Postprint: Effects of Activated Humic Acid-Urea Application on Soil Nitrogen Use Efficiency and Its Controlling Factors in Wheat-Maize Rotation

Authors: Liu Yanli, Fangjun Ding, Juan Zhang, Qi Xingchao, Gu Duanyin, Qinquan Wu, Li Chengliang

Date: 2017-11-07T00:00:00+00:00

Abstract

Humic acid-urea is a novel organic-inorganic compound fertilizer developed in recent years, which exhibits significant yield-enhancing effects; however, its utilization efficiency and environmental regulatory factors in wheat-maize rotation systems remain unclear. This study, through field plot and laboratory incubation experiments, using no-fertilizer treatment (Control) and urea-only treatment (Urea) as controls, investigated the effects of humic acid-urea direct blending treatment (U+HA1), humic acid-urea activated treatment (U+HA2), and humic acid-urea activated catalytic treatment (U+HA3) on wheat and maize growth, soil physicochemical properties, nitrogen fertilizer use efficiency, soil nitrogen transformation, and soil urease content. The results showed that grain yields of wheat and maize under the activated humic acid-urea treatment increased by 15%~28% and 8%~10%, respectively, compared with the Urea treatment. Application of activated humic acid-urea significantly reduced soil bulk density, pH, and the median particle size of soil particles, while increasing the specific surface area, electrical conductivity, organic carbon content, and mineral nitrogen content of the soil. In the wheat season, nitrogen fertilizer recovery efficiency under the activated humic acid-urea treatment was significantly increased by 37%~91% compared with the Urea treatment, and the increase in the maize season was 78%~93%. The nitrogen agronomic use efficiency and partial factor productivity of wheat and maize under the activated humic acid-urea treatment were both higher than those under the Urea treatment. Furthermore, regression analysis indicated that the in-season nitrogen recovery efficiency of activated humic acid-urea decreased with increasing soil nitrification ratio, mineralization amount of organic nitrogen, and urease content, while it increased with increasing specific surface area of soil particles. This study clarified that the activated humic acid-urea treatment had better yield-increasing effects on wheat and maize and could improve soil physicochemical properties, among which the humic acid-urea activated catalytic treatment (U+HA3) showed the best performance. The research results provide fundamental data for the further research, development, and promotion of activated humic acid-urea fertilizer.

Full Text

Effect of Activated Humic Acid-Urea on Nitrogen Use Efficiency and Its Driving Factors Under Wheat-Maize Rotation System

LIU Yanli[1], DING Fangjun[2], ZHANG Juan[3], QI Xingchao[1], GU Duanyin[2], WU Qinquan[2], LI Chengliang[1]

[1] National Engineering Laboratory for Efficient Soil and Fertilizer Utilization / College of Resources and Environment, Shandong Agricultural University, Tai' an 271018, China
[2] Engineering Technology Research Center for Efficient Humic Acid Utilization in Shandong Province / Fertilizer Science Tech. Co., Ltd., Shandong Agricultural University, Tai' an 271000, China
[3] Shandong Institute of Product Quality Inspection, Jinan 250100, China

Abstract

Humic acid-urea is an innovative organic-inorganic compound fertilizer that has demonstrated significant yield-increasing effects in recent years. However, its nitrogen use efficiency and environmental regulatory factors under wheat-maize rotation systems remain unclear. This study investigated the effects of different humic acid-urea treatments on wheat and maize growth, soil physicochemical properties, nitrogen use efficiency, soil nitrogen transformation, and soil urease content through field positioning and laboratory incubation experiments, using no-fertilizer (Control) and urea-only (Urea) treatments as controls. The treatments included: direct mixing of humic acid and urea (U+HA1), activated humic acid-urea (U+HA2), and activated and catalyzed humic acid-urea (U+HA3). The results showed that activated humic acid-urea treatments increased wheat and maize grain yields by 15%-28% and 8%-10%, respectively, compared with the Urea treatment. Activated humic acid-urea application significantly reduced soil bulk density, pH, and median particle diameter while increasing soil specific surface area, electrical conductivity, organic carbon content, and mineral nitrogen content. During the wheat season, nitrogen recovery efficiency under activated humic acid-urea treatments increased by 37%-91% compared with the Urea treatment, while the increase during the maize season ranged from 78%-93%. Both nitrogen agronomic efficiency and partial factor productivity were higher under activated humic acid-urea treatments than under the Urea treatment. Furthermore, regression analysis revealed that nitrogen recovery efficiency decreased with increasing soil nitrification ratio, organic nitrogen mineralization amount, and urease content, but increased with increasing soil particle specific surface area. This study clarifies that activated humic acid-urea application produces better yield-increasing effects on wheat and maize, improves soil physicochemical properties, with the activated and catalyzed humic acid-urea treatment (U+HA3) showing the best performance. These results provide fundamental data for the further development and promotion of activated humic acid-urea fertilizers.

Keywords: Activated humic acid-urea; Wheat; Maize; Grain yield; Soil physicochemical properties; Nitrogen fertilizer use efficiency

Introduction

Nitrogen fertilizer application is a primary measure for maintaining soil fertility and crop productivity in agricultural production [1-3]. However, improper application leads to nitrogen losses through volatilization, leaching, and runoff, resulting in reduced nitrogen use efficiency. Therefore, improving nitrogen use efficiency represents an important research focus for agricultural and fertilizer scientists [4-5]. Currently, enhancing nitrogen use efficiency through novel fertilizers has attracted widespread attention and recognition [6-8]. Combined application of nitrogen fertilizer with organic manure has significantly improved nitrogen use efficiency [9-10], forming the basis for developing and promoting humic acid-urea as a new fertilizer type that holds significant importance for improving nitrogen use efficiency.

Humic acid-urea, as a novel organic-inorganic compound fertilizer, forms through complexation between humic acid molecules and urea nitrogen, which can slow urea nitrogen release rates and thereby improve its utilization efficiency [11-12]. Previous research has investigated the fertilization effects of humic acid-urea. Studies on corn (Zea mays) growth demonstrated that compared with conventional urea, humic acid-urea fertilizer inhibited soil urease activity, extended nitrogen supply duration during the corn growth period, and consequently improved nitrogen use efficiency [13]. Experiments on different humic acid application rates improving wheat (Triticum aestivum) growth and soil nutrient content in various soils indicated that moderate application rates provided better promotion effects on wheat growth and soil nutrient content [14]. Additionally, humic acid-urea application promoted soil aggregate structure formation, which benefits soil nitrogen retention and crop nitrogen absorption, thereby improving urea nitrogen utilization efficiency [15]. To date, however, few reports have addressed soil nitrogen transformation and its effects on nitrogen use efficiency following activated humic acid-urea application.

Research and development of humic acid-urea fertilizers have revealed that fertilizer efficiency is related to the activation degree of humic acid [16]. The activation process increases free humic acid content, enhancing its application effects and agricultural value. However, the fertilization effects of humic acid-urea fertilizers prepared through different activation methods require further investigation. Therefore, this study employed humic acid-urea fertilizers subjected to direct mixing, activation, and activation-catalysis treatments, using no-fertilizer and urea-only treatments as controls. Through field positioning and laboratory incubation experiments, we investigated crop growth, basic soil physicochemical properties, nitrogen transformation processes, and nitrogen use efficiency after applying different activated humic acid-urea fertilizers, and explored the variation characteristics and controlling factors of urea nitrogen use efficiency. These results will clarify the contributions of different activated humic acid-urea fertilizer applications to improving nitrogen use efficiency and provide supporting data for fertilizer development and promotion.

1.1 Experimental Site Description

The experiment was conducted at the Feicheng Agricultural Demonstration Park in Shandong Province (Chaoquan, Feicheng, Tai' an City, Shandong), which features a warm temperate continental semi-humid monsoon climate with an average annual rainfall of 903.2 mm. The tested soil type was sandy loam cinnamon soil. The experiment began with wheat in 2012, following a wheat-maize rotation system with wheat cultivar 'Shannong 0536' and maize cultivar 'Shannong 2000' . The fertilizers applied included urea (N 46%), calcium superphosphate (P_2O_5 16%), and potassium chloride (K_2O 60%). The free humic acid contents of humic acid after urea activation treatment and activation-catalysis treatment were 18.2% and 18.8%, respectively, representing increases of 26.4% and 30.6% compared with untreated raw humic acid (free humic acid content 14.4%). All fertilizers and materials were provided by Shandong Agricultural University Fertilizer Science & Technology Co., Ltd. Basic soil physicochemical properties before the experiment are shown in Table 1 .

The experiment consisted of four treatments under a seasonal nitrogen application rate of 225 $kg(N) \cdot hm^{-2}$, using urea-only treatment (Urea) as the control. The humic acid-urea direct mixing treatment (U+HA1) (total nitrogen content 27.9%) served as the humic acid-urea treatment. Additional treatments included activated humic acid-urea (U+HA2) and activated and catalyzed humic acid-urea (U+HA3). A no-nitrogen treatment was also established as a blank control (Control), making five treatments total. The U+HA2 activation treatment involved heating urea and ammonium sulfate at a 10:1 ratio until molten, then adding humic acid at 65% of the urea amount, mixing uniformly, and cooling to solidify, yielding a total nitrogen content of 27.8%. The U+HA3 activation-catalysis treatment involved mixing urea and ammonium sulfate at a 10:1 ratio, heating until molten, adding humic acid at 65% of the urea amount, mixing uniformly, subjecting to ultrasonic treatment for homogenization, and cooling to solidify, yielding a total nitrogen content of 27.9%.

During the wheat season, two-thirds of the total nitrogen amount for all treatments was applied as base fertilizer, broadcast on the soil surface at the end of September 2012 and then incorporated through tillage; the remaining one-third was top-dressed during the wheat grain-filling stage (mid-May 2013) through fertigation. During the maize season, two-thirds of the total nitrogen amount was applied as base fertilizer, broadcast on the soil surface in early June 2013 and incorporated through tillage; the remaining one-third was top-dressed at the maize trumpet stage through broadcasting. Phosphorus fertilizer was applied at 135 $\mathrm{kg}(\mathrm{P_2O_5}) \cdot \mathrm{hm}^{-2}$ and potassium fertilizer at 105 $\mathrm{kg}(\mathrm{K_2O}) \cdot \mathrm{hm}^{-2}$ for all treatments, broadcast on the soil surface at the end of September 2012 and incorporated through tillage. Wheat was sown on October 21, 2012, at a seeding rate of 202.5 $\mathrm{kg} \cdot \mathrm{hm}^{-2}$; maize was sown on June 15, 2013, at a density of 60,000 plants $\cdot\, \mathrm{hm}^{-2}$. The experimental plot area was 30 m^2, arranged in a randomized block design with three replications. Wheat and maize were managed throughout the growth period strictly according to high-yield farmland management practices, following the Technical Regulations for High-Yield, High-Quality, and High-Efficiency Wheat Cultivation (DB37/T190–93) and the Technical Regulations for High-Yield, High-Quality, and High-Efficiency Summer Maize Production (DB37/T538–2005). The study period was from September 2012 to September 2013.

After wheat and maize harvest in 2013, grain and straw yields were recorded separately for each plot after sun-drying. Following harvest, five mixed soil samples were randomly collected from each plot, along with undisturbed soil samples, which were brought back to the laboratory and air-dried. The air-dried mixed samples were used for determining soil nutrient indicators, while undisturbed samples were used for measuring soil physical properties.

Soil bulk density was determined using the core method; soil pH was measured using a pH meter in distilled water suspension; soil electrical conductivity was measured using a conductivity meter; soil cation exchange capacity (CEC) was determined using neutral ammonium acetate extraction; soil organic carbon content was measured using concentrated sulfuric acid-potassium dichromate oxidation with external heating; soil total nitrogen content was determined using the semi-micro Kjeldahl method; soil ammonium and nitrate nitrogen contents were extracted with 0.02 $\mathrm{mol} \cdot \mathrm{L}^{-1}$ $\mathrm{CaCl_2}$ solution and measured using flow analysis; soil urease content was determined using a colorimetric method. Plant samples were digested with $\mathrm{H_2SO_4}$-$\mathrm{H_2O_2}$, and total nitrogen content was determined using the Kjeldahl method, following procedures outlined in "Soil Agricultural Chemistry Analysis Methods"edited by Lu Rukun [17]. Soil particle size distribution was measured using a laser particle size analyzer (BT-9300H, Dandong Bettersize Instruments Co., Ltd.). The main parameter median particle diameter (D50), representing the average particle diameter, indicates the dominant soil particle composition. The dispersion degree of particle size distribution, reflecting the range of soil particle size distribution, was characterized using (D90-D10)/D50, where D10 represents the particle diameter at 10% of the distribution and D90 represents the particle diameter at 90% of the distribution.

Nitrogen use efficiency was calculated based on crop yield, straw yield, and nitrogen content in grain and straw using the following formulas [5]:

Nitrogen recovery efficiency (RE, %) = (Aboveground nitrogen uptake in nitrogen-applied plot at harvest - Aboveground nitrogen uptake in no-nitrogen plot at harvest) / Nitrogen fertilizer application rate × 100 (1)

Nitrogen partial factor productivity (PP, $\text{kg} \cdot \text{kg}^{-1}$) = Yield in nitrogen-applied plot / Nitrogen fertilizer application rate (2)

Nitrogen agronomic efficiency (AE, $\text{kg} \cdot \text{kg}^{-1}$) = (Grain yield in nitrogen-applied plot - Grain yield in no-nitrogen plot) / Nitrogen fertilizer application rate (3)

1.3 Soil Organic Nitrogen (SON) Mineralization Incubation Experiment

Fresh soil samples from the no-fertilizer treatment were passed through a 2 mm sieve and used as test samples. Soil water content was adjusted to 65% of field capacity, and fertilizers were uniformly mixed at twice the rate used in the field experiment. Ten grams of each treatment soil sample were placed in 100 mL centrifuge tubes, covered with plastic film to maintain soil moisture, and incubated in a 28°C incubator, with 15 replicates per treatment. Samples were taken on days 1, 3, 7, 14, and 21 after incubation. Each time, three centrifuge tubes per treatment were removed, 50 mL of 0.02 $\text{mol} \cdot \text{L}^{-1}$ CaCl_2 solution was added, shaken for 1 hour, filtered, and appropriate amounts of filtrate were taken to determine ammonium and nitrate nitrogen contents using a flow injection analyzer.

Soil organic nitrogen mineralization amount was calculated as the difference in mineral nitrogen content (sum of ammonium and nitrate nitrogen) before and after incubation. Soil organic nitrogen mineralization rate (%) = Soil nitrogen mineralization amount / Soil total nitrogen content × 100% [18].

Nitrification was calculated through ammonium nitrogen transformation during mineralization. The soil nitrification ratio was calculated as follows:

Soil nitrification ratio (%) = Nitrate nitrogen content / Total mineral nitrogen content × 100% (4)

1.4 Data Statistical Analysis

Data processing was performed using Microsoft Excel 2003. Statistical analysis of differences among treatments was conducted using SPSS 17.0 software for ANOVA and LSD significance tests ($P < 0.05$). Graphing was performed using Origin 8.5 software.

2.1 Grain Yield, Straw Yield, and Nitrogen Content in Grain and Straw Under Different Fertilization Treatments

Grain yield is a common indicator for measuring fertilization effects, reflecting the economic benefits of fertilization. Significant differences in wheat and maize grain yields were observed among different fertilization treatments ($P < 0.05$) (Fig. 1a [Figure 1: see original paper]). Wheat grain yield ranged from 4,048 to 7,319 $kg \cdot hm^{-2}$, while maize grain yield ranged from 6,045 to 9,518 $kg \cdot hm^{-2}$. Humic acid-urea treatments increased wheat and maize grain yields by 15.06%-27.69% and 7.84%-9.55%, respectively, compared with the Urea treatment, and by 62.92%-80.81% and 54.97%-57.44%, respectively, compared with the Control treatment (Fig. 1A). The Urea treatment showed significant yield increases compared with the Control treatment for both wheat and maize. Among different humic acid-urea fertilizers, the U+HA3 treatment significantly increased wheat grain yield compared with the other two activation treatments, with increases of 6.32% over U+HA2 and 10.98% over U+HA1.

Maize straw yield showed the same variation pattern as grain yield among different fertilization treatments, ranging from 6,133 to 9,222 $kg \cdot hm^{-2}$, similar to grain yield. Humic acid-urea treatments produced the highest maize straw yield, significantly higher than both Urea and Control treatments, though differences among different humic acid-urea activation treatments were not significant (Fig. 1B). Wheat straw yield differed significantly among treatments, following the order: U+HA3 > U+HA2 > U+HA1, Urea > Control. Wheat straw yield ranged from 2,283 to 5,400 $kg \cdot hm^{-2}$, significantly lower than maize straw yield (Fig. 1B).

Wheat grain nitrogen content across treatments ranged from 14.4 to 23.9 $g \cdot kg^{-1}$, while straw nitrogen content ranged from 2.7 to 5.6 $g \cdot kg^{-1}$. The no-fertilizer treatment was significantly lower than fertilized treatments. U+HA2 and U+HA3 treatments showed significantly higher wheat grain total nitrogen content than U+HA1 and Urea treatments (Fig. 2 [Figure 2: see original paper]A). Compared with wheat, maize grain nitrogen content was significantly lower, ranging from 10.6 to 13.3 $g \cdot kg^{-1}$. Maize straw nitrogen content ranged from 4.6 to 7.0 $g \cdot kg^{-1}$, higher than wheat straw, particularly under humic acid-urea activation treatments (Fig. 2B). Differences in maize grain and straw nitrogen contents among fertilization treatments were not significant, but all were significantly higher than the Control treatment (Fig. 2B).

2.2 Basic Soil Physicochemical Properties Under Different Fertilization Treatments

After wheat and maize cultivation, all three humic acid-urea treatments significantly reduced soil bulk density compared with the Control treatment, though differences among treatments were not significant. Soil pH values did not differ significantly among humic acid-urea treatments but were significantly lower than both Control and Urea treatments (Table 2). Humic acid-urea treatments significantly increased soil electrical conductivity compared with the Control treatment, with no significant differences among humic acid-urea treatments, though U+HA3 showed significantly higher conductivity than the Urea treatment. Soil organic carbon content across treatments ranged from 8.18 to 10.52 $g \cdot kg^{-1}$, showing the same variation pattern as soil electrical conductivity. Soil mineralized nitrogen content differed significantly among treatments, following the order: U+HA3 > U+HA2, U+HA1 > Urea > Control. The Control treatment showed significantly lower soil available phosphorus content than nitrogen-fertilized treatments, though differences among nitrogen-fertilized treatments were not significant. Humic acid-urea treatments significantly increased soil available potassium content compared with the Control treatment, but the Urea treatment did not differ significantly from either the Control or activated humic acid-urea treatments.

Soil particle size distribution results showed that different fertilization treatments significantly affected median particle diameter D50 (Fig. 3 [Figure 3: see original paper]), which reflects the dominant soil particle composition. The variation pattern of median particle size was: U+HA3 (23.5 μm) < U+HA2 (30.5 μm) < U+HA1 (35.6 μm) < Control (47.4 μm) < Urea (59.3 μm). Using (D90-D10)/D50 to characterize the range of soil particle size distribution, the order among treatments was: U+HA3 (7.11), U+HA2 (6.62), U+HA1 (5.07), Urea (3.29), Control (1.66), indicating that fertilization expanded the soil particle size distribution range, with humic acid-urea activation treatments having greater effects than the Urea treatment. Fertilization treatments significantly increased soil particle specific surface area (Fig. 3), with humic acid-urea activation treatments showing significant increases compared with the Urea treatment, and U+HA3 showing better effects than other activation treatments.

2.3 Soil Nitrogen Forms and Contents Under Different Fertilization Treatments After Wheat and Maize Harvest

Differences in soil nitrate nitrogen content among treatments were significant. After wheat harvest, soil nitrate nitrogen content under U+HA1 treatment was significantly higher than under Urea treatment, while differences among U+HA2, U+HA3, and Urea treatments were not significant, with the Control treatment showing the lowest content, significantly different from fertilized treatments (Table 3). Differences in soil ammonium nitrogen and total nitrogen contents after wheat harvest were not significant among treatments. Compared with soil nitrogen contents after wheat harvest, all treatments showed the lowest values after maize harvest in the Control treatment, followed by the Urea treatment, with humic acid-urea treatments showing higher values. Among different activation treatments after maize harvest, U+HA2 treatment showed significantly lower soil nitrate nitrogen content than the other two activation treatments, but its soil ammonium nitrogen content was higher, with no significant difference from U+HA3 treatment but significantly higher than U+HA1 treatment (Table 3). Soil mineralized nitrogen content differed significantly among treatments, following the order: U+HA3 > U+HA2, U+HA1 > Urea > Control (Table 3).

2.4 Soil Nitrogen Mineralization and Nitrification Ratios Under Different Fertilization Treatments

Significant differences in soil urease activity were observed among treatments (Fig. 4 [Figure 4: see original paper]). The Urea treatment showed the highest soil urease activity, significantly different from Control, U+HA2, and U+HA3 treatments. U+HA3 treatment showed lower soil urease activity than the other two activation treatments, though differences among the three activation treatments were not significant (Fig. 4). The Control treatment did not differ significantly from U+HA3 treatment in soil urease activity.

The mineralization ratio of soil organic nitrogen in the Control treatment decreased with increasing incubation time. Differences in soil organic nitrogen mineralization ratio among fertilization treatments were not significant in the early incubation stage. With extended incubation time, the Urea treatment showed significantly increased soil organic nitrogen mineralization ratio compared with other treatments, reaching the highest ratio of 3.79% at the end of incubation, followed by U+HA1 treatment at 2.98%. Other treatments showed lower mineralization ratios with no significant differences among them (Fig. 5 [Figure 5: see original paper]A). The cumulative mineralization amount of soil organic nitrogen showed the same variation pattern as the mineralization ratio (Fig. 5B): Urea > U+HA1 > U+HA3, U+HA2 > Control. The cumulative mineralization amount ranged from 23.03 to 37.36 $\mathrm{mg \cdot kg^{-1}}$. These results indicate that application of activated and catalyzed humic acid-urea fertilizers significantly inhibited soil organic nitrogen mineralization, while U+HA1 treatment showed weaker inhibition, though its mineralization amount was lower than the Urea treatment. Correlation analysis of factors affecting soil organic nitrogen mineralization ratio revealed a positive correlation between soil available phosphorus content and soil organic nitrogen mineralization ratio, with a correlation coefficient of 0.4559. This result is consistent with Li Huixin et al. [18], who found that soil available phosphorus is a major nutrient element for soil microbial activity and promotes soil organic nitrogen mineralization.

Soil nitrification ratio under different fertilization treatments increased with incubation time, stabilizing in the later incubation stage (Fig. 6 [Figure 6: see original paper]). Notably, the Urea treatment showed significantly higher increases in soil nitrification ratio during early incubation compared with other treatments, maintaining higher nitrification ratios than other treatments throughout incubation, ultimately reaching 84%. The Control treatment showed the lowest nitrification ratio at 53%. Compared with the Urea treatment, activated humic acid-urea treatments reduced soil nitrification ratio by 24%-29%.

2.5 Nitrogen Use Efficiency Under Different Fertilization Treatments for Wheat and Maize

Nitrogen recovery efficiency reflects crop recovery efficiency of fertilizer nitrogen applied to soil. In this study, nitrogen recovery efficiency under Urea treatment was 32.91% for wheat, while humic acid-urea treatments showed significantly higher nitrogen recovery efficiency, with U+HA2 and U+HA3 treatments increasing by 20% and 39%, respectively, compared with U+HA1 treatment (Fig. 7A). For maize, nitrogen recovery efficiency under Urea treatment was 22.91%, significantly lower than humic acid-urea treatments. Differences among different activation treatments were not significant, ranging from 40.82% to 44.22% (Fig. 7 [Figure 7: see original paper]B), representing increases of 78%-93% compared with the Urea treatment.

Nitrogen partial factor productivity refers to crop grain yield per unit of fertilizer nitrogen input. For wheat, partial factor productivity under Urea treatment was 25.48 $\mathrm{kg \cdot kg^{-1}}$, while humic acid-urea treatments U+HA1, U+HA2, and U+HA3 increased by 15%, 20%, and 28%, respectively, compared with urea-only treatment (Fig. 7A). For maize, Urea treatment showed 38.61 $\mathrm{kg \cdot kg^{-1}}$, with humic acid-urea treatments increasing by 8%-10% compared with Urea treatment, though differences among humic acid-urea treatments were not significant. Maize nitrogen partial factor productivity was higher than wheat due to significantly higher maize yields (Fig. 1).

Nitrogen agronomic efficiency refers to increased crop grain yield per unit of nitrogen applied. In this study, wheat nitrogen agronomic efficiency differed significantly among treatments, with Urea treatment showing the lowest value at 7.48 $\mathrm{kg \cdot kg^{-1}}$ and U+HA3 treatment showing the highest at 14.54 $\mathrm{kg \cdot kg^{-1}}$ (Fig. 7A). The difference between U+HA3 and U+HA2 treatments was not significant, but U+HA3 showed significant increases compared with U+HA1 treatment. All maize treatments showed higher nitrogen agronomic efficiency than wheat season, though differences among activation treatments were not significant, all showing significant improvements compared with Urea treatment (Fig. 7B).

2.6 Regression Analysis Between Nitrogen Use Efficiency and Other Related Indicators Under Different Fertilization Treatments

Regression analysis showed that nitrogen recovery efficiency exhibited significant negative linear relationships with soil nitrification ratio, mineralized amount of soil organic nitrogen, and soil urease content (Table 4). Soil particle specific surface area showed a positive linear correlation with soil nitrogen recovery efficiency. This indicates that nitrogen recovery efficiency tends to decrease with increasing soil nitrification ratio, soil organic nitrogen mineralization amount, and soil urease content, but increase with increasing soil particle specific surface area (Table 4).

Table 4 Regression analysis of nitrogen recovery efficiency and other soil indexes in different fertilization treatments

Dependent variable (y)	Independent variable (x)	Regression equation
Nitrogen recovery efficiency	Soil nitrification rate	$y = -0.44x + 97.42$
Nitrogen recovery efficiency	Mineralized quantity of soil organic nitrogen	$y = -0.47x + 51.42$
Nitrogen recovery efficiency	Soil urease activity	$y = -2.97x + 571.94$
Nitrogen recovery efficiency	Soil particle specific surface	$y = 39.16x - 603.58$

Discussion

In this study, the three activated humic acid-urea treatments increased wheat grain yield by 15.06%-27.69% and maize grain yield by 7.84%-9.55% compared with the Urea treatment. The Urea treatment showed significant yield increases compared with the Control treatment for both crops. Similar results were reported by Li Zhaojun et al. [13], who found that humic acid long-lasting urea significantly increased maize grain yield. This was attributed to humic acid long-lasting urea inhibiting soil urease activity during early maize growth, making soil nitrogen supply rate consistent with maize growth and nitrogen demand, while also increasing leaf chlorophyll content and photosynthesis intensity at different growth stages. Tahir et al. [14] found that humic acid application at 60 $\mathrm{mg \cdot kg^{-1}}$ (soil) significantly promoted wheat plant height, fresh root weight, and dry matter, while increasing plant nitrogen uptake, particularly notable in non-calcareous soils, mainly because humic acid can chelate unavailable soil nutrients and regulate soil pH.

This study found that humic acid-urea application significantly reduced soil bulk density and pH while increasing soil electrical conductivity, organic carbon content, and mineralized nitrogen content (Table 2). Additionally, it decreased median soil particle diameter D50 and increased the range of soil particle size distribution and soil specific surface area (Fig. 3). Compared with the Urea treatment, humic acid-urea application significantly reduced soil urease activity, indicating that humic acid delayed urea hydrolysis and transformation, indirectly prolonging urea effectiveness and promoting crop growth. U+HA2 and U+HA3 treatments significantly inhibited soil organic nitrogen mineralization, while U+HA1 treatment showed weaker inhibition, though its mineralization amount was lower than the Urea treatment (Fig. 5). Many researchers [11-12,19] have reported similar results, finding that humic acid application reduced soil nitrogen supply rate because humic acid-urea complexation decreased nitrogen nutrient release rate and soil urease activity. These factors collectively indicate that humic acid-urea application improved soil physicochemical properties, slowed transformation of nitrogen fertilizer forms such as urea, enhanced soil capacity for adsorbing and storing different nitrogen forms (Table 3), extended fertilizer effectiveness period, improved fertilizer use efficiency, and ultimately increased crop yield.

This study employed three fertilizer use efficiency indicators because different efficiency indicators reflect different fertilization effects [16,20]. Fertilizer recovery efficiency is a comprehensive indicator reflecting crop utilization of fertilizer, concentrating multiple factors affecting fertilizer use efficiency, such as soil properties, crop characteristics, tillage patterns, management practices, fertilizer amount, type, and application timing [4-5,20-22]. Since fertilization techniques and soil fertility conditions were identical, fertilization treatments had significant effects on nitrogen use efficiency (Fig. 7). Humic acid-urea treatments significantly increased nitrogen recovery efficiency by 36.73%-91.43% compared with the Urea treatment. Different activation treatments also had varying effects on nitrogen use efficiency; compared with direct humic acid-urea mixing, activated and catalyzed humic acid-urea treatment showed better promotion of nitrogen recovery efficiency. The main influencing factor for these results is that activated humic acid-urea is an organic-inorganic compound fertilizer; existing research has shown that combined organic-inorganic fertilizer application can improve inorganic fertilizer use efficiency [8-9]. Additionally, the different effects of activation treatments on nitrogen recovery efficiency may result from differences in free humic acid content among treatments. After urea activation and activation-catalysis treatments, free humic acid contents were 18.2% and 18.8%, respectively, representing increases of 26.4% and 30.6% compared with untreated raw humic acid (free humic acid content 14.4%).

In this study, nitrogen recovery efficiencies of U+HA1, U+HA2, and U+HA3 treatments in the wheat season were 45%, 54%, and 63%, respectively, higher than the average wheat nitrogen recovery efficiency of 40.5% reported by Zhang Fusuo et al. [5] for Shandong and Shanxi regions (2001-2005). The Urea treatment in this study showed nitrogen recovery efficiency of 32.91%, lower than the reported average. The difference may be because the reported values involved combined nitrogen, phosphorus, and potassium application with multiple nitrogen top-dressings. This result falls within the internationally reported appropriate range of 30%-50% for nitrogen recovery efficiency in cereal crops [21]. All maize treatments in this study showed lower nitrogen recovery efficiency than wheat, with Urea treatment at only 22.91% and humic acid-urea treatments at 41%-44%, with no significant differences among activation treatments. This may be because returning wheat straw to the field in the previous season increased soil available nitrogen content, reducing nitrogen fertilization effects [23]. Significant differences in nitrogen recovery efficiency among treatments mainly resulted from different fertilization treatments affecting soil nitrogen transformation processes, different nitrogen form contents, and other soil properties. Regression analysis between nitrogen recovery efficiency and related influencing factors showed that soil nitrification ratio, organic nitrogen mineralization amount, and soil urease content all had negative effects on nitrogen recovery efficiency. Higher nitrogen recovery efficiency under activated humic acid-urea treatments may be due to: (1) humic acid-urea application improved soil physicochemical properties such as bulk density, specific surface area, and organic carbon content, enhancing soil nitrogen retention capacity; (2) humic acid-urea treatment inhibited soil urease activity, reducing urea decomposition rate (Fig. 4), making urea transformation consistent with crop nitrogen demand patterns; (3) activated humic acid-urea treatments significantly inhibited soil organic nitrogen mineralization rate and nitrification ratio (Figs. 5 and 6), similar to Sharif et al. [24]. In summary, humic acid-urea prolonged urea effectiveness, thereby improving its use efficiency. Nitrogen recovery efficiency increased with soil particle specific surface area, possibly because increased specific surface area promoted soil nitrogen adsorption and retention, reduced nitrogen loss, increased crop nitrogen absorption and utilization, and consequently improved nitrogen recovery efficiency.

Improved soil nitrogen use efficiency promoted crop yield formation. Although differences in soil ammonium and nitrate nitrogen contents among activated humic acid-urea treatments were not significant, soil mineralized nitrogen content differed significantly among treatments after maize harvest (Table 3), showing consistent variation patterns with crop yield. Free humic acid contents differed among different activation treatments in this study. External organic carbon entering soil promoted soil organic carbon mineralization, increasing soil available nutrient content [25]. However, nitrogen recovery efficiency showed a negative linear relationship with soil organic nitrogen mineralization amount, possibly because activated humic acid-urea addition inhibited urease activity and soil nitrification ratio, ultimately reducing urea decomposition rate. This study did not monitor fertilizer release characteristics during the crop growth period, which should be addressed in future research.

Humic acid-urea treatments increased wheat and maize grain yields by 15%-28% and 8%-10%, respectively, compared with the Urea treatment, and by 63%-81% and 55%-57%, respectively, compared with the Control treatment. The activated and catalyzed humic acid-urea treatment (U+HA3) significantly increased wheat grain yield compared with the other two activation treatments, with increases of 6% over U+HA2 and 11% over U+HA1. Variation trends in wheat and maize straw yields among different fertilization treatments were similar to those of grain yields. Activated humic acid-urea showed more pronounced application effects on wheat.

All three humic acid-urea treatments significantly reduced soil nitrification ratio, organic nitrogen mineralization amount, and urease content while increasing soil particle specific surface area. Humic acid-urea treatments significantly improved nitrogen recovery efficiency, nitrogen agronomic efficiency, and partial factor productivity, with the activated and catalyzed humic acid-urea treatment (U+HA3) showing the best performance.

References

[1] Liu E K, Zhao B Q, Hu C H, et al. Effects of long-term nitrogen, phosphorus and potassium fertilizer applications on maize yield and soil fertility[J]. Plant Nutrition and Fertilizer Science, 2007, 13(5): 789-794

[2] Liu Z J, Li S T, Zhou W, et al. Effects of various nitrogen managements on grain yield, nitrogen uptake and economic benefits of spring maize in Northeast China[J]. Soil and Fertilizer Sciences in China, 2012(3): 59-64

[3] Zhong Y Q W, Yan W M, Shangguan Z P. Soil carbon and nitrogen fractions in the soil profile and their response to long-term nitrogen fertilization in a wheat field[J]. Catena, 2015, 135: 38-46

[4] Yan X, Jin J Y, He P, et al. Recent advances in technology of increasing fertilizer use efficiency[J]. Scientia Agricultura Sinica, 2008, 41(2): 450-459

[5] Zhang F S, Wang J Q, Zhang W F, et al. Nutrient use efficiencies of major cereal crops in China and measures for improvement[J]. Acta Pedologica Sinica, 2008, 45(5): 915-924

[6] Shi Y F, Zhang M, Zhang Z H, et al. Effects of different types of nitrogen fertilization on yield, N use efficiency and apparent budget of soil nitrogen in summer maize[J]. Journal of Soil and Water Conservation, 2009, 23(6): 95-98

[7] Xu Y, Liu Z H, Jiang L H, et al. Effects of different nitrogen regulation on nitrogen fertilizer use of winter wheat and content of soil Nitrate-N[J]. Journal of Soil and Water Conservation, 2010, 24(4): 90-93

[8] Du W, Zhao B Q, Lin Z A, et al. Effect and mechanism of organic-inorganic compound fertilization on chemical fertilizer utilization I. Effect of urea compounded with organic material on maize yield and fertilizer utilization[J]. Plant Nutrition and Fertilizer Science, 2012, 18(3): 579-586

[9] Liu Y L. Changes in soil properties and its relation with soil productivity under long-term fertilization in paddy fields[D]. Nanjing: Nanjing Agricultural University, 2007

[10] Liang B, Zhao W, Yang X Y, et al. Effects of N application on N content and N uptake by crops in soils under different long-term fertilization managements in wheat-maize rotation system[J]. Acta Pedologica Sinica, 2012, 49(4): 748-757

[11] Yan S D, Liu L J, Hong J P. Effect of humic acid-urea complex on urea transformation and nitrogen release[J]. Chinese Journal of Eco-Agriculture, 2008, 16(1): 109-112

[12] Juan Y H, Chen L J, Wu Z J, et al. Response of urea-N transformation to humic acid[J]. Chinese Journal of Soil Science, 2011, 42(1): 112-116

[13] Li Z J, Ma G R, Wang S G, et al. Transformation of long-lasting UHA in soil and its effect on maize yield[J]. Chinese Journal of Eco-Agriculture, 2005, 13(4): 121-123

[14] Tahir M M, Khurshid M, Khan M Z, et al. Lignite-derived humic acid effect on growth of wheat plants in different soils[J]. Pedosphere, 2011, 21(1): 124-131

[15] Vlčková Z, Grasset L, Antosŏvá B, et al. Lignite pre-treatment and its effect on bio-stimulative properties of respective lignite humic acids[J]. Soil Biology and Biochemistry, 2009, 41(9): 1894-1901

[16] Zhang Y X, Suo Q Y, Hu X Y, et al. Effect of activation conditions on content of water soluble humic acid in lignite[J]. Humic Acid, 2011(3): 10-12

[17] Lu R K. Soil Agricultural Chemistry Analysis Method[M]. Beijing: Chinese Agricultural Science Press, 2000

[18] Li H X, Hu F, Liu M Q, et al. Characteristics of nitrogen mineralization and nitrification in red soil[J]. Soils, 2000, 32(4): 194-197

[19] Cheng L, Zhang B L, Wang J, et al. Research progress of humic-acid containing fertilizer[J]. Soil and Fertilizer Sciences in China, 2011(5): 1-6

[20] Miao J G, Jin J Y, Qiu S J, et al. Effect of ecological intensification nutrient management on the yield and nitrogen use efficiency of spring maize[J]. Plant Nutrition and Fertilizer Science, 2012, 18(3): 571-578

[21] Dobermann A. Nitrogen use efficiency-state of the art[R]. IFA International Workshop on Enhanced-Efficiency Fertilizers. Frankfurt, Germany, 28-30 June 2005

[22] Jiang L N, Liu P, Qi B Y, et al. Effects of different nitrogen application amounts and seedling densities on nitrogen accumulation and transport in winter wheat at anthesis stage[J]. Chinese Journal of Eco-Agriculture, 2016, 24(2): 131—141

[23] Yan C P, Pei X X, Wang J A, et al. Effects of straw returned to soil and N application on growth, water and nitrogen use efficiency of winter wheat[J]. Chinese Journal of Eco-Agriculture, 2011, 19(2): 271—275

[24] Sharif M, Khattak R A, Sarir M S. Effect of different levels of lignitic coal derived humic acid on growth of maize plants[J]. Communications in Soil Science and Plant Analysis, 2002, 33(19/20): 3567-3580

[25] Liu Y L, Ding F J, Gu D Y, et al. Characteristics of wheat-maize yield and soil organic carbon/nitrogen mineralization in cinnamon soil with application of different activation humic acid-urea[J]. Soils, 2015, 47(1): 42–48

Note: Figure translations are in progress. See original paper for figures.