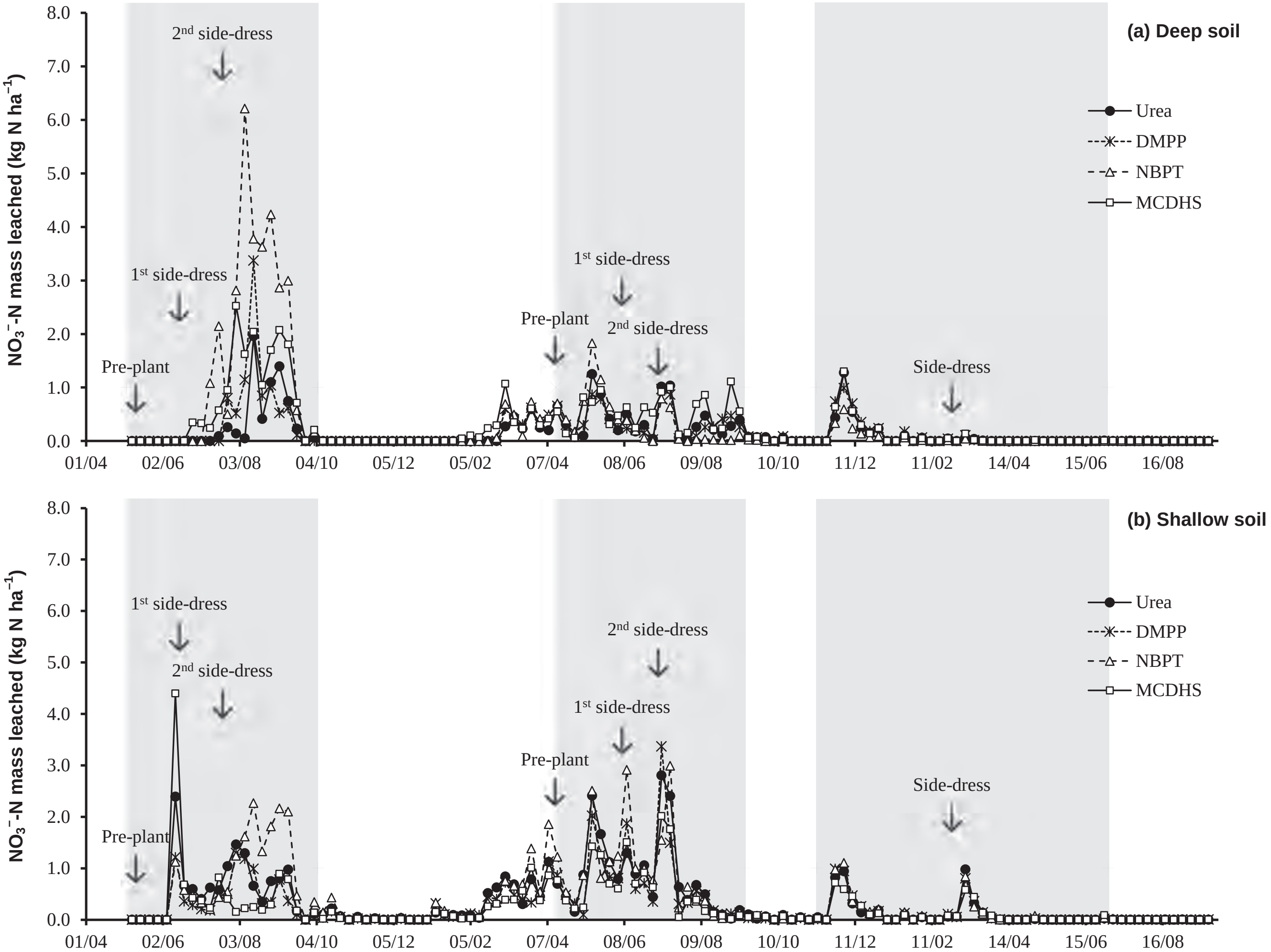

Figure 2: Averages of weekly nitrate mass leached (kg N ha^{-1} week^{-1}, $n = 3$) for the different fertilizer treatments (Urea, DMPP, NBPT, and MCDHS) for the Deep (a) and Shallow soil (b). The shadow area shows the period between seeding and harvest for each crop (Maize 1, Maize 2, and Wheat).

slightly higher in NBPT than in Urea (11% lower) and MCDHS (9% lower) in the Shallow soil.

No significant differences among treatments were observed in the total N uptake (total aboveground biomass N) of maize and wheat in the Deep soil (Tab. 7). However, some significant differences were found in the Shallow soil for maize. The MCDHS treatment presented (in the second maize crop) lower N uptake than the Urea (19%) and NBPT (21%) treatments. NBPT treatment always ranked as the top treatment in terms of total N uptake in the two soils, although the differences were not always significant.

In maize, NUE was higher than 1 for all treatments (except for MCDHS in Shallow soil– Maize 2; Tab. 7), indicating a relevant contribution of the soil to maize N nutrition. In the Deep soil, this contribution is remarkable because the soil contribution is equivalent, at least, to 51–82% of that of N fertilizer. Averaging over crops and years, NUE was significantly higher in the Deep soil (1.44 kg N kg^{-1} N applied) than in the Shallow soil (0.94 kg N kg^{-1} N applied). No significant differences in NUE among fertilizer treatments were observed in the Deep soil for maize or wheat. In the Shallow soil, averaging over the two maize years, NBPT presented a 17% higher NUE than MCDHS, although the difference was significant only in 2016 (Maize 2). Similarly, Urea also showed a 15% higher NUE than MCDHS in maize, but the difference was only significant in 2016. In wheat, the NUE of NBPT was 17% higher than that of the MCDHS treatment.

4 Discussion

Soil mineral nitrogen responded according to the management practices. SMN increased from harvest to the subsequent seeding as in the study of *Arregui* and *Quemada* (2006), presumably due to organic matter mineralization. The Deep soil presented higher SMN change (119 kg N ha^{-1}) during the intercrop period from the harvest of Maize 1 (October 2015) to the sowing of Maize 2 (April 2016) than Shallow soil (27 kg N ha^{-1}). That important increase in SMN, especially in the Deep soil, could be explained by the high number of short rainfall events (51 days with precipitation lower than 5 mm and one day with precipitation higher than 25 mm) and scarcity of events of drainage. The high soil water content in the

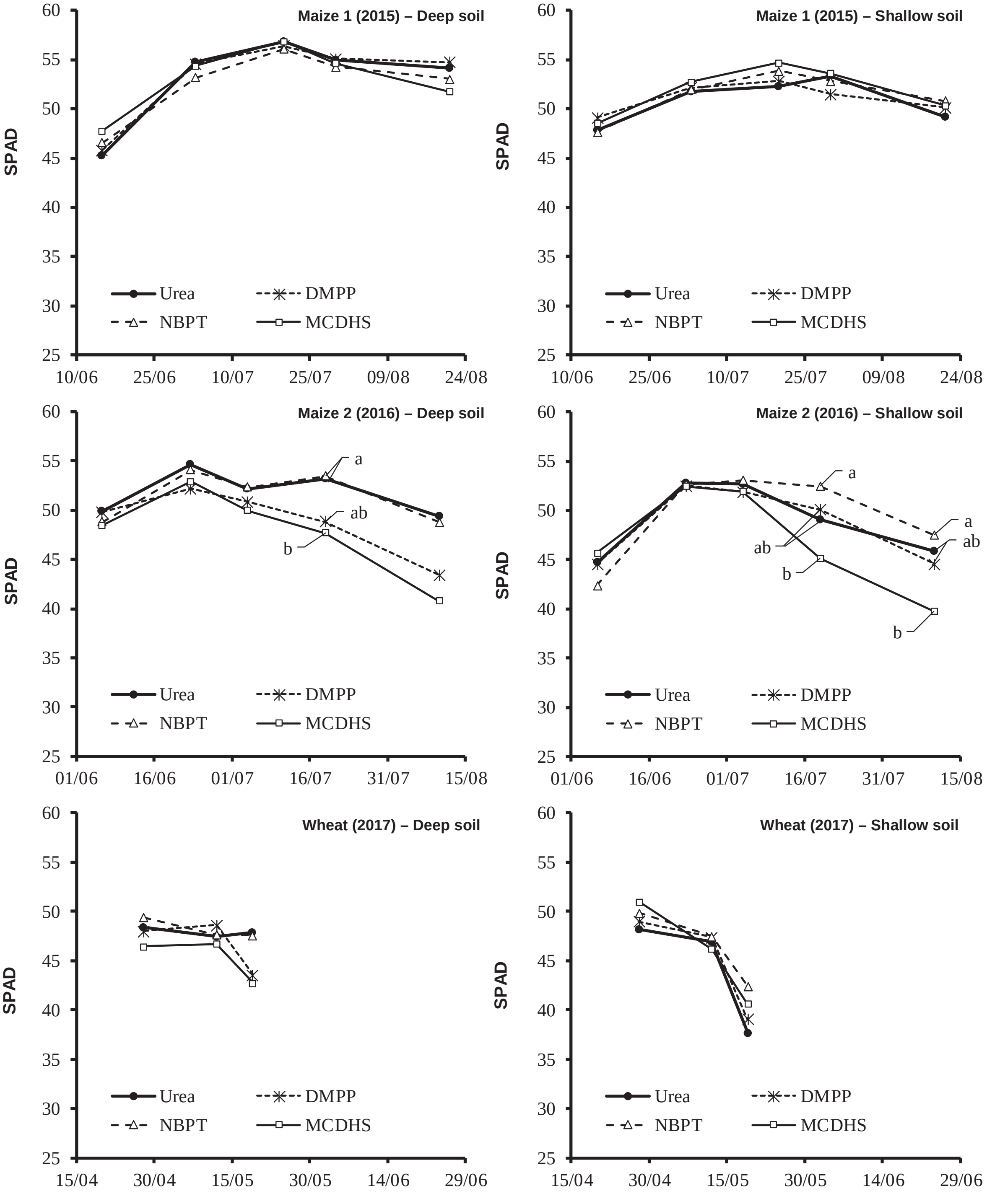

Figure 3: Averages of chlorophyll meter readings (SPAD, $n = 3$) for the different fertilizer treatments (Urea, DMPP, NBPT, and MCDHS) in different maize (V6, V10, V13, VT, and R3) and wheat (GS-65, GS-71, and GS-75) stages. Different letters indicate significant differences between treatments.

topsoil during spring could promote high N mineralization rates. This happened despite the removal of maize crop residues each year, which indicates the high mineralization rate that could be expected in some soils under irrigated Mediterranean conditions. SMN values after the harvest were prone to be small, which indicates a good adjustment of N fertilizer rates. This good adjustment may be the reason for the absence of significant differences in residual SMN among treatments at the end of the experiment in contrast to the residual SMN effect of inhibitors reported by other studies. *Alonso-Ayuso* et al. (2016) found, in two consecutive years of maize cultivation, a higher residual SMN after the application of ammonium nitrate sulfate blended with the nitrification inhibitor DMPP compared to the application of the same N fertilizer

Table 6: Average values (*n* = 3) of grain yield (Mg ha^{-1}) and total aboveground biomass (Mg ha^{-1}) of maize (Maize 1 and Maize 2) and Wheat in the different fertilizer treatments (Urea, DMPP, NBPT, and MCDHS) for the two soil types (Deep and Shallow). For each soil type, values followed by the same letter in a column are not significantly different.

	Grain yield (Mg ha^{-1})				**Total aboveground biomass (Mg ha^{-1})**			
	Maize 1	**Maize 2**	**Wheat**	**Maize 1+2**	**Maize 1**	**Maize 2**	**Wheat**	**Maize 1+2**
Deep soil								
Urea	20.9	17.2	8.7	38.1	35.3	30.8	18.2	65.2
DMPP	20.7	16.3	8.9	36.9	33.9	30.0	19.2	63.9
NBPT	21.1	18.0	8.8	39.1	35.3	31.5	19.7	66.8
MCDHS	20.1	16.4	8.5	36.3	33.3	28.9	19.1	62.2
Shallow soil								
Urea	17.5	14.6	6.7 a	32.1 ab	28.7	26.7	15.1	55.4 ab
DMPP	18.8	14.4	6.0 b	33.0 ab	28.6	26.6	14.8	55.1 ab
NBPT	19.6	15.4	6.3 ab	34.8 a	29.3	28.1	15.2	57.3 a
MCDHS	17.3	12.4	6.2 ab	29.7 b	27.7	23.8	14.6	51.4 b

Table 7: Average values (*n* = 3) of N in grain (%), N in total aboveground biomass (kg ha^{-1}), and NUE (kg N kg^{-1} N applied) for maize (Maize 1 and Maize 2) and wheat in the different fertilizer treatments (Urea, DMPP, NBPT, and MCDHS) and the two soil types (Deep and Shallow). For each soil type, values followed by the same letter in a column are not significantly different.

	Grain N (%)			**Total aboveground biomass N (kg ha^{-1})**			**NUE (kg N kg^{-1} N applied)**		
	Maize 1	**Maize 2**	**Wheat**	**Maize 1**	**Maize 2**	**Wheat**	**Maize 1**	**Maize 2**	**Wheat**
Deep soil									
Urea	1.37	1.37	1.61	353	303	155	1.67	1.75	1.03
DMPP	1.36	1.36	1.61	328	272	157	1.55	1.57	1.04
NBPT	1.36	1.36	1.73	360	315	171	1.71	1.82	1.14
MCDHS	1.33	1.38	1.60	318	276	149	1.51	1.59	1.00
Shallow soil									
Urea	1.30	1.30	1.41 b	270	241 a	103	1.14	1.15 a	0.69 ab
DMPP	1.26	1.26	1.47 ab	248	229 ab	93	1.10	1.09 ab	0.62 ab
NBPT	1.25	1.31	1.56 a	269	247 a	104	1.15	1.18 a	0.70 a
MCDHS	1.23	1.23	1.43 b	254	195 b	90	1.08	0.93 b	0.60 b

without NI. According to that study, the higher long-term life of NH_4^+ in the soil solution associated with NIs produced a larger non-exchangeable NH_4^+ fixation that could be conserved and released in the subsequent years to meet crop demands. The three-year rotation of this study does not suggest a significant effect in residual SMN using NI coupled with urea compared to standard urea. The considerably higher maize grain yields observed in this study (17.5 Mg ha^{-1}) compared to 10 Mg ha^{-1} in the study of *Alonso-Ayuso* et al. (2016) could drive to higher N crop uptake decreasing the chance for ammonium fixation by the clay particles of the soil, even with the comparatively higher doses of N applied in this experiment (average 208 kg N ha^{-1} *vs*. 170 kg N ha^{-1} in the above-mentioned study). Besides, the total N plant uptake after three years of cropping in the DMPP treatment (664 kg N ha^{-1}) was similar to or even lower than that in the Urea treatment (712 kg N ha^{-1}).

No significant differences in the mass of leached nitrate were observed with the addition of inhibitors. The good adjustment of irrigation to crop needs using very well defined crop coefficient values may explain the non-significant differences in N leaching among fertilizer treatments. As suggested by *Diez* et al. (2000), the mass of N leached depends strongly on the amount of drainage and, to a lesser extent, on variation in drainage nitrate concentration. However, in this study, a high percentage of the variability in the mass of nitrate drained among treatments and crops (97% and 72% in Shallow and Deep soils, respectively) was explained by differences in nitrate concentration in the drained water, and a smaller effect was associated with differences in volume drained (55% and 48% for Shallow and Deep soils). Similarly, *Díez* et al. (2010) could not find an effect of stabilized N-fertilizers on nitrate leaching when the water requirements of maize were adjusted and the drainage was low (71 mm during the crop-growing season). This experiment corroborates that result since the study had a similar volume of drainage for the maize crop season (55 mm in Deep soil and 91 mm in Shallow soil from sowing to harvest).

According to the meta-analysis of *Yang* et al. (2016), the more N fertilizer is applied, the greater reduction in soil N leaching should be expected from using NIs. In this study, N doses were calculated taking into account the potential N uptake and the SMN available at pre-planting, and the N rates were low compared to those used by farmers in the region (*Jiménez-Aguirre* et al., 2014). Maize residues were removed from plots due to the practical difficulty of incorporating maize residues into the soil because of the small size of the lysimeters preventing the use of heavy machinery. This fact could have promoted sub-optimal N conditions during the second and third crop seasons.

Maize SPAD values were similar to those in other studies at nearby locations (*Berenguer* et al., 2009); although they tended to be lower in the second growing season. SPAD readings in the wheat crop in this study were higher than the critical value described by *Arregui* et al. (2006), suggesting acceptable nutritional N-status during the vegetative period, although the low grain N content indicates N-deficit at later stages, affecting grain quality. Grain yields of maize and wheat were in the upper range of the yields normally obtained by growers in the region (*Berenguer* et al., 2009; *Isla* et al., 2015), especially the maize during the first growing season.

In a recent paper, *Rose* et al. (2018) suggest that the fertilizers frequently called enhanced efficiency N fertilizers (EENFs), which include fertilizers with nitrification and urease inhibitors, only allow higher yields compared to standard fertilizers when sub-optimal N rates are used. This makes sense since the agronomic advantage of EENFs compared to conventional fertilizers mainly relies on a significant reduction in N losses with subsequent improvement of the nutritional N-status of crops. In this maize–maize–wheat rotation, no significant advantage of using different stabilized fertilizers in terms of yield, total aboveground biomass, or NUE was observed, although the rates of N applied could be considered optimal to sub-optimal (average of 208 and 150 kg N ha^{-1} for maize and wheat, respectively) or at least clearly below the normal rates used by farmers in the region. Other authors have described no differences in grain yield, biomass yield, and aboveground N uptake between fertilizers with and without inhibitors in maize crop. Thus, *Guardia* et al. (2017) did not see differences between Urea and Urea+NBPT, and *Díez-López* et al. (2008) did not see differences between Urea and Urea+DMPP. During the wheat season and in Shallow soil, the grain yield was 0.7 Mg ha^{-1} lower in the DMPP than in the Urea treatment, although in both treatments N doses were the same and equally applied in one side-dress application at tillering stage. That contrasts with the results of the meta-analysis of *Hu* et al. (2014) in which NIs did not affect yield at the same number of N fertilizer applications in winter wheat. It can only be hypothesized that an increase in ammonia volatilization associated with the use of NIs (*Pan* et al., 2016) may have reduced the N availability in some critical stages inducing yield decrease.

In the case of maize, this study compares not only the effect of the addition of nitrification or urease inhibitors to urea but also the differences in N management: a single application for stabilized fertilizers *versus* two split applications for urea. Due to the higher price of these special fertilizers compared to the price of urea, their adoption by farmers must imply some advantage in practical terms. The main advantage supplied by stabilized N-fertilizers in this study was their ability to provide in maize, using a single side-dress application of N, similar yield, nitrogen uptake, and NUE as the conventional urea treatment in two side-dress applications. However, the exception was the tendency for lower performance of MCDHS in Shallow soil during the second year when N in total aboveground biomass and NUE were significantly different from those of Urea. The results for wheat indicate no significant advantage in terms of yield of using stabilized fertilizers compared to conventional urea, although there is a tendency for a higher NUE with NBPT, especially in the Shallow soil.

5 Conclusions

According to the results obtained in this experiment, under optimal irrigation and adjusted N rates the use of stabilized N-fertilizers presents relatively limited advantages in terms of yield and N leaching. However, the use of DMPP or NBPT allows the reduction in the number of side-dress applications in maize, which can be of interest from a practical point of view to simplify fertilizer management. On the other hand, the new urease inhibitor MCDHS decreased yield, N uptake, and NUE compared to the other evaluated stabilized N-fertilizers in most cases.

Acknowledgments

This study was funded by the *Spanish Ministry of Economy, Industry, and Competitiveness* (AGL2013-49062-C4-3-R project). N. Mateo-Marín was granted with a *FPI-INIA 2015 predoctoral fellowship* (*CPD-2015-0044*) by the *Spanish National Institute for Agricultural Research*. The stabilized fertilizers were supplied by Compo Expert S.L., EuroChem Agro Iberia S.L., and Fertinagro Biotech S.L. Thanks to *European funds* (Group A10-17R, Aragón Government). Thanks to the field and laboratory personnel of the Soil and Irrigation Department of CITA.

References

Abalos, D., *Jeffery, S.*, *Sanz-Cobena, A.*, *Guardia, G.*, *Vallejo, A.* (2014): Meta-analysis of the effect of urease and nitrification inhibitors on crop productivity and nitrogen use efficiency. *Agric. Ecosyst. Environ.* 189, 136–144.

Allen, R. G., *Pereira, L. S.*, *Raes, D.*, *Smith, M.* (1998): Crop Evapotranspiration: Guidelines for Computing Crop Water Requirements. FAO, Rome, Italy.

Alonso-Ayuso, M., *Gabriel, J. L.*, *Quemada, M.* (2016): Nitrogen use efficiency and residual effect of fertilizers with nitrification inhibitors. *Eur. J. Agron.* 80, 1–8.

Arregui, L. M., *Lasa, B.*, *Lafarga, A.*, *Irañeta, I.*, *Baroja, E.*, *Quemada, M.* (2006): Evaluation of chlorophyll meters as tools for N fertilization in winter wheat under humid Mediterranean conditions. *Eur. J. Agron.* 24, 140–148.

Arregui, L. M., *Quemada, M.* (2006): Drainage and nitrate leaching in a crop rotation under different N-fertilizer strategies: application of capacitance probes. *Plant Soil* 288, 57–69.

Ayers, R. S., *Westcot, D. W.* (1985): Water Quality for Agriculture. FAO, Rome, Italy.

Barros, R., *Isidoro, D.*, *Aragüés, R.* (2012): Irrigation management, nitrogen fertilization and nitrogen losses in the return flows of La Violada irrigation district (Spain). *Agric. Ecosyst. Environ.* 155, 161–171.

Berenguer, P., *Santiveri, F.*, *Boixadera, J.*, *Lloveras, J.* (2009): Nitrogen fertilisation of irrigated maize under Mediterranean conditions. *Eur. J. Agron.* 30, 163–171.

Carrasco, I., *Villar, J. M.* (2001): Field evaluation of DMPP as a nitrification inhibitor in the area irrigated by the Canal d'Urgell (Northeast Spain), in Horst, W. J., Schenk, M. K., Bürkert, A., Claassen, N., Flessa, H., Frommer, W. B., Goldbach, H., Olfs, H.-W., Römheld, V., Sattelmacher, B., Schmidhalter, U., Schubert, S., von Wirén, N., Wittenmayer, L. (eds.): Plant Nutrition—Food Security and Sustainability of Agro-Ecosystems. Springer, Dordrecht, The Netherlands, pp. 764–765.

Cavero, J., *Barros, R.*, *Sellam, F.*, *Topcu, S.*, *Isidoro, D.*, *Hartani, T.*, *Lounis, A.*, *Ibrikci, H.*, *Cetin, M.*, *Williams, J. R.*, *Aragüés, R.* (2012): APEX simulation of best irrigation and N management strategies for off-site N pollution control in three Mediterranean irrigated watersheds. *Agric. Water Manag.* 103, 88–99.

Díez-López, J. A., *Hernaiz-Algarra, P.*, *Arauzo-Sánchez, M. A.*, *Carrasco-Martín, I.* (2008): Effect of a nitrification inhibitor (DMPP) on nitrate leaching and maize yield during two growing seasons. *Spanish J. Agric. Res.* 6, 294–303.

Díez, J. A., *Arauzo, M.*, *Hernaiz, P. J.*, *Pascua, A. S.*, *Vallejo, A.* (2010): Comparison of nitrification inhibitors to restrict nitrate leaching in a maize crop irrigated under Mediterranean conditions. *Spanish J. Agric. Res.* 8, 481–492.

Diez, J. A., *Caballero, R.*, *Roman, R.*, *Tarquis, A.*, *Cartagena, M. C.*, *Vallejo, A.* (2000): Integrated fertilizer and irrigation management to reduce nitrate leaching in central Spain. *J. Environ. Qual.* 29, 1539–1547.

Egea, C., *Alarcón, A. L.* (2004): Seguimiento y control de la fertirrigación nitrogenada mediante el producto inhibidor de la nitrificación 3,4-dimetilpirazolfosfato, en cultivo de melón tipo verde. *Agrochimica* 48, 77–88.

Guardia, G., *Cangani, M. T.*, *Andreu, G.*, *Sanz-Cobena, A.*, *García-Marco, S.*, *Álvarez, J. M.*, *Recio-Huetos, J.*, *Vallejo, A.* (2017): Effect of inhibitors and fertigation strategies on GHG emissions, NO fluxes and yield in irrigated maize. *Field Crop. Res.* 204, 135–145.

Hu, Y., *Schraml, M.*, *von Tucher, S.*, *Li, F.*, *Schmidhalter, U.* (2014): Influence of nitrification inhibitors on yields of arable crops: A meta-analysis of recent studies in Germany. *Int. J. Plant Prod.* 8, 33–50.

Huérfano, X., *Fuertes-Mendizábal, T.*, *Duñabeitia, M. K.*, *González-Murua, C.*, *Estavillo, J. M.*, *Menéndez, S.* (2015): Splitting the application of 3,4-dimethylpyrazole phosphate (DMPP): Influence on greenhouse gases emissions and wheat yield and quality under humid Mediterranean conditions. *Eur. J. Agron.* 64, 47–57.

Isla, R., *Cavero, J.*, *Yagüe, M. R.*, *Quílez, D.* (2006): Balances de Nitrógeno en Cultivo de Maíz de Regadío en Aragón, in Ministerio de Educación y Ciencia (ed.): Monografía INIA, No 22. Balance de Nitrógeno En Sistemas de Cultivo de Cereal de Invierno y de Maíz En Varias Regiones Españolas. Ministerio de Educación y Ciencia, Madrid, Spain, pp. 107–124.

Isla, R., *Salmerón, M.*, *Cavero, J.*, *Yagüe, M. R.*, *Quílez, D.* (2015): Utility of the end-of-season nitrate test for nitrogen sufficiency of irrigated maize under Mediterranean semi-arid conditions. *Spanish J. Agric. Res.* 13, 1–12.

Jiménez-Aguirre, M. T., *Isidoro, D.*, *Barros-García, R.* (2014): Effect of irrigation modernization on water and nitrogen use efficiency. EGU General Assembly, April 27–May 02, 2014, Vienna, Austria.

Martínez-Cob, A. (2008): Use of thermal units to estimate corn crop coefficients under semiarid climatic conditions. *Irrig. Sci.* 26, 335–345.

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.

Peña-Haro, S., *Llopis-Albert, C.*, *Pulido-Velazquez, M.*, *Pulido-Velazquez, D.* (2010): Fertilizer standards for controlling groundwater nitrate pollution from agriculture: El Salobral-Los Llanos case study, Spain. *J. Hydrol.* 392, 174–187.

Quemada, M., *Baranski, M.*, *Nobel-de Lange, M. N. J.*, *Vallejo, A.*, *Cooper, J. M.* (2013): Meta-analysis of strategies to control nitrate leaching in irrigated agricultural systems and their effects on crop yield. *Agric. Ecosyst. Environ.* 174, 1–10.

Rawluk, C. D. L., *Grant, C. A.*, *Racz, G. J.* (2001): Ammonia volatilization from soils fertilized with urea and varying rates of urease inhibitor NBPT. *Can. J. Soil Sci.* 81, 239–246.

Ritchie, S. W., *Hanway, J. J.*, *Benson, G. O.* (1986): How a Corn Plant Develops. Iowa State University of Science and Technology, and Cooperative Extension Service, Ames, IA, USA.

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.

Serna, M. D., *Bañuls, J.*, *Quiñones, A.*, *Primo-Millo, E.*, *Legaz, F.* (2000): Evaluation of 3,4-dimethylpyrazole phosphate as a nitrification inhibitor in a *Citrus*-cultivated soil. *Biol. Fertil. Soils* 32, 41–46.

Snyder, C. S., *Bruulsema, T. W.*, *Jensen, T. L.*, *Fixen, P. E.* (2009): Review of greenhouse gas emissions from crop production systems and fertilizer management effects. *Agric. Ecosyst. Environ.* 133, 247–266.

Timilsena, Y. P., *Adhikari, R.*, *Casey, P.*, *Muster, T.*, *Gill, H.*, *Adhikari, B.* (2015): Enhanced efficiency fertilisers: a review of formulation and nutrient release patterns. *J. Sci. Food Agric.* 95, 1131–1142.

United Nations (2015): Sustainable Development Goals. Available at: https://www.un.org/sustainabledevelopment/sustainable-development-goals/ (last accessed December 03, 2019).

Yang, M., *Fang, Y.*, *Sun, D.*, *Shi, Y.* (2016): Efficiency of two nitrification inhibitors (dicyandiamide and 3, 4-dimethypyrazole phosphate) on soil nitrogen transformations and plant productivity: a meta-analysis. *Sci. Rep.* 6. DOI: https://doi.org/10.1038/srep22075.

Zadoks, J. C., *Chang, T. T.*, *Konzak, C. F.* (1974): A decimal code for the growth stages of cereals. *Weed Res.* 14, 415–421.

Zerulla, W., *Pasda, G.*, *Hähndel, R.*, *Wissemeier, A. H.* (2001): The New Nitrification Inhibitor DMPP (ENTEC®) for Use in Agricultural and Horticultural Crops—An Overview, in Horst, W. J., Schenk, M. K., Bürkert, A., Claassen, N., Flessa, H., Frommer, W. B., Goldbach, H., Olfs, H.-W., Römheld, V., Sattelmacher, B., Schmidhalter, U., Schubert, S., von Wirén, N., Wittenmayer, L. (eds.): Plant Nutrition—Food Security and Sustainability of Agro-Ecosystems. Springer, Dordrecht, The Netherlands, pp. 754–755.

Zhang, X., *Davidson, E. A.*, *Mauzerall, D. L.*, *Searchinger, T. D.*, *Dumas, P.*, *Shen, Y.* (2015): Managing nitrogen for sustainable development. *Nature* 528, 51–59.