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Full Length Article

Effect of Urease and Nitrification Inhibitors on Nitrogen Transformation and Nitrogen Use Efficiency of Rain-Fed Summer Maize (*Zea mays*) at Loess Plateau of China

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Abstract

Urease and nitrification inhibitors are used to increase N use efficiency (NUE) and decrease N loss. Their efficiencies are dependent on soil properties and climatic conditions. Rain-fed summer maize crop was grown at three different sites (Yangling, Zhouzhi-1 and Zhouzhi-2) in the Loess Plateau of China, having different soil properties and climatic conditions. The study aimed to evaluate the efficiency of urea treatment with N-(n-butyl) thiophosphoric triamide (NBPT) and a mixture of NBPT + dicyandiamide (DCD) on soil N transformation, N uptake and NUE. Control (no N), Urea (220 kg N ha^{-1}), U + NBPT (1.0%) and U+ NBPT + DCD (10%) were applied through the band placement method in two splits (7:3). The application of urea treatment with NBPT alone and a mixture of NBPT + DCD maintained the soil mineral-N content for a longer time, which resulted in more N uptake by the plant, and eventually improved NUE. Urea treatment with inhibitors increased maize grain yield (15–20%) compared to plain urea and (37.7–48%) unfertilized control. The highest plant N uptake was observed in maize fertilized by urea treatment with NBPT + DCD, followed by U + NBPT. Urea treatment with NBPT and NBPT + DCD increased NUE by 13.8–21.6 and 14.3–22.6%, respectively, compared to unfertilized control. The NUE was higher in maize fertilized with urea plus inhibitors, compared to urea alone. Consequently, the application of urea treatment with NBPT and a mixture of NBPT + DCD represented the best approach for improving N uptake, NUE and yield of the maize crop. © 2023 Friends Science Publishers

Keywords: Nitrogen accumulation; Nitrogen uptake; Nitrogen management; NBPT; DCD

Introduction

Nitrogen (N) is a key essential nutrient to maintain a higher crop production and global economic sustainability of agricultural systems (Zhang *et al.* 2013; Santos *et al.* 2020). Synthetic N fertilizers have been extensively used in the world (Miao *et al.* 2015; Santos *et al.* 2020). Urea is leading among the leading nitrogenous fertilizers, nearly 60% all over the world (Davis *et al.* 2016). Currently, it is a prominent source of chemical N fertilizer in China, accounting for 50–60% of total N fertilizer consumption and the most common N source for maize (*Zea mays* L.) crop fertilization (Li *et al.* 2015, 2017). Due to the complex nature of soil N transformation, accompanied by sub-optimal management practices of N fertilizers, usage of urea generally results in low N use efficiency (30–50%), contributing towards higher economic losses to farmers and hazards to the environment (Cui *et al.* 2010; Liu *et al.* 2015). Consequently, farmers are facing dual challenges to decrease N losses and improve N use efficiency (NUE) to sustain crop productivity (Liu *et al.* 2013; Tan *et al.* 2017). Enhancement of NUE in crop production is one of the significant ways of addressing the challenges of food security, environmental pollution and climate change (Davidson *et al.* 2015; Zhang *et al.* 2015).

Over last three decades, agricultural activities of northern China have relied on the increased use of N fertilizers to maintain the increasing crop yield (Gu *et al.* 2015). However, the response of N fertilizer varies considerably under different climatic conditions, soil physicochemical properties, fertilization methods, agronomic practices and water regimes, which affect N loss and NUE (Mohanty *et al.* 1999; Qiao *et al.* 2015).

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Ammonia (NH_3) emission is considered a principal source of N loss after urea fertilization, especially in alkaline and calcareous soils (Li *et al.* 2015, 2017). Therefore, it is crucial to decrease N losses, thereby enhancing the NUE. The N-(n-butyl) thiophosphoric triamide (NBPT) is considered as most effective urease inhibitor (UI) and is extensively used in many cropping systems (Sanz-Cobena *et al.* 2008, 2012; Zaman *et al.* 2009). NBPT efficiently blocked the urease enzymes thereby effectively delaying urea hydrolysis (Cantarella *et al.* 2018), resulting in increased N availability for plant uptake (Rose *et al.* 2018; Meng *et al.* 2023). Furthermore, the use of NBPT with urea has the potential to be the most appropriate strategy to decrease N loss and enhance NUE, thereby increasing crop productivity (Singh *et al.* 2013; Martins *et al.* 2017; Fu *et al.* 2020).

Generally, nitrate (NO_3^-) is the most abundant N form in upland soils and prone to highly vulnerable to leaching and denitrification losses, contributing to reduced NUE. A meta-analysis has reported that nitrification inhibitors (NIs) enhanced plant N recovery (34–93%), and subsequently increased grain (6–13%) and straw biomass (12–18%) yield (Qiao *et al.* 2015). Among the NIs, Dicyandiamide (DCD) has been extensively used in agriculture for agronomic enhancement of N use efficiency (Tian *et al.* 2015; Raza *et al.* 2019). Furthermore, the use of DCD with N fertilizers might prolong ammonium (NH_4^+) in the soil, which enables more plant N uptake (Asing *et al.* 2008; Li *et al.* 2009; Meng *et al.* 2023) and decreased N losses through NO_3^- leaching and nitrous oxide (N_2O) emissions contributing increased NUE (Cahalan *et al.* 2015; Yang *et al.* 2016; Montalbán *et al.* 2021).

Although NIs are effective in improving NUE, however, one drawback of using them is increased NH_3 volatilization, which is a significant N loss pathway in arable lands (Soares *et al.* 2012; Qiao *et al.* 2015). Some studies reported that the combined use of inhibitors affects the urea hydrolysis by UIs and ammonia oxidation by NIs, thereby enhancing effectiveness of N fertilizer use, is a "win-win" strategy to reduce N losses, increase crop yield and NUE for attaining economic benefits from the agricultural system (Abalos *et al.* 2016; Martins *et al.* 2017; Zhao *et al.* 2017). Furthermore, the use of inhibitors with N fertilizers produced additional revenue of $ 109.49–163 ha^{-1} Y^{-1} for maize farms (Qiao *et al*. 2015; Yang *et al*. 2016). The urease and nitrification inhibitors are environment-friendly approaches and are not considered harmful to animals or humans (Nugrahaeningtyas *et al.* 2022).

Some studies reported that the use of DCD with NBPT has no positive effect on N uptake, crop yield and NUE (Ding *et al.* 2011; Kawakami *et al.* 2012; Montalbán *et al.* 2021). It may be related to the differences in soil physicochemical properties (soil pH, soil texture, soil temperature, moisture content, and soil organic matter), climatic factors (precipitation and air temperature) and management factors (time and method of application) (Francisco *et al.* 2011; Suter *et al.* 2013; Abalos *et al.* 2014). Soils with medium to coarse textured and pH ≤ 6 have a positive response to inhibitors in increasing crop production and NUE (Abalos *et al.* 2014, 2016). The efficiency of NBPT and a mixture of NBPT+DCD are affected by soil moisture and climatic conditions (Sanz-cobena *et al.* 2012). Furthermore, NIs and UIs immediately decompose within a few days after fertilization and the efficiency becomes lower under high temperatures (>20°C) (Soares *et al.* 2012; Zhao *et al.* 2017). However, more studies are needed to evaluate the effectiveness of NBPT and a mixture of NBPT + DCD in different soil and environmental conditions.

In this context, it was hypothesized that different soils and climatic conditions of the Loess Plateau affect the urease and NIs efficiency. Therefore, this field experiment was conducted at three different sites of the Loess Plateau to evaluate the effect of urea treatment with NBPT alone and urea treatment with a mixture of NBPT + DCD. The objectives of the current study were to (1) evaluate the efficiency of NBPT alone and a mixture of NBPT + DCD with urea on soil N transformation; (2) assess the effectiveness of urea treatment with NBPT alone and urea treatment with NBPT+DCD on N uptake, yield and NUE of maize crop in different soil properties under different environmental conditions of Loess Plateau, China.

Materials and Methods

Research site description

The two study sites (Yangling and Zhouzhi) were located at the south of Loess Plateau, Shaanxi, China. This zone has semi-humid continental monsoon climate and almost 65–75% of precipitation happens during June–October. Maize and wheat crop rotation is the principal cropping system of this region. Yangling (34°30′N, 108°02′E) had mean annual precipitation of 600 mm, and air temperature of 12.9°C (1957–2013). The Yangling soil is Eum-Orthic Anthrosol (Udic Haplustalf based on USDA system). Zhouzhi (34°13′N, 108°34′E) had mean annual precipitation of 713 mm, and air temperature of 13.2°C (1957–2013). The Zhouzhi soil is cinnamon (Udic Haplustalf based on USDA system) in nature. The total precipitation at Yangling 349 mm and Zhouzhi 305 mm occurred during current maize growing season (Fig. 1). The major soil physicochemical properties of the top (0–20 cm) surface layer of the selected sites is shown in Table 1.

Experimental design and field management

Experimental material: Summer maize (cultivar "Zhengdan 958") crop was grown (June–October 2018) in three different farmer's fields (one at Yangling, two at Zhouzhi) at two counties. The crop was sown on June 1st, 2018, by drilling method with space between row to row 60 cm and harvested on October 25th, 2018. The plant population 75,000 plants ha^{-1} (7.5 plants sq m^{-1}) were maintained. There were four treatments in each site, including control (no N fertilizer), urea (U), U + NBPT and U + NBPT + DCD. The N rate (220 kg ha^{-1}) was same for all fertilized treatments, on the basis of local recommendations (Raza *et al*. 2019; Wang *et al*. 2022). The NBPT and DCD rates were 1 and 10% on the w/w basis of applied N, respectively. The NBPT and DCD were manually mixed with urea like coating one day before fertilization. Field trials were organized in randomized complete block design, with three replications. The size of each plot was 5 × 4 (20 m^2), with one-meter protection area.

Treatments: Fertilizer treatments were applied in two splits through sub-surface band placement (7 ± 1 cm depth), 70% at 30 days after sowing (V5 leaf growth stage) and 30% at 24 days after first fertilization (V10 leaf growth stage). The experimental fields were plowed with deep plow machine at first, and it was again tilled up to 10–15 cm soil profile using rotavator to homogenize the soil, and properly leveled one week before sowing. The Maize (*Zea mays* L.) crop (cultivar "*Zhengdan 958*") was grown and entirely dependent on natural rainfall. No extra irrigation was applied, same as local farmers' management.

Sample collection and analysis

Soil sampling and analysis: Soil profile samples (0–200 cm, 20 cm intervals) were collected from each site before cultivation and after harvesting of the crop, using a stainless steel auger (diameter: 5 cm). The primary physical and chemical properties of soil (0–20 cm) were determined before starting the experiment. Total N was measured by Kjeldahl digestion method and soil organic carbon (SOC) with wet soil digestion with H_2SO_4-$K_2Cr_2O_7$ method (Zhang *et al.* 2017). Available P (Olsen P) and K were determined by extraction with 0.5 mol L^{-1} $NaHCO_3$ and 1 mol L^{-1} NH_4OA_C, respectively (Lu *et al.* 2016). Soil particles sand, silt and clay distribution were measured by Mastersizer 2000E lasar diffractometer (Malven, UK) as according to Sochan *et al.* (2012).

For the determination of soil pH, electric conductivity (EC) and mineral-N, soil samples were randomly collected (depth: 0–20 cm) from individual plot at different intervals (2, 6, 10, 14, 20, 26, 30, 34, 40, 46, 60 and 75 days) during maize crop growth season. After each sampling, samples were thoroughly mixed after removing visible roots and litters, sealed immediately in separate marked plastic bags and stored in the freezer until analysis and other air dried for measuring soil pH and EC. A 5 g sub-sample (oven dry equivalent) was extracted with 50 mL of 1 *M* KCL solution and was used for the analysis of mineral-N by using continuous flow analyzer (Bran and Luebbe AA3, Norderstedt Australia). Soil pH (1:2.5 soil: deionized water ratio) and EC (1:5 soil: water ratio) were measured by using glass electrode Mettler Tloedo 320-S pH meter and DDS-307 EC meter followed by shaking for 30 minutes on a rotary shaker, respectively.

Plant sampling and measurements: The leaf green pigment content of the maize crop was measured by using a portable Minota chlorophyll detector (SPAD-502, Osaka 590-8551, Japan). Approximately, 3–4 leaves of 5 plants randomly were measured in each plot. The chlorophyll contents were measured in four different growth stages *i.e*., early rapid growth stage (V7), peak vegetative stage (V12), tasseling stage (V15) and maturity stage (VT).

After maturity, whole plots were manually harvested from ground level for the measurement of yield, and fifteen plants were randomly selected from each plot for other analysis. Plant heights were recorded at the time of maturity. The grains from fifteen randomly selected plants were manually separated and weighed. The remaining plant material (leaf and stem) was also detached and weighed. The plant components were thoroughly washed with tap water, followed by rinsing with distilled water and dried up to constant weight at a 65°C for 48 h in an electric oven and reweighed. The plant components were crushed into a fine powder (particle size: 0.15–0.25 mm) with the help of stainless-steel grinder, weighed and digested with H_2SO_4 and H_2O_2 for the determination of N concentration. Total N in each plant part was analyzed by the Kjeldahl digestion method and results were used to calculate total N uptake by multiplying the N concentration by dry matter for each treatment. NUE indicators were estimated by using N uptake values. NUE and partial factor productivity (PFP) were calculated by following formulas:

$$\text{NUE (\%)} = \frac{\text{(N uptake in the fertilized plot} - \text{N uptake in the control plot)}}{\text{N applied rate}} \times 100 \qquad (1)$$

$$\text{PFP (kg grain kg}^{-1}\text{ N applied)} = \frac{\text{grain yield in the fertilized plot}}{\text{N applied rate}} \qquad (2)$$

Statistical analysis

All the experimental data were compiled by using Microsoft Excel 2013 and analyzed by Statistix (version 8.1). Analysis of variance (ANOVA) evaluated data, and the means were compared using least significant difference (LSD) at a $P < 0.05$ level. Graphs were prepared using OriginPro software (version 2016).

Results

Changes in soil pH and EC

The higher soil pH was recorded in urea treatment with NBPT + DCD and control treatments, compared to other fertilized treatments. The soil pH level significantly decreased in urea alone and urea treatment with NBPT after the first week up to 20 days after fertilization (DAF) and also similar trend was observed in second fertilization at all three fields (Fig. 2).

The highest EC was observed in soil amended with urea. Contrarily, NBPT + DCD treated urea decreased soil

Table 1: Basic soil physicochemical properties of selected experimental sites

Parameter	Yangling	Zhouzhi-1	Zouzhi-2
pH	8.03	7.97	8.02
SOC (g kg^{-1})	11.8	5.5	6.2
Total N (g kg^{-1})	0.74	0.36	0.32
NH_4^+-N (mg kg^{-1})	5.2	4.2	3.5
NO_3^--N (mg kg^{-1})	23.9	12.8	2.6
Olsen P (mg kg^{-1})	35.6	16.8	17.4
Available K (mg kg^{-1})	265.9	100.1	114.1
EC (dS m^{-1})	0.168	0.167	0.134
Texture class	Silt loam	Loam	Silt clay loam
Particle size distribution Sand %	18.4	31.6	15.5
Silt %	56.6	47.9	54.4
Clay %	25.0	20.5	30.1

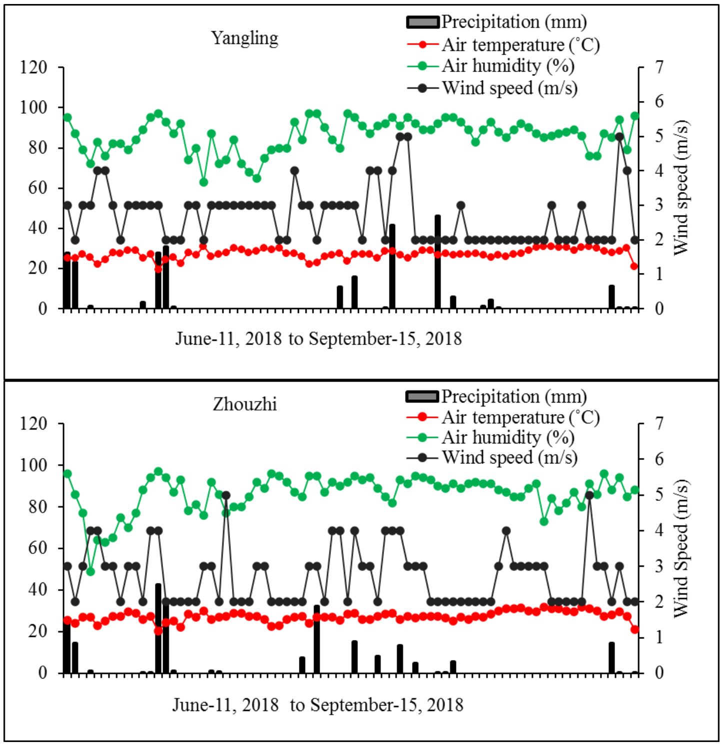

Fig. 1: Daily precipitation (mm), Average temperature (°C), relative humidity (%) and wind speed (m/s) during maize crop growing season of different two sites. Dash lines indicate fertilizer application timings.

EC throughout the maize growing season. The EC in the urea and U + NBPT treatments gradually increased 10 DAF, reached at peak 20 DAF and subsequently decreased. However, Lower EC was recorded in U + NBPT + DCD at all fields as compared to other fertilized treatments (Fig. 3).

Changes in mineral-N in soil

The changes in mineral-N were observed in the fertilized soils in all three sites (Yangling, Zhouzhi-1, and Zhouzhi-2) during maize growing season (Figs. 4 and 5). The sharp increase was recorded in soil NH_4^+-N content, soon after urea application, which reflects rapid urea hydrolysis. At Yangling field, the highest soil NH_4^+-N was recorded from urea treatment on 10th and 6th day for first and second fertilization, respectively (Fig. 4). However, the maximum soil NH_4^+-N was observed on 10th day for first and second fertilization at Zhouzhi-1 and Zhouzhi-2 fields. The use of NBPT with urea significantly reduced soil NH_4^+-N by slowed down hydrolysis as compared to other fertilized treatment throughout growing season at all three fields ($P < 0.05$). However, the combined use of NBPT + DCD with urea, produced slightly more soil NH_4^+-N, than U + NBPT (Fig. 4).

Soil NO_3^--N content increased gradually in urea alone and urea treated with inhibitors as compared to the control treatment during maize crop growing season (Fig. 5). The highest soil NO_3^--N was recorded on 14 DAF in all three fields, and afterward gradually decreased. The use of DCD with U + NBPT

Table 2: Yield dry matter, yield increase and different agronomic parameters of maize crop under different treatments after harvesting

Sites	Treatment	Yield (DM kg ha^{-1})		Yield increase (%)			
		Straw yield	Grain yield	Straw yield	Grain yield	1000 seed weight (g)	Plant height (cm)
Yangling	Control	4490c	3720c	-	-	287.28b	249.50b
	Urea	4790b	4760b	9.82b	27.90b	313.44a	257.20a
	U + NBPT	5180a	5500a	15.30ab	47.64ab	317.05a	261.47a
	U + NBPT + DCD	5300a	5510a	17.88a	48.03a	323.49a	261.77a
Zhouzhi-1	Control	3360b	2610b	-	-	253.39b	242.90b
	Urea	3810a	3250a	13.30a	24.26a	270.31a	260.80a
	U + NBPT	4000a	3650a	19.03a	39.48a	273.76a	264.73a
	U + NBPT + DCD	4030a	3650a	19.89a	39.53a	272.00a	263.00a
Zhouzhi-2	Control	2990b	2510c	-	-	245.15b	212.80b
	Urea	3670a	3070b	22.37a	22.17b	265.15a	249.93a
	U + NBPT	3840a	3460a	28.24a	37.66a	269.38a	253.20a
	U + NBPT + DCD	3890a	3510a	29.93a	39.81a	269.33a	255.80a

Different letters in a column indicate significant difference at $P < 0.05$

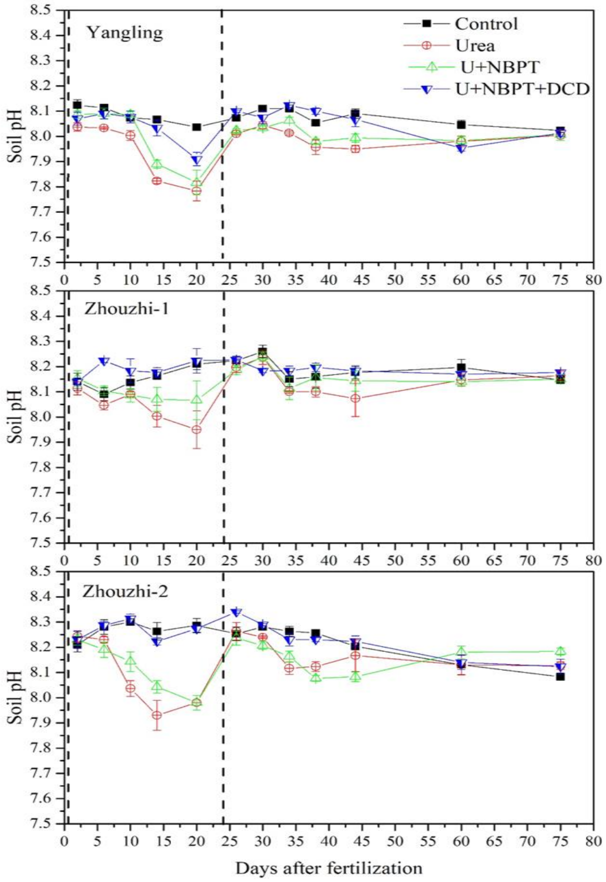

Fig. 2: Changes in soil pH after application of urea and treated urea of three different experimental sites. Vertical bars represent standard error (SE), n = 3. Dash lines indicate fertilizer application timings

significantly reduced soil NO_3^--N throughout the growing season. Urea treated with NBPT + DCD inhibited the urea hydrolysis and nitrification process, thus slightly decreased nitrate leaching (Fig. 6).

Changes in plant height, seed index and SPAD values

Plant height (cm) and seed index (1000 seed weight) were significantly influenced by the application of urea, use of NBPT alone and mixture of NBPT + DCD with urea (Table 2). The increased plant height and higher seed index was recorded in fertilized treatments compared to control at all three fields ($P < 0.05$). However, no significant difference was noticed between the application of urea alone, U+ NBPT and U + NBPT + DCD.

The temporal variation in SPAD chlorophyll content values were observed during different growth stages at all three fields (Fig. 7). The combined use of NBPT + DCD

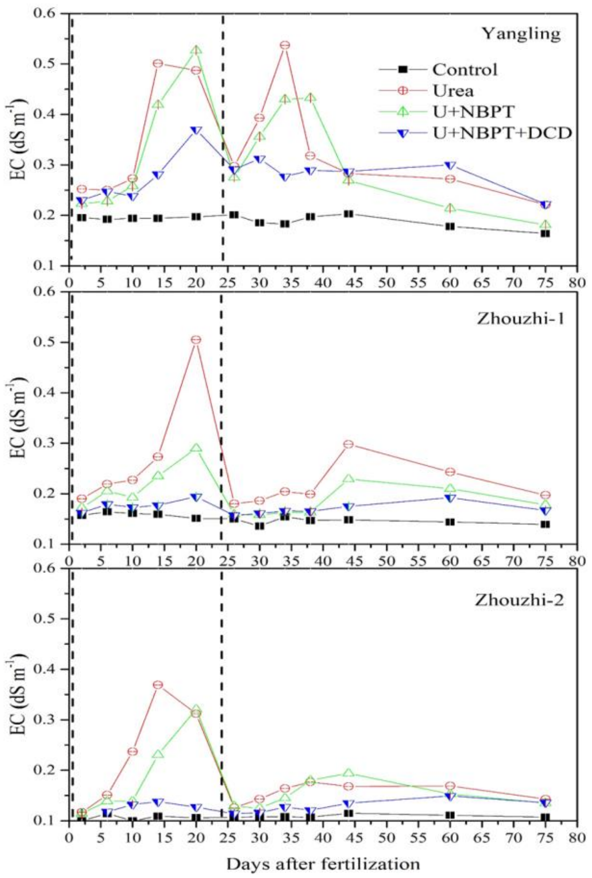

Fig. 3: Changes in soil EC after application of urea and treated urea of three different experimental sites. Vertical bars represent standard error (SE), n = 3. Dash lines indicate fertilizer application timings

with urea treatment slightly increased SPAD chlorophyll content at vegetative (V12), tasseling (V15) and maturity (VT) growth stages, as compared to urea treatment. However, the urea treatment with NBPT + DCD significantly enhanced SPAD chlorophyll content, compared to other treatments at tasseling (V15) and maturity (VT) growth stages of Zhouzhi-1 field. All fertilized treatments had significant effects on SPAD chlorophyll during all growth stages, compared to unfertilized control at all three fields ($P < 0.05$). Highest SPAD chlorophyll content was observed during peak vegetative growth stage (V12), however, lowest was observed at maturity stage (VT) followed by early growth stage (V7).

Changes in plant dry weight and grain yields

Grain and straw dry matter were significantly affected by the urea application, and use of NBPT and NBPT + DCD with urea (Table 2). Maximum maize grain and straw yield were recorded in U + NBPT + DCD followed by U+NBPT at all three fields. Moreover, no significant difference was noticed in the U + NBPT and U + NBPT + DCD.

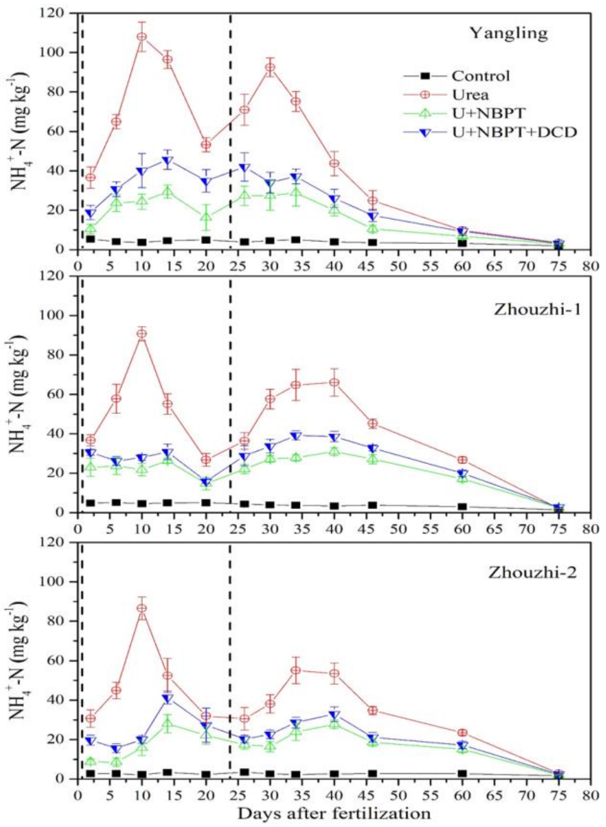

Fig. 4: Changes in NH_4^+-N after application of urea and treated urea of three different experimental sites. Vertical bars represent standard error (SE), n = 3. Dash lines indicate fertilizer application timings

Yield increase percentage was calculated by subtracting control yield form fertilized yield divided by the control yield. The maximum grain yield and straw biomass were found in U + NBPT + DCD, followed by U + NBPT at all three fields. Moreover, the increase in grain yield (15–20%) was observed in NBPT and NBPT + DCD treated urea, as compared to urea alone (Table 2). At Yangling field, the highest grain yield (5510 DM kg ha^{-1}) was recorded with an increase of 37.7–48% in U + NBPT + DCD, followed by U+NBPT as compared to unfertilized control treatment. Moreover, a maximum grain yield increase (20%) was recorded at Yangling field in U + NBPT and U + NBPT + DCD as compared to urea alone treatment (Table 2).

Changes in N uptake and N use efficiency

The N uptake by different plant organs (stem, leaf, and grain) was significantly affected by the application of urea, urea treatment with NBPT and NBPT + DCD ($P < 0.05$). Highest N uptake was recorded in U + NBPT + DCD followed by U + NBPT compared to unfertilized control at all three fields. The maximum N uptake was observed in grain and lower in stem across all three fields (Table 3). The

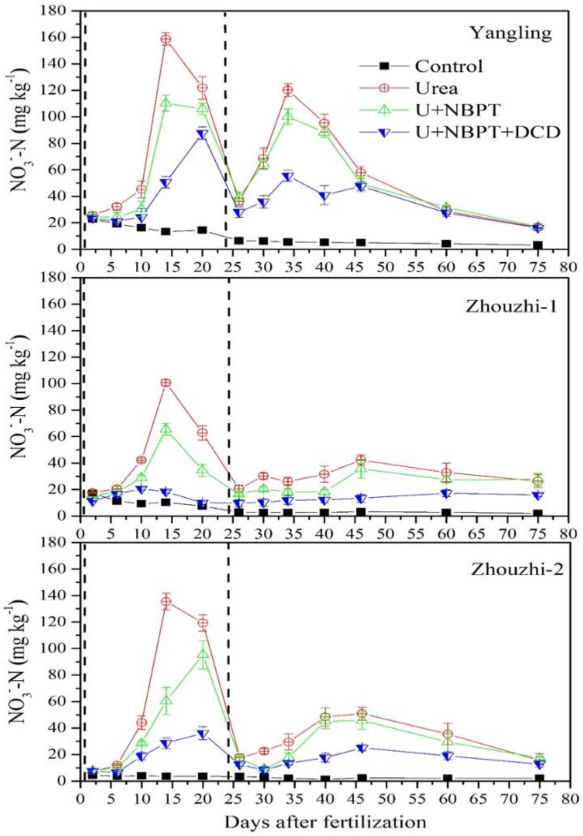

Fig. 5: Changes in NO_3^--N after application of urea and treated urea of different three experimental sites. Vertical bars represent standard error (SE), n = 3. Dash lines indicate fertilizer application timings

temporal variations in above ground biomass, N were taken up by maize crop was observed in all three fields (Table 3). Urea treated with NBPT and NBPT + DCD increased N uptake (79.3–136.1 and 80.1–139.6 N kg ha^{-1}), respectively. The maximum N uptake was recorded in U + NBPT + DCD, followed by U + NBPT at Yangling field.

Application of U+NBPT and U+NBPT+DCD had significantly improved N use efficiency compared to urea alone ($P < 0.05$), while no significant difference was observed between U + NBPT and U + NBPT + DCD (Table 3). The highest N use efficiency was recorded in U + NBPT + DCD (14.3–22.6%), followed by U + NBPT (13.8–21.6%). However, lowest N use efficiency (9–15.5%) was recorded in urea alone as compared to unfertilized control treatment across all fields. Urea treatment with NBPT and NBPT + DCD produced higher N use efficiency at Zhouzhi-2 field, followed by the Yangling. However, lower N use efficiency was observed at Zhouzhi-1 field for all fertilized treatments (Table 3).

Higher partial factor productivity (PFP) was recorded in U + NBPT and U + NBPT + DCD, compared to unfertilized control (Table 3). Application of urea treatment with NBPT + DCD increased PFP (15.97–25.1 kg grain kg^{-1} N applied), followed by U + NBPT (15.7–24.99 kg grain kg^{-1} N applied). The maximum PFP was increased by the application of urea treatment with NBPT + DCD and U + NBPT at Yangling field. However, minimum PFP was observed at Zhouzhi-2 for all fertilized treatments, contrast with Yangling and Zhouzhi-1 (Table 3).

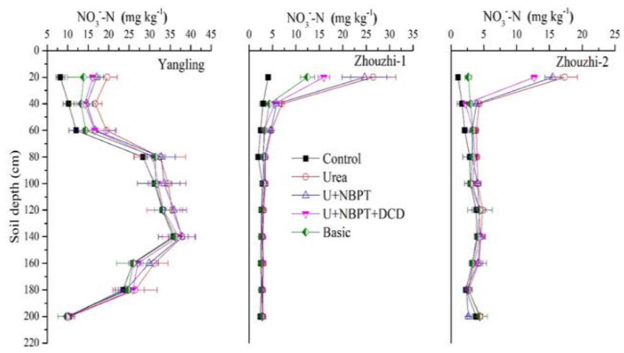

Fig. 6: Changes in NO_3^--N accumulation in soil profile after application of urea and treated urea of different three experimental sites. Vertical bars represent standard error (SE), n = 3

Discussion

Application of NBPT significantly affected soil N transformation, thereby increased N uptake, crop yield, and ultimately improved NUE. The change in soil pH during first week of fertilization was due to the production of NH_4^+-N in the soil, afterward gradually decreased, because of the nitrification of the NH_4^+-N by soil microbes. A short-term rise in soil pH always happens after urea application due to urea hydrolysis (Zaman *et al.* 2009; Zaman and Nguyen 2012). The application of NBPT with urea inhibited urea hydrolysis, resulting in decreased NH_4^+-N content and low rise of soil pH throughout the maize growing season (Sanz-Cobena *et al.* 2008; Zaman *et al.* 2009; Meng *et al.* 2023), which is associated with release of hydroxyl (OH^-) ions (Singh *et al.* 2013). Observations of other studies confirm these results that application of urea treatment with NBPT decreased NH_4^+-N content (Zaman *et al.* 2009, 2013; Suter *et al.* 2013; Montalbán *et al.* 2021).

The use of NBPT increased EC and NO_3^--N content throughout maize growing season, compared to U+NBPT+DCD (Figs. 3 and 5). There is a close relationship between NO_3^--N content and EC of the soil under aerobic conditions. The gradual decrease in NH_4^+-N and increase in NO_3^--N concentrations, demonstrate the happening of nitrification after urea hydrolysis (Martins *et al.* 2017; Lan *et al.* 2018).

Plant growth and yield were significantly influenced by the use of NBPT with urea. N is one of the major components of chlorophyll, and the chlorophyll content depends on increase and decrease of plant N uptake (Makino and Osmond 1991). The application of urea

Table 3: N uptake by different plant parts, above ground biomass, Nitrogen use efficiency and partial factor productivity of maize crop under different treatments in three different experimental sites after harvesting

		N uptake (N kg ha^{-1})					
Sites	Treatment	Leaf	Stem	Grain	Above ground biomass	NUE (%)	PFP (N kg kg^{-1})
Yangling	Control	22.30c	21.21c	51.50c	95.00c	-	-
	N-220	25.41b	23.72b	68.52b	117.65b	10.30b	21.65b
	N + NBPT	29.00a	26.44a	80.62a	136.06a	18.66a	24.99ab
	N + NBPT + DCD	29.72a	28.37a	81.53a	139.63a	20.28a	25.06a
Zhouzhi-1	Control	11.27b	7.758b	29.85c	48.881b	-	-
	N-220	16.42ab	10.73ab	41.56b	68.71a	9.01a	14.76a
	N + NBPT	18.03a	12.53a	48.72a	79.29a	13.82a	16.57a
	N + NBPT + DCD	18.40a	12.24a	49.63a	80.27a	14.27a	16.57a
Zhouzhi-2	Control	7.64b	6.34c	23.68c	37.663c	-	-
	N-220	18.73a	14.46b	38.62b	71.80b	15.52b	13.97b
	N + NBPT	20.14a	17.81a	47.32a	85.27a	21.64a	15.73a
	N + NBPT + DCD	20.70a	18.21a	48.39a	87.30a	22.56a	15.97a

Different letters in a column indicate significant difference at $P < 0.05$.

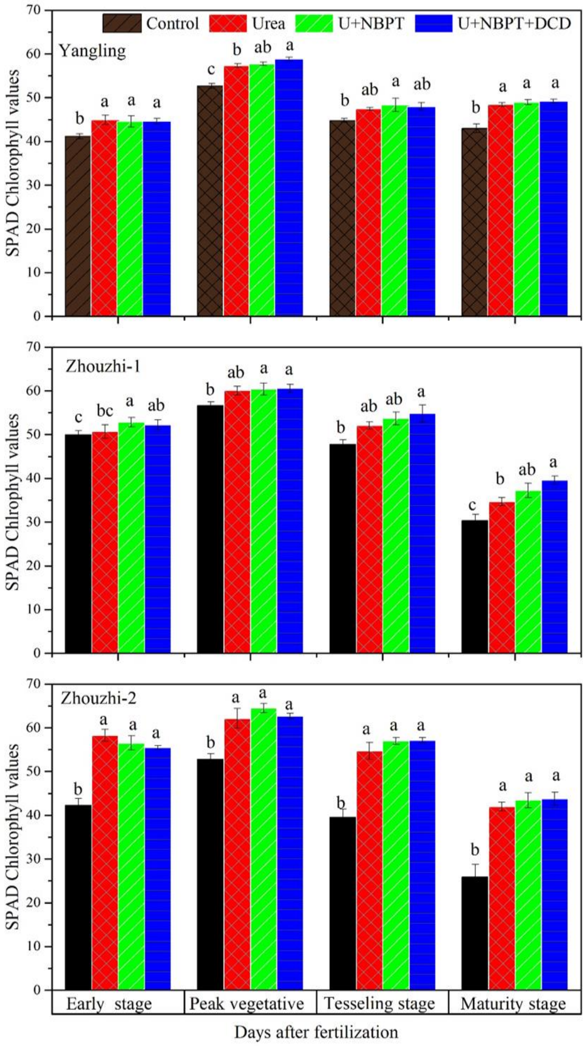

Fig. 7: SPAD chlorophyll values after application of urea and treated urea of three different experimental sites. Values followed be same letters in each column do not differ significant differently. Vertical bars represent standard error (SE), n = 3

treatment with NBPT was significantly increased SPAD chlorophyll content throughout all growth stages across all fields (Fig. 7). Our results are confirmed by other studies, reported that the addition of NBPT with urea had increased leaf chlorophyll content (Kawakami *et al.* 2012; Zuki *et al.* 2020).

In our study, the addition of NBPT with urea significantly increased the crop yield, N uptake and NUE, compared to urea and control treatments ($P < 0.05$). Application of NBPT delayed urea hydrolysis for both fertilization, that enables to plant uptake N in NH_4^+-N form, which is more efficient than uptake in the form of NO_3^--N, as less energy consumed to convert NH_4^+-N into plant proteins, compared to NO_3^--N (Zaman *et al.* 2009). The use of NBPT with urea intensely reduced ammonia (NH_3) volatilization ranged 80–93% in the Chinese Loess Plateau (Ahmed *et al.* 2018), thereby increased N uptake and NUE (Kawakami *et al.* 2012; Singh *et al.* 2013). The increase in N uptake could be related to reduced N losses, which conserve N in the soil (Zaman *et al.* 2013; Rose *et al.* 2018). The N conservation in the soil ultimately increased NUE and crop yield (Abalos *et al.* 2014; Ramalingappa *et al.* 2023).

Application of urea treatment with NBPT was resulted in significant increase in maize grain yield (37.7–47.6, 15–19.7%), compared to control and urea treatments, respectively (Table 2). Almost same results were obtained with the application of NBPT treated urea increased (10–15%) maize grain yield compared to urea (Ding *et al.* 2011; Martins *et al.* 2017; Montalbán *et al.* 2021). Furthermore, different meta-analysis have been reported that the use of NBPT with urea significantly increased crop yield (10%) and NUE (12%) (Abalos *et al.* 2014; Silva *et al.* 2017; Meng *et al.* 2023). The other studies described that the use of NBPT with urea have potential to increase N uptake and crop yield (Ding *et al.* 2015; Zhao *et al.* 2017; Fu *et al.* 2020). In addition, NBPT treated urea demonstrated higher N uptake than urea alone (Singh *et al.* 2013; Ramalingappa *et al.* 2023).

The use of NBPT with urea significantly increased N uptake (33%), N recovery efficiency (48–61%) and crop yield (27–38%) (Dawar *et al.* 2011), which resulted in 1.8 times more NUE than urea alone (Hube *et al.* 2016). Moreover, the use of NBPT with urea has potential to increase uptake (17–22%) and improve NUE (29–41%) as compared to urea treatment (Kawakami *et al.* 2012, 2013; Sanz-Cobena *et al.* 2012; Ding *et al.* 2015; Fu *et al.* 2020). Application of Limus (75% NBPT + 25% NPPT) with urea increased N recovery efficiency ranged 11–17%, compared to urea alone (Li *et al.* 2015, 2017). The results of the present study stated that use of NBPT with urea could be an appropriate approach for increasing yield, N uptake and improving NUE of the maize crop.

The combined use of NBPT + DCD with urea delayed urea hydrolysis and nitrification process, result in increased soil pH, which is due to the presence of NH_4^+-N in the soil (Zaman *et al.* 2009). In addition, the inclusion of DCD with U + NBPT retained high soil NH_4^+-N concentration and pH for a long time (Soares *et al.* 2012). Our results are agreed with other studies, reported that the high NH_4^+-N content remained in the soil for a longer time by the use of DCD with U + NBPT (Zaman and Nguyen, 2012; Montalbán *et al.* 2021), possibly due to nitrification (Lan *et al.* 2018).

Generally, the trend of EC was more or less similar pattern to changes in soil NO_3^--N. The changes in soil EC associated with NO_3^--N, probably due to nitrification. The soil NO_3^--N was significantly lower in the urea treatment with NBPT + DCD across all fields, due to NI reduced nitrification (Lan *et al.* 2018). The results of the current study are agreed with the observations of previous studies (Ding *et al.* 2011; Rose *et al.* 2018). Application of NBPT+DCD with urea had slow down the nitrification and decreased soil NO_3^--N on the surface, thereby influenced the nitrate leaching (Zaman and Nguyen, 2012; Montalbán *et al.* 2021). Addition of DCD with U+NBPT affected nitrate accumulation in the soil profile (Zaman *et al.* 2009, 2013). Although, in our study, nitrate leaching was not significantly observed, might be due to the short duration and less precipitation.

The slow release of NH_4^+-N and NO_3^--N in the soil, less susceptible to N loss and provide greater opportunity for more N uptake by plant, thereby improving NUE and crop yield (Zaman *et al.* 2009; Zaman and Nguyen, 2012; Rose *et al.* 2018). Urea treatment with NBPT+ DCD also increased SPAD chlorophyll content throughout all growth stages across all fields (Fig. 7). Our results are agreed, that the combined use of NBPT + DCD with urea increased chlorophyll content (Kawakami *et al.* 2013). Moreover, application of UIs and NIs with N fertilizer significantly enhanced chlorophyll content (Zuki *et al.* 2020; Meng *et al.* 2023). Reduction in nitrification is possible to protect N against denitrification and NO_3^--N leaching and provide more chance to plant N uptake (Zaman and Nguyen, 2012; Montalbán *et al.* 2021). Our results are confirmed, that combined use of NBPT + DCD with urea slightly increased crop yield, N uptake and NUE compared to use of NBPT (Ding *et al.* 2015; Rose *et al.* 2018). Furthermore, observations of other studies revealed that use of DCD with U + NBPT did not significantly increase crop yield, compared to U + NBPT (Ding *et al.* 2011; Fu *et al.* 2020). Furthermore, combined use of UIs and NIs with N fertilizer significantly increased crop yield compared to single use of N fertilizer (Zuki *et al.* 2020; Meng *et al.* 2023). The inclusion of NBPT with DCD considerably decreased 75–90% NH_3 volatilization in different soils under different climatic conditions (Ahmed *et al.* 2018), thus improving the bioavailability of N, thereby increasing 8–13% plant dry matter, then urea alone (Zaman and Nguyen 2012). Furthermore, Sanz-Cobena *et al.* (2012), also reported that combination of NBPT + DCD with urea increased N uptake of maize crop 38.5 and 18% compared to control (no fertilization) and urea treatments, respectively. In addition, a meta-analysis reported that mixture of NBPT + DCD with urea increased 14.7% NUE (Abalos *et al.* 2014). Application of inhibitors (UI + NI) also increased grain yield (23–30%), N uptake (47%) and N recovery efficiency (30%) of maize crop (Martins *et al.* 2017; Meng *et al.* 2023). In addition, the combined use of UI and NI along with urea demonstrate higher NUE of maize crop (22 and

37%) compared to urea and control treatment, respectively (Zhao *et al.* 2017; Montalbán *et al.* 2021). Such increase in crop yield, N uptake and NUE can be attributed to combined use of inhibitors (Meng *et al.* 2023), probably delayed urea hydrolysis and nitrification (Zaman *et al.* 2009), provide chance for efficient N uptake by plants with the conversion of N into proteins (Rose *et al.* 2018). The combined application of NI and UI with urea also represented the best strategy tended to enhancement in crop production (Abalos *et al.* 2014, 2016; Zuki *et al.* 2020).

The highest crop yield and N uptake were recorded at Yangling field which might be due to high soil fertility status, such as high residual NPK content and more amount of precipitation. These results agree with previous observations, that yield response was intensely influenced by residual fertility status and soil water content (Cui *et al.* 2008; Abalos *et al.* 2016). In addition, it was reported that high crop yield is correlated with residual soil NO_3^--N (Li *et al.* 2015; Miao *et al.* 2015). Moreover, the difference in precipitation (44 mm) could be the other reason, which plays a vital role in increasing crop yield and NUE (Zhao *et al.* 2017).

The positive response of inhibitors in terms of increased crop production, N uptake and NUE were mainly differentiated by the soil texture and soil moisture content. In our study, soils with high soil clay and silt, and low sand contents resulted in more effectiveness of inhibitors, compared to high sand content. This efficiency is probably related to the ability of clay and silt content to retain NH_4^+-N fixation in the soil and reduce N loss. The soil clay content keeps NH_4^+-N concentration long time in the soil matrix by water coming through precipitation, resulting in more N uptake by plants, thereby increasing crop yield and NUE (Francisco *et al.* 2011; Li *et al.* 2017). In addition, UIs and NIs are effective in fine-textured soils, and probably lower N losses through leaching (Abalos *et al.* 2014).

The current study demonstrated that soil properties such as basic fertility status and climatic factors (precipitation, air temperature) could have effects on increasing yield and NUE of the maize crop. Overall, these findings indicate that the application of NBPT alone and the combination of NBPT + DCD with urea could be an integrated strategy to increase crop yield, N uptake and NUE of maize crops in different soil properties under various environmental factors.

Conclusion

Application of urea treatment with NBPT and a mixture of NBPT + DCD increased urea N retention, in different edaphic and environmental circumstances conducive to N loss. The sole application of NBPT with urea increased nitrate leaching compared to U + NBPT + DCD. However, the combined application of NBPT + DCD with urea delayed urea hydrolysis and nitrification processes, thereby alleviating N losses by NO_3^--N leaching and gas emission. In this manner N remained in the soil for a long time, providing an opportunity to more plant N uptake, resulting in improved NUE and crop yield. This integrated strategy had increased above-ground dry matter, N uptake and NUE of the maize crop, and could be helpful for more economic benefits to growers and the environmental performance of the agricultural system. The current study concluded that the combination of NBPT + DCD with urea would be the more reasonable approach to overcome N losses, thereby enhancing NUE and crop yield.

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Author Contributions

MA and JZ planned experiment, WY and M L interpreted results, M A, SR and ASE made the write up, MA statistically analyzed the data and SR made illustrations.

Conflicts of Interest

All authors have read and approved the final manuscript and declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding authors.

Ethics Approval

Not applicabe to this paper

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