

Article

The Application of Humic Acid Urea Improves Nitrogen Use Efficiency and Crop Yield by Reducing the Nitrogen Loss Compared with Urea

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Abstract: Enhancing nitrogen (N) use efficiency (NUE) and reducing urea N losses are major challenges in ensuring sustainable agriculture. The aim of this study was to investigate the effect of humic acid urea on N losses, soil microbial nutrient balance and NUE through leaching experiments, soil incubation experiments and field experiments of maize-wheat rotation. We set up four N gradients (240 kg N hm⁻², 216 kg N hm⁻², 192 kg N hm⁻², 168 kg N hm⁻²) and two N fertilizer types (urea and humic acid urea) to make up five treatments, with no N application as the control. The results showed that humic acid urea reduced the fertilizer N losses by 25.51%, 23.07% and 23.08% in the three pathways of N leaching, NH₃ volatilization and N₂O emission, respectively, compared with urea. Humic acid urea significantly increased soil ammonium N, nitrate N and available phosphorus contents, and brought the enzyme stoichiometry ratio closer to 1:1:1, which promoted microbial nutrient balance. Application of humic acid urea significantly increased yield, NUE and annual net economic profit of maize and wheat. Among all treatments, the application of humic acid urea at 216 kg N hm⁻² maximized NUE, reduced environmental pollution and increased yield.

Keywords: NH₃ volatilization; N₂O emission; inorganic N leaching; microbial nutrient balance; yield



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1. Introduction

Nitrogen (N) is an essential element for plant growth and contributes most to crop yield increase [1]. About 70% of the N fertilizer applied in global agricultural production is urea [2]. Farmers need to apply more and more urea to increase yields due to the imbalance between the growing population and the demand for food. In China, the N fertilizer application in agricultural practices increased from 7.07 million tons in 1977 to 26.21 million tons in 2005 (a 271% increase) [3]. However, excessive application of urea will reduce nitrogen use efficiency (NUE) and lead to environmental problems, such as greenhouse gas emissions and groundwater contamination. Studies have shown that N recovery in soil-plant systems rarely exceeds 50% [4], and most of the applied N fertilizer is lost through leaching and gaseous emissions [5]. The main reason is that the N from urea is easily hydrolyzed to ammonium N (NH₄⁺-N). The input of excess NH₄⁺-N, due to inappropriate urea application, can disrupt the balance of ammonium N (NH₄⁺-N) in the soil solid-liquid-gas phase, resulting in N loss as ammonia (NH₃) volatilization [6]. NH₃ is an atmospheric pollutant that can cause soil acidification and water eutrophication, with agricultural practices accounting for approximately 90% of global NH₃ emissions [7]. A significant portion of the NH₄⁺-N from urea hydrolysis is not used by plants in time and is converted to nitrate N (NO₃⁻-N) by nitrification. This leads to the accumulation of NO₃⁻-N in deep soils, increasing the risk of N leaching and groundwater contamination [8]. Meanwhile, nitrification and denitrification increase N losses as nitrous oxide (N₂O) [9].

N_2O is a greenhouse gas that can cause ozone layer depletion and has 298 times the warming potential of CO_2 on a 100-year time scale [10]. Meanwhile, agriculture accounts for 60% of global anthropogenic N_2O emissions [11]. Excessive urea application can lead to serious environmental problems. Therefore, we should choose the right fertilizer to prolong the fertilization effect, improve the NUE and reduce the negative environmental effects.

Humic acid is an N fertilizer synergist that plays an active role in promoting plant growth, prolonging fertilizer efficiency and reducing N losses [12]. Firstly, humic acid promotes root growth and development, causing increased root biomass [13]. Moreover, humic acid can promote root H^+ -ATPase activity and improve root uptake of soil nutrients, thus promoting above-ground crop growth [14]. Secondly, the application of humic acid facilitates the formation of soil macroaggregates and improves the soil physical structure, which enhances the ability of soil to retain water and fertilizer, thus prolonging the fertilization effect [15]. Finally, Dong et al. [16] showed that humic acid inhibits urease activity to reduce the rate of urea hydrolysis. This keeps the soil NH_4^+ -N content at a low level, which reduces the risk of NH_3 volatilization and potential nitrification, leading to lower N losses. In addition, humic acid has shown positive effects in controlling soil acidification and increasing P effectiveness [17,18]. Therefore, humic acid has a very important role in agricultural production as an N fertilizer synergist.

Although numerous studies have revealed the positive role of humic acid in agricultural production, humic acid is not convenient as a synergist for application alone or in combination with N fertilizers. The development of efficient humic acid urea using organic-inorganic compound technology is necessary [19,20]. Shen et al. [21] found that urea can chemically react with humic to combine the N in urea with amino, carboxyl and hydroxyl groups in the humic acid, which reduces the hydrolysis rate of urea to prolong the fertilization effect. Studies have shown that the application of humic acid urea significantly improved maize yield and recovery of fertilizer N [22]. Liu et al. [23] found that application of humic acid urea in coastal saline soils increased soil nutrient effectiveness and maize yield. Therefore, the development of humic acid urea using organic-inorganic complexes is feasible and effective. We hypothesized that (1) humic acid urea can reduce the hydrolysis rate of urea, which decreases the impact of urea nitrogen loss in the agro-environment. (2) Humic acid urea can promote microbial nutrient balance to alleviate soil nutrient limitation and improve soil quality. (3) Humic acid urea can prolong the fertilization effect, which enhances the NUE of crops. Through leaching experiments, soil incubation experiments and field experiments, this study aimed to investigate: (1) whether humic acid urea could mitigate the environmental impact of agricultural N application by reducing N losses through three pathways: NH_3 volatilization, N leaching and N_2O emissions; (2) what effect humic acid urea had on the dynamics of soil nutrients, enzyme activity and enzyme stoichiometry during crop growth; and (3) to determine the optimum N application rate of humic acid urea through yield and NUE of maize and wheat.

2. Materials and Methods

2.1. Materials

The test fertilizers were provided by Kingenta Ecological Engineering Group Co., Ltd. (Linshu, China) where the test fertilizers were urea (46% N), double superphosphate (44% P_2O_5), potassium sulfate (50% K_2O) and humic acid urea (45% N and 1% humic acid). Humic acid was extracted from weathered coal, which contained 70% water-soluble humic acid and 20% K_2O , and prepared with urea by hot fusion to form humic acid urea [21]. The test soil was collected in December 2020 from the cultivated soil (0–20 cm) of Zhaizi Village, Tai'an City, Shandong Province, China, and the soil type was Typic Hapludalf soil [24]. Soil samples were retrieved to remove stones and roots, air-dried and prepared for use. The hydrogen ion concentration (pH) of the test soil was 7.29; electrical conductance (EC) was $707.25 \mu\text{S cm}^{-1}$; bulk density (BD) was 1.12 g cm^{-3} ; soil organic carbon (SOC) and total nitrogen (TN) were 12.50 and 1.09 g kg^{-1} ; NH_4^+ -N, NO_3^- -N, available phosphorous (AP) and available Kalium (AK) were 2.16, 13.32, 26.64 and 50.81 mg kg^{-1} , respectively.

2.2. Leaching Experiment

The leaching experiment was carried out in the laboratory (25 °C). The leaching device was a PVC cylindrical pipe with an inner diameter of 10 cm and a height of 60 cm. The bottom of the device was lined with one layer of 200 mesh nylon net and 2 cm thick quartz sand with a particle size of 1 to 2 mm. The wall of the tube was coated with a layer of petroleum jelly to reduce the edge effect of the soil column [25]. The flow rate of water was controlled by a medical infuser during leaching. Based on the local soil BD, the leaching device was filled with a mixture of soil (3960 g) and N fertilizer to form a 45 cm high soil column to simulate the soil root layer [26]. The soil column BD was measured to be 1.127 g cm^{-3} .

Three treatments with three replicates each were established: (1) no N application (CK); (2) urea at 240 kg N hm^{-2} (UN); and (3) humic acid urea at 240 kg N hm^{-2} (HUN). Intermittent drenching method was used to collect the leachate. Before leaching, the soil column was saturated with water by adding water (1500 mL) and placed at 25 °C for 1 day. For the first leaching, 400 mL of water was added to the soil column. The 24-h leachate was collected and its volume was measured. The soil column was weighed after 6 days at 25 °C. At this time, the soil moisture content was approximately 60% after natural evaporation and the second leaching was performed. Nine consecutive leaching sessions were carried out on days 2, 8, 14, 20, 26, 32, 38, 44 and 50, each with the same process. The $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ contents of the leachate were determined using continuous flow injection analysis (AA3-A001-02E, SEAL Analytical Limited, Norderstedt, Germany).

The leaching amounts of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ were calculated by Equation (1). The leaching amount of inorganic nitrogen (IN) was the sum of the leaching amounts of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$. The cumulative leaching amounts of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and IN were calculated by Equation (2) [27].

$$Q_i = C_i \times V_i \quad (1)$$

Q_i (mg) was the leaching amount of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and IN from the i -th leachate. C_i (mg L^{-1}) was the content of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the i -th leachate. V_i (L) was the volume of the i -th leachate.

$$Q = \left(\sum_{i=1}^n Q_i \right) \div S \quad (2)$$

Q (mg m^{-2}) was the cumulative leaching amount of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and IN. n was the number of leaching times. S (m^2) was the cross-sectional area of the leaching device.

2.3. Incubation Experiment

The two incubation experiments were conducted in the laboratory incubator from December 2020 to January 2021. For all incubation experiments, 500 g of air-dried soil passed through a 2 mm sieve was weighed and evenly mixed with N fertilizer, and transferred to a 1.5 L airtight container. N was applied at a level of 240 kg N hm^{-2} and incubated at 25 ± 1 °C in the dark. The soil water content was kept at 70% of the field moisture capacity during the incubation period. Prior to incubation, all containers were preincubated for 1 day at 25 ± 1 °C to activate soil microorganisms and ensure uniform water diffusion [28]. The two incubation experiment treatments with four replicates each were identical to the leaching experiment (CK, UN and HUN).

2.3.1. Ammonium Volatilization

NH_3 volatilization was determined by the closed room method, and a cup with 20 mL of 2% H_3BO_3 solution was placed inside the container to absorb NH_3 . The experiment was continuously incubated for 15 days. During the incubation period, the H_3BO_3 solution was taken out every 24 h and titrated with H_2SO_4 using bromocresol green-methyl red as an indicator to determine the absorbed NH_3 . After sampling, the chamber was ventilated for 30 min. NH_3 volatilization flux was calculated using Equation (3) [28].

$$\text{FNH}_3 = (c \times V) \div (t \times S) \quad (3)$$

FNH_3 ($mg\ m^{-2}\ h^{-1}$) was the NH_3 volatilization flux. c ($mg\ L^{-1}$) was the NH_4^+ -N content in the absorbent solution. V (L) was the volume of the absorbent solution. t (h) was the sampling interval time. S (m^2) was the area of the closed room.

2.3.2. N_2O Emission

N_2O emissions were determined using a ventilation room-gas chromatography method. A manual pump was used to exchange the internal air prior to sampling. At 0, 30, 60 and 90 min after closure, gas samples were collected using a 50 mL syringe and the sampling time was recorded. The containers were kept sealed throughout the process. This procedure was repeated every 24 h during the incubation period. Gas samples were measured on the same day using a gas chromatograph (Shimadzu GC-2010 Plus, Japan). N_2O emission fluxes were calculated by Equation (4) [29].

$$FN_2O = \rho \times \frac{dc}{dt} \times H \times \frac{273}{273 + T} \quad (4)$$

FN_2O ($mg\ m^{-2}\ h^{-1}$) was the N_2O emission flux. ρ ($1.25\ kg\ m^{-3}$) was the density of N_2O in the standard state. dc/dt ($mg\ h^{-1}$) was the change in N_2O content in the room per unit time. H (m) was the height of the room. T ($^{\circ}C$) was the temperature in the room.

2.3.3. Cumulative N-Gas Emissions and N Losses from Fertilizer

The cumulative emissions of a gas could be calculated by Equation (5) [30].

$$CE = \sum_{i=1}^n \left(\frac{F_i + F_{i-1}}{2} \right) \times (t_i - t_{i-1}) \quad (5)$$

CE ($mg\ m^{-2}$) was the cumulative emission of a gas. F_i and F_{i-1} ($mg\ m^{-2}\ h^{-1}$) were the i -th and $i-1$ -th emission fluxes of a gas, respectively. $t_i - t_{i-1}$ (h) was the time interval between the i -th and $i-1$ -th sampling events. n was the number of gas sampling events.

N loss from fertilizer was calculated by Equation (6) [10].

$$\%N = \frac{m_N - m_0}{m_{fertilizer-N}} \quad (6)$$

$\%N$ was the percentage of N loss from N fertilizer. m_N (mg) was the cumulative N loss from N application treatment. m_0 (mg) was the cumulative N loss from no N application treatment. $m_{fertilizer-N}$ (mg) was the weight of N in the applied N fertilizer.

2.4. Field Experiment of Maize-Wheat Rotation

2.4.1. Experiment Site and Method

The maize-wheat field fertilization experiment was conducted from June 2017 to June 2019 in Zhaizi village, Tai'an City, Shandong Province, China ($36^{\circ}15'89''$ N, $117^{\circ}15'20''$ E, Figure 1). The study site had a temperate continental monsoon climate with the same soil type and basic physicochemical properties as the test soil described above. The study site had been in a maize-wheat rotation for 20 years prior to the fertilization experiment, with fertilization levels of $240\ kg\ N\ hm^{-2}$, $120\ kg\ P_2O_5\ hm^{-2}$ and $90\ kg\ K_2O\ hm^{-2}$.

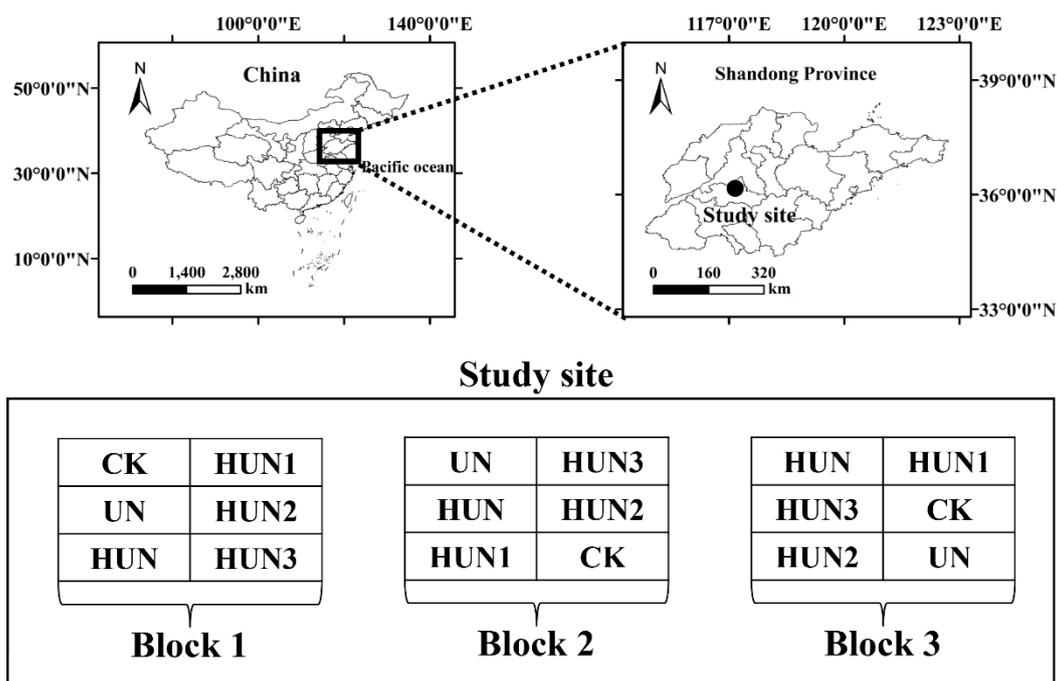


Figure 1. Location diagram of the study site. This map was created using Arcmap 10.8 software (<https://developers.arcgis.com/>, accessed on 2 November 2022).

The experiment applied a randomized block design with five experimental treatments (UN: urea at 240 kg N hm^{-2} , HUN: humic acid urea at 240 kg N hm^{-2} , HUN1: humic acid urea at 216 kg N hm^{-2} , HUN2: humic acid urea at 192 kg N hm^{-2} and HUN3: humic acid urea at 168 kg N hm^{-2}) and no N application (CK) as a control, for a total of six treatments. All treatments were applied with double superphosphate ($44\% \text{ P}_2\text{O}_5$) and potassium sulfate ($50\% \text{ K}_2\text{O}$) at $120 \text{ kg P}_2\text{O}_5 \text{ hm}^{-2}$ and $90 \text{ kg K}_2\text{O hm}^{-2}$ levels, with three replications of each treatment. The experiment was conducted in 18 plots of $4 \times 10 \text{ m}^2$ each with 1 m protection rows. Maize was seeded at a spacing of 60 cm between rows and 35 cm between plants, and the variety was Delinong 988, with all fertilizers applied at the base. Wheat was seeded at a rate of 187.5 kg hm^{-2} and the variety was Shannong 28. The 50% N fertilizer and all P and K fertilizers were applied at the base, and 50% N fertilizer was applied at the jointing stage. All plots were managed in the same way, and maize and wheat straws were returned to the field.

2.4.2. Soil Sampling and Analysis

Soil samples were collected at the 2019 wheat jointing, booting, filling and maturity stages in each plot by the five-point sampling method, and the soil from the five points was mixed into one soil sample. Sampling depths were 0–20, 20–40 and 40–60 cm, with 216 mixed soil samples. The mixed soil samples were taken back to the laboratory to remove roots and stones, and were divided into two parts. One of the samples was immediately stored in a refrigerator at $-80 \text{ }^\circ\text{C}$.

The enzyme activities ($\mu\text{mol h}^{-1} \text{ g}^{-1}$) were determined by the method of Deforest et al. [31]. The 50-mM acetic acid-sodium acetate buffer was made by mixing sodium acetate trihydrate with deionized water. This solution pH was adjusted with NaOH according to the average soil pH of the study site ($\text{pH} = 7.29$). Four 4-methylumbelliferone (MUB)-based fluorogenic enzyme substrates were used to determine enzyme activities: 4-MUB- β -D-glucopyranoside for β -1,4-glucosidase (βG), 4-MUB- β -D-cellobioside for cellobiohydrolase (CBH), 4-MUB-N-acetyl- β -D-glucosaminide for β -1,4-N-acetyl-glucosaminidase (NAG) and 4-MUB-phosphate for alkaline phosphatase (ALP). The enzyme activity in 96-well plates was determined by the fluorometric method. The 96-well plates were incubated in the dark ($25 \text{ }^\circ\text{C}$, 4 h) and then measured using a microplate reader (Synergy HTX, USA). $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ (mg kg^{-1})

were extracted with 1 mol L⁻¹ KCl using continuous flow injection analysis (AA3-A001-02E, SEAL Analytical Limited, Norderstedt, Germany).

The other sample, after air-drying and crushing, was sieved using 2 mm and 0.18 mm sieves. SOC content (g kg⁻¹) was determined by the potassium dichromate external heating method using soil samples screened by a 0.18 mm sieve. Soil AP content (mg kg⁻¹) was extracted with 0.5 mol L⁻¹ NaHCO₃ and determined by UV spectrophotometer at 700 nm using soil samples screened with a 2 mm sieve.

Enzyme C: N, C: P and N: P ratios, calculated by Equations (7)–(9) [32].

$$\text{Enzyme C : N ratio} = \ln \beta G \div \ln \text{NAG} \quad (7)$$

$$\text{Enzyme C : P ratio} = \ln \beta G \div \ln \text{ALP} \quad (8)$$

$$\text{Enzyme N : P} = \ln \text{NAG} \div \ln \text{ALP} \quad (9)$$

2.4.3. Plant Sampling and Analysis

The yield, aboveground biomass and plant N content of maize and wheat were measured at maturity stage from 2018 to 2019. Three 1 × 1 m² quadrats were selected in each plot, and the ear number and grain number per ear of wheat and maize in each quadrat were measured, and above-ground plant samples from each quadrat were mowed and brought back to the laboratory for weighing. Maize and wheat grain samples were dried and the hundred grain weight was determined. The aboveground plant samples were dried and the moisture content was determined. These samples were crushed and digested with H₂SO₄-H₂O₂, and the plant N content was determined by Kjeldahl method [33].

2.4.4. Calculation of Yield and Nitrogen Use Efficiency

Maize and wheat yields were measured from the ear number, grain number per ear and hundred grain weight determined by the quadrat method using Equation (10), cumulative N uptake by plant was calculated using Equation (11), and NUE was calculated using Equation (12) [34].

$$\text{Yield (kg hm}^{-2}\text{)} = \text{ear number (ear m}^{-2}\text{)} \times \text{grain number per ear (grain ear}^{-1}\text{)} \times \text{hundred grain weight (kg)} \div 100 \times 10000 \times 0.85 \quad (10)$$

$$\text{Cumulative N uptake by plant} = \text{aboveground plant N content (\%)} \times \text{aboveground biomass (kg hm}^{-2}\text{)} \quad (11)$$

$$\begin{aligned} \text{NUE(\%)} &= [\text{cumulative N uptake by plants in N treatment (kg hm}^{-2}\text{)} \\ &\quad - \text{cumulative N uptake by plants in no N treatment (kg hm}^{-2}\text{)}] \\ &\quad \div \text{Total N of fertilizer in N treatment (kg hm}^{-2}\text{)} \times 100\% \end{aligned} \quad (12)$$

The annual total economic profit (TEP) and annual net economic profit (NEP) of maize and wheat were calculated by Equations (13) and (14), respectively.

$$\text{TEP (\$ hm}^{-2}\text{)} = \text{yield (kg hm}^{-2}\text{)} \times \text{average grain price (\$ kg}^{-1}\text{)} \quad (13)$$

$$\text{NEP (\$ hm}^{-2}\text{)} = \text{TEP (\$ hm}^{-2}\text{)} - [\text{fertilizer expenditure (\$ hm}^{-1}\text{)} + \text{Labor expenditure (\$ hm}^{-1}\text{)} + \text{seed expenditure (\$ hm}^{-1}\text{)}] \quad (14)$$

2.5. Statistical Analysis

All data were normally distributed and tested for homogeneity of variance. One-way analysis of variance (ANOVA) and least significant difference (LSD) post hoc tests ($\alpha = 0.05$) were used to determine the effect of humic acid urea, compared to urea, on N leaching, NH₃ volatilization, N₂O emission and soil chemical-biological properties. Four replicate experiments were conducted for the incubation experiment, and three replicate experiments were conducted for the leaching experiment and the field experiment. Independent samples

t-test was used to determine the effect of humic acid urea and urea on fertilizer N loss. All the above analyses were performed in the statistical software SPSS 23 (International Business Machines Corporation, Armonk, NYC, USA) and the graphs were plotted using Origin 2018 (OriginLab, Northampton, MA, USA).

3. Results

3.1. Effect of Humic Acid Urea on Nitrogen Leaching in the Leaching Experiment

Compared to the UN, the HUN increased the risk of NH_4^+ -N leaching, with a 14.68% increase in cumulative NH_4^+ -N leaching (Figure 2). The first four cumulative NO_3^- -N leaching of the two N fertilizers accounted for 31.25%–33.38% of the total cumulative NO_3^- -N leaching. The cumulative NO_3^- -N leaching in the last five times sharply increased with the increase of incubation days. The HUN reduced the risk of NO_3^- -N leaching and the cumulative NO_3^- -N leaching loss was reduced by 13.32% compared to the UN. IN leaching loss was dominated by NO_3^- -N. After the fifth leaching, the cumulative IN leaching in the HUN started to become lower than that in the UN. The total cumulative IN leaching of HUN was 8.89% reduced compared with that of UN.

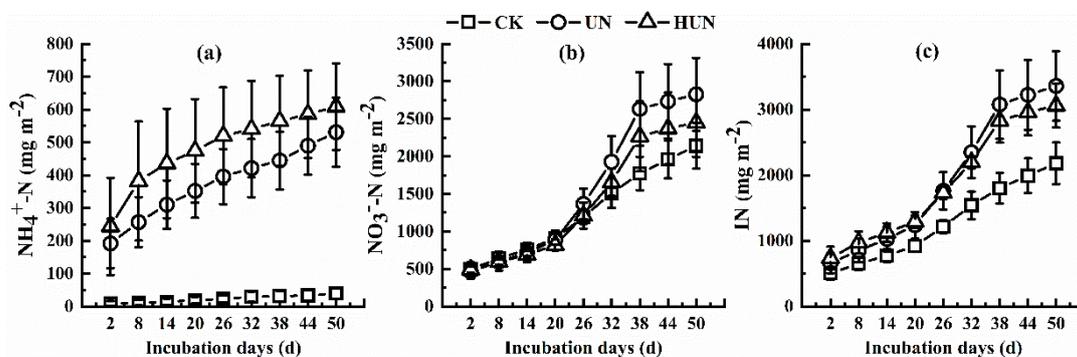


Figure 2. Effects of different treatments on cumulative leaching loss of NH_4^+ -N (a), NO_3^- -N (b) and IN (c). The vertical coordinate corresponding to the point is the average value. The length of the error bars is the standard deviation. NH_4^+ -N: ammonium nitrogen. NO_3^- -N: nitrate nitrogen. IN: inorganic nitrogen. CK: no nitrogen, UN: urea at 240 kg N hm⁻², HUN: humic acid urea at 240 kg N hm⁻².

3.2. Effects of Humic Acid Urea on NH_3 Volatilization and N_2O Emission in the Incubation Experiment

The effect of N addition on NH_3 volatilization flux was mainly concentrated in the first 7 days (Figure 3a). Compared with UN, HUN significantly reduced NH_3 volatilization flux and cumulative NH_3 volatilization, with a 22.96% reduction after 15-day cumulative NH_3 volatilization (Figure 3b). N addition had a small effect on N_2O emission flux in the first 5 days (Figure 4a). The peak N_2O emissions for each treatment occurred mainly between 5 and 15 days. HUN was effective in reducing cumulative N_2O emissions (Figure 4b). Compared to the UN, the 35-day cumulative N_2O emissions were 22.13% lower for HUN.

The main pathway of N loss from N fertilizer was NH_3 volatilization, followed by N leaching (Table 1). Compared with UN, the total N loss from HUN was reduced by 21.72%, and the N loss from HUN was reduced by 25.51%, 23.07% and 23.08% in the three pathways of leaching, NH_3 volatilization and N_2O emission, respectively. Among them, HUN significantly reduced NH_3 volatilization compared to UN ($p < 0.05$).

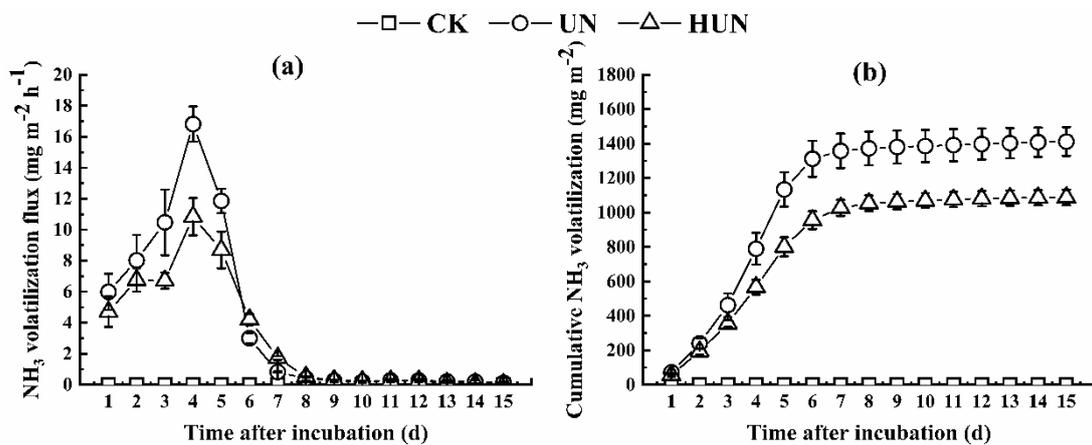


Figure 3. Effects of different treatments on NH₃ volatilization flux (a) and cumulative ammonia volatilization (b). The vertical coordinate corresponding to the point is the average value. The length of the error bars is the standard deviation. NH₃: ammonia, CK: no nitrogen, UN: urea at 240 kg N hm⁻², HUN: humic acid urea at 240 kg N hm⁻².

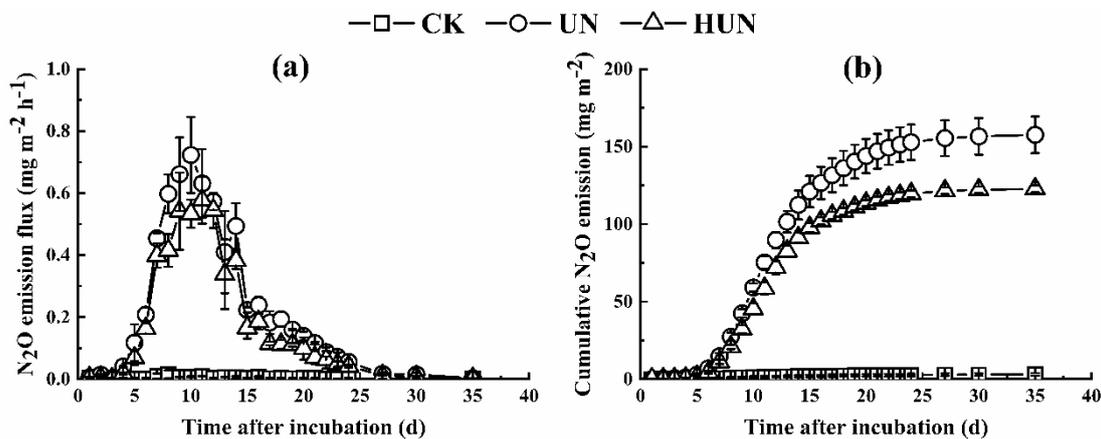


Figure 4. Effects of different treatments on N₂O emission flux (a) and cumulative N₂O emission (b). The vertical coordinate corresponding to the point is the average value. The length of the error bars is the standard deviation. N₂O: nitrous oxide, CK: no nitrogen, UN: urea at 240 kg N hm⁻², HUN: humic acid urea at 240 kg N hm⁻².

Table 1. The effects of different treatments on nitrogen loss in nitrogen fertilizer.

Treatment	N Loss Pathway			Total N Loss (%)
	IN Leaching (%)	NH ₃ Volatilization (%)	N ₂ O Emission (%)	
UN	4.90 ± 2.20a	7.11 ± 0.59a	0.78 ± 0.06a	12.80 ± 2.26a
HUN	3.65 ± 1.39a	5.47 ± 0.31b	0.60 ± 0.01b	10.02 ± 1.41a

Note: Data are expressed as mean ± standard deviation. Different letters indicate significant differences between different treatments at $p < 0.05$. IN: inorganic nitrogen. UN: urea, 240 kg N hm⁻². HUN: humic acid urea, 240 kg N hm⁻².

3.3. Effect of Humic Acid Urea on Soil Nutrient Content in the Field Experiment of Maize-Wheat Rotation

SOC content marginally varied with urea application during wheat growth, while SOC content with humic acid urea application showed an increasing trend from the jointing to booting stage and then leveled off (Figure 5). On the whole, humic acid urea had almost no effect on SOC content compared to urea. The soil NH₄⁺-N content of all treatments showed a decreasing trend at the wheat jointing stage, then reached the lowest at the wheat booting stage, and finally gradually increased and leveled off. Compared with the UN, HUN and HUN1 could increase the soil NH₄⁺-N content by 27.26% and 5.41% on average,

respectively. Soil NO_3^- -N content increased with wheat growth in all treatments, and HUN and HUN1 increased soil NO_3^- -N content compared with UN, with an average increase of 17.51% and 2.83%, respectively. Meanwhile, the increase in soil NO_3^- -N content by application of humic acid urea compared with urea was mainly in 0–20 and 20–40 cm soil layers, while the difference was smaller in the 40–60 cm soil layers. The AP content of all treatments showed a trend of decreasing, increasing and then decreasing with wheat growth in the 0–20 and 40–60 cm soil layers. The HUN, HUN1, HUN2 and HUN3 increased soil AP content by an average of 24.68%, 12.50%, 3.35% and 16.57%, respectively, compared to UN.

3.4. Effect of Humic Acid Urea on Soil Enzyme Activity and Enzyme Stoichiometry in the Field Experiment of Maize-Wheat Rotation

Humic acid urea application inhibited βG enzyme activity (Figure 6a). βG enzyme activity of HUN, HUN1, HUN2 and HUN3 was reduced by an average of 24.04%, 10.58%, 31.32% and 16.11%, respectively, compared to UN. Humic acid urea increased CBH enzyme activity and it increased with the amount of humic acid urea applied (Figure 6b). Compared to UN, the CBH enzyme activities of HUN and HUN1 increased by 32.24% and 13.81% on average, respectively. The NAG enzyme activity of all treatments showed a decrease at the wheat booting stage, and then increased and leveled off (Figure 6c). On the whole, the lower the amount of humic acid urea applied, the greater the increase in NAG enzyme activity. Compared to UN, NAG enzyme activity decreased by 5.09% in HUN, while NAG enzyme activity increased by 0.66%, 17.34% and 31.65% in HUN1, HUN2 and HUN3, respectively. HUN and HUN1 increased ALP enzyme activity compared to UN by an average of 13.23% and 15.62%, respectively (Figure 6d).

The enzyme C: N and C: P ratios of all treatments were greater than 1, suggesting that soil microorganisms were more limited by N and P compared to C (Figure 7). Compared with UN, the enzyme C: N ratios of HUN, HUN1, HUN2 and HUN3 were reduced by 1.58%, 0.91%, 2.79% and 4.50% on average, respectively, and their enzyme C: P ratios were reduced by 4.11%, 2.02%, 1.41% and 1.73% on average, respectively. The enzyme N: P ratios of all treatments were less than 1, indicating that soil microorganisms were more P-limited than N. The HUN2 and HUN3 increased the enzyme N: P ratio by an average of 1.82% and 3.14%, respectively, compared to the UN, showing that a small amount of humic acid urea application could alleviate microbial P limitation.

3.5. Effect of Humic Acid Urea on Yield, NUE and NEP in the Field Experiment of Maize-Wheat Rotation

Maize yield of HUN1 and wheat yield of both HUN and HUN1 were significantly higher than that of UN ($p < 0.05$) (Table 2). Compared to UN, HUN, HUN1 and HUN2 increased maize yield by 7.94%, 14.23% and 4.86% and wheat yield by 33.08%, 15.57% and 9.69%, respectively. Compared with UN, the NUE of maize in HUN and HUN1 increased by 18.11% and 35.33%, respectively, and that of wheat in HUN, HUN1, HUN2 and HUN3 increased by 60.55%, 77.51%, 25.21% and 24.77%, respectively. Despite the higher price of humic acid urea than urea, the application of humic acid urea still increased the NEP of maize-wheat rotation (Table 3). HUN, HUN1 and HUN2 increased the NEP by 8.36%, 16.53% and 5.92% at the maize stage and 38.35%, 18.06% and 11.66% at the wheat stage, respectively, compared with the UN. Among the treatments, HUN1 had the highest NUE and increased yield and NEP compared to UN.

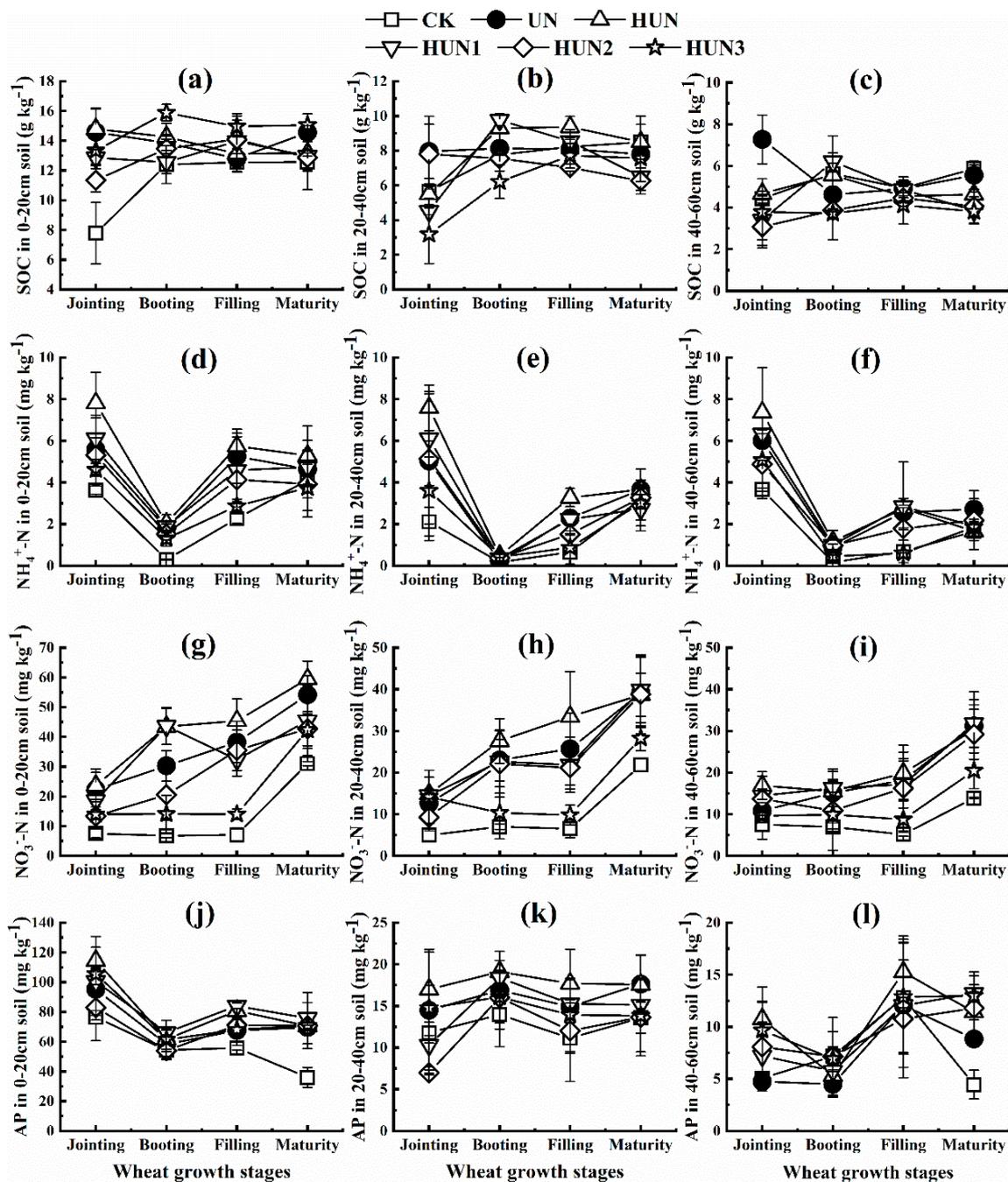


Figure 5. Effects of different treatments on soil nutrient content in wheat growth stages. The SOC contents of 0–20, 20–40 and 40–60 cm were shown as (a–c), respectively. The NH₄⁺-N contents of 0–20, 20–40 and 40–60 cm were shown as (d–f), respectively. The NO₃⁻-N contents of 0–20, 20–40 and 40–60 cm were shown as (g–i), respectively. The AP contents of 0–20, 20–40 and 40–60 cm were shown as (j–l), respectively. The vertical coordinate corresponding to the point is the average value. The length of the error bars is the standard deviation. SOC: soil organic carbon. NH₄⁺-N: ammonium nitrogen. NO₃⁻-N: nitrate nitrogen. AP: available phosphorus. CK: no nitrogen. UN: urea, 240 kg N hm⁻². HUN: humic acid urea, 240 kg N hm⁻². HUN1: humic acid urea, 216 kg N hm⁻². HUN2: humic acid urea, 192 kg N hm⁻². HUN3: humic acid urea, 168 kg N hm⁻².

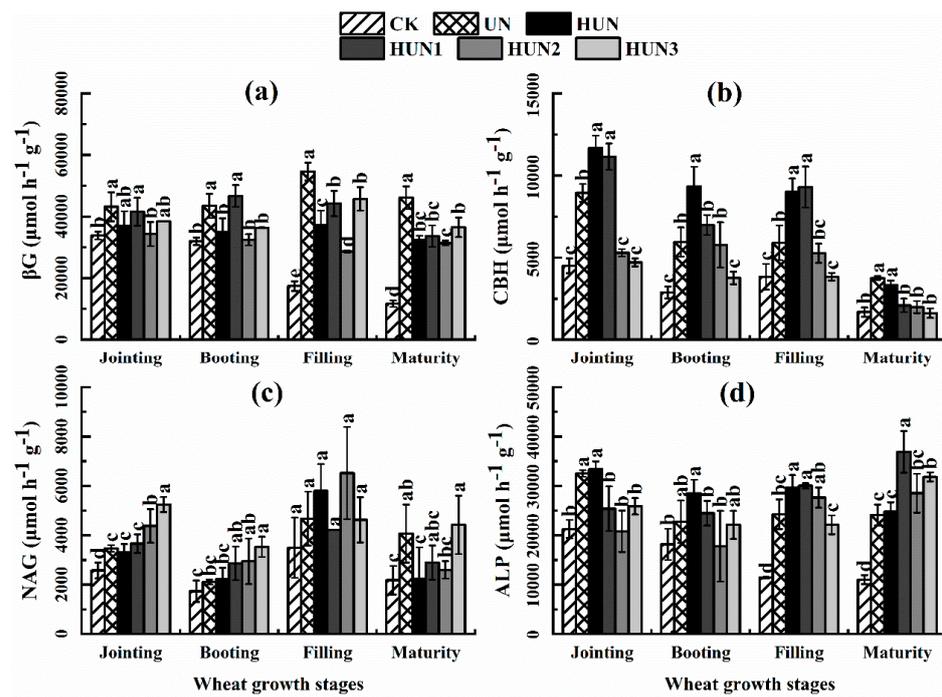


Figure 6. Effects of different treatments on βG (a), CBH (b), NAG (c) and ALP (d) in wheat growth stages. The lengths of the bars are average values. The length of the error bars is the standard deviation. Different letters indicate significant differences between different treatments at $p < 0.05$ for the same wheat growth stages. βG : β -1,4-glucosidase. CBH: β -D-1,4-cellobiosidase. NAG: β -1,4-N-acetyl-glucosaminidase. ALP: alkaline phosphatase. CK: no nitrogen. UN: urea, 240 kg N hm^{-2} . HUN: humic acid urea, 240 kg N hm^{-2} . HUN1: humic acid urea, 216 kg N hm^{-2} . HUN2: humic acid urea, 192 kg N hm^{-2} . HUN3: humic acid urea, 168 kg N hm^{-2} .

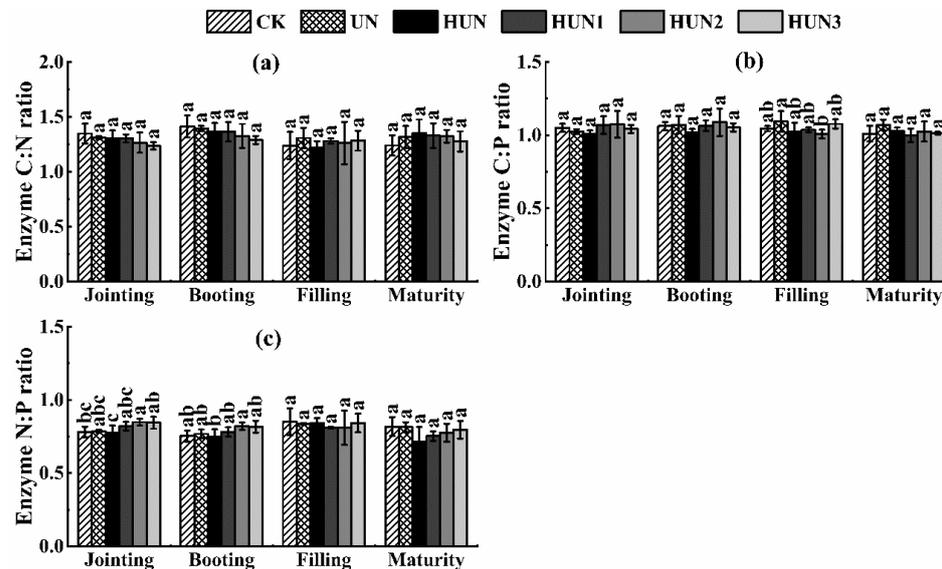


Figure 7. Effects of different treatments on enzyme C:N ratio (a), enzyme C:P ratio (b) and enzyme N:P ratio (c) in wheat growth stages. The lengths of the bars are average values. The length of the error bars is the standard deviation. Different letters indicate significant differences between different treatments at $p < 0.05$ for the same wheat growth stages. C: carbon. N: nitrogen. P: phosphorus. CK: no nitrogen. UN: urea, 240 kg N hm^{-2} . HUN: humic acid urea, 240 kg N hm^{-2} . HUN1: humic acid urea, 216 kg N hm^{-2} . HUN2: humic acid urea, 192 kg N hm^{-2} . HUN3: humic acid urea, 168 kg N hm^{-2} .

Table 2. The effects of different treatments on yield, nitrogen accumulation and NUE in maize-wheat rotation.

Treatment	Maize			Wheat		
	Yield (kg·hm ⁻²)	N Accumulation (kg·hm ⁻²)	NUE (%)	Yield (kg·hm ⁻²)	N Accumulation (kg·hm ⁻²)	NUE (%)
CK	5355.85 ± 266.43c	112.93 ± 12.18d		7612.61 ± 470.30d	68.57 ± 3.74c	
UN	7465.38 ± 236.77b	195.89 ± 13.04a	34.56 ± 5.43bc	8078.30 ± 517.01cd	129.40 ± 9.62b	25.35 ± 4.01c
HUN	8058.45 ± 228.73ab	210.91 ± 3.19a	40.82 ± 1.33ab	10,750.33 ± 504.24a	166.25 ± 16.80a	40.70 ± 7.00ab
HUN1	8527.90 ± 59.05a	213.96 ± 15.67a	46.77 ± 7.25a	9335.90 ± 275.83b	165.76 ± 6.56a	45.00 ± 3.04a
HUN2	7828.41 ± 442.62ab	175.01 ± 6.08b	32.33 ± 3.17c	8860.89 ± 727.78bc	129.51 ± 16.75b	31.74 ± 8.72bc
HUN3	7381.10 ± 762.17b	140.44 ± 2.46c	16.37 ± 1.46d	8619.59 ± 370.33bc	121.70 ± 7.89b	31.63 ± 4.70bc

Note: Data are expressed as mean ± standard deviation. Different letters indicate a significant difference at $p < 0.05$ between different treatments. NUE: nitrogen use efficiency. CK: no nitrogen. UN: urea, 240 kg N hm⁻². HUN: humic acid urea, 240 kg N hm⁻². HUN1: humic acid urea, 216 kg N hm⁻². HUN2: humic acid urea, 192 kg N hm⁻². HUN3: humic acid urea, 168 kg N hm⁻².

Table 3. The effects of different treatments on TEP and NEP in maize-wheat rotation.

Treatment	Maize		Wheat	
	TEP (\$ hm ⁻²)	NEP (\$ hm ⁻²)	TEP (\$ hm ⁻²)	NEP (\$ hm ⁻²)
CK	2058.79 ± 102.42c	1704.3 ± 102.42c	2907.41 ± 359.24b	2536.83 ± 359.24b
UN	2869.69 ± 91.01b	2392.08 ± 91.01b	3085.26 ± 394.91b	2583.35 ± 394.91b
HUN	3097.67 ± 87.92ab	2592.01 ± 87.92ab	4105.77 ± 385.16a	3573.94 ± 385.16a
HUN1	3278.12 ± 22.7a	2787.58 ± 22.7a	3565.57 ± 210.69ab	3049.86 ± 210.69ab
HUN2	3009.24 ± 170.14ab	2533.81 ± 170.14ab	3384.15 ± 555.91b	2884.57 ± 555.91ab
HUN3	2837.29 ± 292.98b	2376.98 ± 292.98b	3291.99 ± 282.87b	2808.54 ± 282.87b

Note: Data are expressed as mean ± standard deviation. Different letters indicate a significant difference at $p < 0.05$ between different treatments. Average market prices in China: maize: 384.40 \$ t⁻¹; wheat: 381.92 \$ t⁻¹; urea: 251.72 \$ t⁻¹; humic acid urea: 309.07 \$ t⁻¹; double superphosphate: 157.33 \$ t⁻¹; potassium sulfate: 356.50 \$ t⁻¹; other costs included labor expenditure and seed expenditure: 263.50 \$ hm⁻². TEP: annual total economic profit. NEP: annual net economic profit. CK: no nitrogen. UN: urea, 240 kg N hm⁻². HUN: humic acid urea, 240 kg N hm⁻². HUN1: humic acid urea, 216 kg N hm⁻². HUN2: humic acid urea, 192 kg N hm⁻². HUN3: humic acid urea, 168 kg N hm⁻².

4. Discussion

4.1. Effect of Humic Acid Urea on Nitrogen Loss

The activity of humic acid is mainly determined by the number of its carboxyl and hydroxyl functional groups [35]. Zhang et al. [22] showed that humic acid can generate stable complexes with urea and provide more hydroxyl functional groups. This enables the humic acid in humic acid urea to still function in its role in promoting plant growth, prolonging fertilizer effectiveness and reducing nitrogen losses. Meanwhile, Shen et al. [21] found that humic acid combines with amino, carboxyl and hydroxyl groups of urea, reducing the rate of N hydrolysis in urea. Since humic acid urea possesses a more sustained N release process, this enables the soil NH₄⁺-N content of the humic acid urea treatment to not increase as rapidly as the urea treatment, leading to a weakening of nitrification and denitrification, and a reduction in N loss [36,37]. Liu et al. [38] investigated that functional groups in humic acid can interact with the thiol group of urease to form complexes, thus inhibiting the activity of urease, which further slows down the rate of N conversion in the soil. Therefore, humic acid urea prolongs fertilizer efficiency and reduces N loss mainly by slowing down the rate of urea hydrolysis and the rate of N fertilizer conversion in the soil. Studies have shown that humic acid addition can promote the formation of soil macroaggregates, improve soil physical structure and enhance soil water holding capacity [15]. This also enables humic acid urea to reduce the risk of soil NH₄⁺-N and NO₃⁻-N leaching. NH₃ volatilization usually accounts for 10%–60% of total N losses in agriculture and is the main pathway for fertilizer N losses [39]. This is consistent with the results of this study. NH₃ volatilization accounted for 55.55% and 54.59% of total fertilizer N losses for UN and HUN, respectively. Meanwhile, compared with UN, HUN reduced N losses by 25.51% and 23.08% in the two N loss pathways of IN leaching and

N₂O emission, respectively. This indicates that humic acid urea can effectively reduce N losses and environmental pollution.

4.2. Effect of Humic Acid Urea on Soil Nutrients and Enzyme Stoichiometry

Mitigation of soil microbial nutrient limitation is essential to improve soil quality. Enzyme stoichiometry are sensitive probes used to study microbial nutrient limitations and is significantly influenced by soil nutrient effectiveness [40]. Numerous studies have shown that humic acid can increase soil macroaggregates and SOC content [41,42]. However, the application of humic acid urea in this study did not significantly increase soil SOC. This may be because humic acid stimulated root growth and triggered the enrichment of microorganisms, which increased microbial utilization of SOC [13,43]. In this study, the enzyme C:N and C:P ratios in all treatments were greater than 1, and these in humic acid urea treatment were closer to 1. According to the view that microorganisms optimize resource allocation to obtain the most limited resources, the greater the microbial investment in an enzyme, the greater the demand for that nutrient, which globally converges the enzyme stoichiometry ratio to 1:1:1 [32,44]. This suggests that humic acid urea application alleviates microbial C limitation and increases microbial utilization of SOC. Meanwhile, humic acid urea increased CBH enzyme activity and decreased β G enzyme activity. This indicates that humic acid urea makes C limitation of soil fungi greater than that of bacteria [45].

In our study, humic acid urea could increase soil NH₄⁺-N and NO₃⁻-N content during wheat growth by inhibiting urea hydrolysis and reducing nitrogen loss, which is consistent with other research results [15]. Meanwhile, application of humic acid urea reduced microbial investment in NAG and brought the enzyme stoichiometry ratio for wheat growth closer to 1:1:1, thus promoting microbial nutrient balance [46]. Dong et al. [47] showed that humic acid did not directly affect the number of soil nitrobacteria, but caused changes in the number of soil nitrobacteria by affecting the rate of urea hydrolysis to NH₄⁺-N. In this study, soil NO₃⁻-N content gradually increased with wheat growth. This indicates that humic acid urea itself does not inhibit soil nitrification. Humic acid urea only reduced the soil NH₄⁺-N content, leading to a weakening of potential nitrification, which reduced the soil NO₃⁻-N content [48].

Humic acid urea promoted microbial nutrient balance also in the way of alleviating P limitation. In this study, humic acid urea treatment increased the ALP enzyme activity and AP content, and brought the enzyme N: P ratio closer to 1. This is due to a large number of oxygen-containing functional groups in humic acid urea [21] that can compete with soil P for sorption sites, leading to an increase in AP content [49]. Meanwhile, Li et al. [50] showed that humic acid had a positive effect on P-solubilizing microbial community and ALP enzyme activity. Therefore, humic acid urea is beneficial to enhance soil nutrients, promote microbial nutrient balance, and improve soil quality in maize and wheat growth.

4.3. Effect of Humic Acid Urea on Yield and NUE

In this study, HUN and HUN1 increased the yield and NUE of maize and wheat, which is consistent with the results of other researchers [22]. This is mainly due to three aspects. First, humic acid urea reduced N losses and increased soil NH₄⁺-N and NO₃⁻-N content to fulfill the N requirements of wheat and maize. Secondly, humic acid urea increased ALP enzyme activity and soil P effectiveness. Studies have shown that humic acid makes soil P in an exchangeable state, effectively preventing P from binding to metal elements, which makes it difficult to be used by crops [18]. This significantly improved the yield and quality of wheat and maize [51]. Finally, humic acid urea could slow down urea hydrolysis and prolong N supply. Ma et al. [52] showed that the key to improving NUE in crops is to extend the nitrogen fertilizer effectiveness, so that the rate of N release from the fertilizer is at the same rate as the plant N requirement.

The positive effect of humic acid urea on yield and NUE in maize and wheat was attributed not only to its improved soil nutrient supply, but also to its promotion of plant growth and nutrient uptake efficiency. Studies have shown that humic acid has a positive

effect on crop chlorophyll content and chloroplast ultrastructure, which improves crop photosynthetic efficiency and shows an obvious dose-effect [53]. Meanwhile, humic acid can be a eustressor that triggers metabolic changes in crops, inducing stimulation of crop root growth and development [54]. Liu et al. [23] found that humic acid promotes nutrient acquisition by maize roots and increases maize yield. Thus, HUN and HUN1 increased the yield and NUE of maize and wheat, which is the positive result of the combined effect on soil nutrient supply and plant nutrient acquisition.

Overall, our results showed that humic acid urea could effectively reduce N losses from all pathways, decrease the environmental pollution caused by N application, promote soil microbial nutrient balance and improve the yield and NUE of maize and wheat. We determined that humic acid urea at 216 kg N hm⁻² level could maximize NUE and NEP under the condition of improving crop yield.

5. Conclusions

In this study, humic acid urea reduced fertilizer N losses in three pathways of N leaching, NH₃ volatilization and N₂O emission by 25.51%, 23.07% and 23.08%, respectively, compared to urea. This mitigated the environmental impact of agricultural N application. Humic acid urea increased the effectiveness of soil N and P, and brought the enzyme stoichiometry closer to 1:1:1. This alleviated soil microbial nutrient limitation and improved soil quality. Application of humic acid urea increased the yield, NUE and NEP of maize and wheat due to reduced N losses and prolonged fertilization effect. Among them, HUN1 increased the yield of maize and wheat by 14.23% and 15.57%, improved NUE by 35.33% and 77.51%, and increased NEP by 16.53% and 18.06%, respectively, compared to UN. Therefore, we recommend the application of humic acid urea at 216 kg N hm⁻² in the Typic Hapludalf soil to maximize NUE, reduce environmental pollution and improve yield and NEP. In the future, more soil types and more detailed soil microbial studies should be applied to study the effects of humic acid urea on NUE and environmental benefits.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture12121996/s1>, agriculture-2040875-supplementary.

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References

1. Li, R.C.; Gao, Y.X.; Chen, Q.; Li, Z.L.; Gao, F.; Meng, Q.M.; Li, T.G.; Liu, A.R.; Wang, Q.; Wu, L.; et al. Blended controlled-release nitrogen fertilizer with straw returning improved soil nitrogen availability, soil microbial community, and root morphology of wheat. *Soil Tillage Res.* **2021**, *212*, 105045. [[CrossRef](#)]
2. Gao, Y.X.; Song, X.; Liu, K.X.; Li, T.G.; Zheng, W.K.; Wang, Y.; Liu, Z.G.; Zhang, M.; Chen, Q.; Li, Z.L.; et al. Mixture of controlled-release and conventional urea fertilizer application changed soil aggregate stability, soil humic acid molecular composition, and nitrogen uptake. *Sci. Total Environ.* **2021**, *789*, 147778. [[CrossRef](#)] [[PubMed](#)]

3. Ju, X.T.; Xing, G.X.; Chen, X.P.; Zhang, S.L.; Zhang, L.J.; Liu, X.J.; Cui, Z.L.; Yin, B.; Christie, P.; Zhu, Z.L.; et al. Reducing environmental risk by improving N management in intensive Chinese agricultural systems. *PNAS* **2009**, *106*, 3041–3046. [[CrossRef](#)]
4. Saha, B.K.; Rose, M.T.; Wong, V.N.L.; Cavagnaro, T.R.; Patti, A.F.; Patti, A.F. A slow release brown coal-urea fertiliser reduced gaseous N loss from soil and increased silver beet yield and N uptake. *Sci. Total Environ.* **2019**, *649*, 793–800. [[CrossRef](#)] [[PubMed](#)]
5. UNECE. 2018. Available online: <https://www.unece.org/env/lrtap/taskforce/tfrn/welcome.html> (accessed on 15 October 2019).
6. Wang, Y.L.; Li, M.N.; Yan, H.J. Ammonia volatilization from urea in alfalfa field with different nitrogen application rates, methods and timing. *Agric. Ecosyst. Environ.* **2021**, *312*, 107344. [[CrossRef](#)]
7. Wang, H.T.; Oertelt, L.; Dittert, K. The addition of magnesium sulfate and borax to urea reduced soil NH₃ emissions but increased N₂O emissions from soil with grass. *Sci. Total Environ.* **2022**, *803*, 149902. [[CrossRef](#)]
8. Meng, X.P.; Guo, Z.Y.; Yang, X.N.; Su, W.N.; Li, Z.M.; Wu, X.R.; Ahmad, I.; Cai, T.; Han, Q.F. Straw incorporation helps inhibit nitrogen leaching in maize season to increase yield and efficiency in the Loess Plateau of China. *Soil Tillage Res.* **2021**, *211*, 105006. [[CrossRef](#)]
9. Wu, P.; Liu, F.; Li, H.; Cai, T.; Zhang, P.; Jia, Z.K. Suitable fertilizer application depth can increase nitrogen use efficiency and maize yield by reducing gaseous nitrogen losses. *Sci. Total Environ.* **2021**, *781*, 146787. [[CrossRef](#)]
10. Kira, O.; Shaviv, A.; Dubowski, Y. Concomitant tracking of NH₃, N₂O and soil mineral-N using steady-state incubation cells to enhance sustainability of urea fertilization approaches. *Geoderma* **2021**, *404*, 115305. [[CrossRef](#)]
11. Wang, H.T.; Ma, S.T.; Shao, G.D.; Dittert, K. Use of urease and nitrification inhibitors to decrease yield-scaled N₂O emissions from winter wheat and oilseed rape fields: A two-year field experiment. *Agric. Ecosyst. Environ.* **2021**, *319*, 107552. [[CrossRef](#)]
12. Araújo, B.R.; Romão, L.P.C.; Doumer, M.E.; Mangrich, A.S. Evaluation of the interactions between chitosan and humics in media for the controlled release of nitrogen fertilizer. *J. Environ. Manag.* **2017**, *190*, 122–131. [[CrossRef](#)] [[PubMed](#)]
13. Silva, M.S.R.D.A.D.; Tavares, O.C.H.; Ribeiro, T.G.; Baldani, V.L.D.; Berbara, R.L.L.; Jesus, E.D.C. Humic acids enrich the plant microbiota with bacterial candidates for the suppression of pathogens. *Appl. Soil Ecol.* **2021**, *168*, 104146. [[CrossRef](#)]
14. Mora, V.; Bacaicoa, E.; Zamarreño, A.M.; Aguirre, E.; Garnica, M.; Fuentes, M. Action of humic acid on promotion of cucumber shoot growth involves nitrate-related changes associated with the root-to-shoot distribution of cytokinins, polyamines and mineral nutrients. *J. Plant Physiol.* **2010**, *167*, 633–642. [[CrossRef](#)]
15. Liu, M.L.; Wang, C.; Liu, X.L.; Lu, Y.C.; Wang, Y.F. Saline-alkali soil applied with vermicompost and humic acid fertilizer improved macroaggregate microstructure to enhance salt leaching and inhibit nitrogen losses. *Appl. Soil Ecol.* **2020**, *156*, 103705. [[CrossRef](#)]
16. Dong, L.H.; Yuan, Q.; Yuan, L.H. Changes of chemical properties of humic acids from crude and fungal transformed lignite. *Fuel* **2006**, *85*, 2402–2407. [[CrossRef](#)]
17. Deng, A.N.; Wu, X.F.; Su, C.L.; Zhao, M.; Wu, B.; Luo, J.H. Enhancement of soil microstructural stability and alleviation of aluminium toxicity in acidic latosols via alkaline humic acid fertiliser amendment. *Chem. Geol.* **2021**, *583*, 120473. [[CrossRef](#)]
18. Yang, F.; Sui, L.; Tang, C.Y.; Li, J.S.; Cheng, K.; Xue, Q. Sustainable advances on phosphorus utilization in soil via addition of biochar and humic substances. *Sci. Total Environ.* **2021**, *768*, 145106. [[CrossRef](#)]
19. Walling, E.; Vaneekhaute, C. Greenhouse gas emissions from inorganic and organic fertilizer production and use: A review of emission factors and their variability. *J. Environ. Manag.* **2020**, *276*, 111211. [[CrossRef](#)]
20. Zhao, J.; Ni, T.; Li, J.; Lu, Q.; Fang, Z.Y.; Huang, Q.W.; Zhang, R.F.; Li, R.; Shen, B.; Shen, Q.R. Effects of organic–inorganic compound fertilizer with reduced chemical fertilizer application on crop yields, soil biological activity and bacterial community structure in a rice–wheat cropping system. *Appl. Soil Ecol.* **2016**, *99*, 1–12. [[CrossRef](#)]
21. Shen, Y.W.; Lin, H.T.; Gao, W.S.; Li, M.L. The effects of humic acid urea and polyaspartic acid urea on reducing nitrogen loss compared with urea. *J. Sci. Food Agric.* **2020**, *100*, 4425–4432. [[CrossRef](#)]
22. Zhang, S.Q.; Yuan, L.; Li, W.; Lin, Z.A.; Li, Y.T.; Hu, S.W.; Zhao, B.Q. Effects of urea enhanced with different weathered coal-derived humic acid components on maize yield and fate of fertilizer nitrogen. *J. Integr. Agric.* **2019**, *18*, 656–666. [[CrossRef](#)]
23. Liu, M.L.; Wang, C.; Wang, F.Y.; Xie, Y.J. Maize (*Zea mays*) growth and nutrient uptake following integrated improvement of vermicompost and humic acid fertilizer on coastal saline soil. *Appl. Soil Ecol.* **2019**, *142*, 147–154. [[CrossRef](#)]
24. Staff, S.S. *Keys to Soil Taxonomy*, 12th ed.; Government Printing Office: Washington, DC, USA, 2014.
25. Zhang, C.; Huang, X.; Zhang, X.W.; Wan, L.; Wang, Z.H. Effects of biochar application on soil nitrogen and phosphorous leaching loss and oil peony growth. *Agric. Water Manag.* **2021**, *255*, 107022. [[CrossRef](#)]
26. Long, G.Q.; Li, L.H.; Wang, D.; Zhao, P.; Tang, L.; Zhou, Y.L.; Yin, X.H. Nitrogen levels regulate intercropping-related mitigation of potential nitrate leaching. *Agric. Ecosyst. Environ.* **2021**, *319*, 107540. [[CrossRef](#)]
27. Xiao, Q.Y.; Dong, Z.X.; Han, Y.; Hu, L.; Hu, D.N.; Zhu, B. Impact of soil thickness on productivity and nitrate leaching from sloping cropland in the upper Yangtze River Basin. *Agric. Ecosyst. Environ.* **2021**, *311*, 107266. [[CrossRef](#)]
28. Zhu, H.; Yang, J.S.; Yao, R.J.; Wang, X.P.; Xie, W.P.; Zhu, W.; Liu, X.Y.; Cao, Y.F.; Tao, J.Y. Interactive effects of soil amendments (biochar and gypsum) and salinity on ammonia volatilization in coastal saline soil. *Catena* **2020**, *190*, 104527. [[CrossRef](#)]
29. Sá, M.M.F.; Schaefer, C.E.G.R.; Loureiro, D.C.; Simas, F.N.B.; Alves, B.J.R.; Mendonça, E.D.S.; Figueiredo, E.B.D.; Panosso, A.R. Fluxes of CO₂, CH₄, and N₂O in tundra-covered and *Nothofagus* forest soils in the Argentinian Patagonia. *Sci. Total Environ.* **2019**, *659*, 401–409. [[CrossRef](#)]
30. Zhang, Y.; Liu, D.H.; Jia, Z.K.; Zhang, P. Ridge and furrow rainfall harvesting can significantly reduce N₂O emissions from spring maize fields in semiarid regions of China. *Soil Tillage Res.* **2021**, *209*, 104971. [[CrossRef](#)]

31. Deforest, J.L. The influence of time, storage temperature, and substrate age on potential soil enzyme activity in acidic forest soils using MUB-linked substrates and l-DOPA. *Soil Biol. Biochem.* **2009**, *41*, 1180–1186. [[CrossRef](#)]
32. Bai, X.J.; Dippold, M.A.; An, S.S.; Wang, B.R.; Zhang, H.X.; Loeppmann, S. Extracellular enzyme activity and stoichiometry: The effect of soil microbial element limitation during leaf litter decomposition. *Ecol. Indic.* **2021**, *121*, 107200. [[CrossRef](#)]
33. Zhang, W.; Xu, Y.D.; Gao, D.X.; Wang, X.; Liu, W.C.; Deng, J.; Han, X.H.; Yang, G.H.; Feng, Y.Z.; Ren, G.X. Ecoenzymatic stoichiometry and nutrient dynamics along a revegetation chronosequence in the soils of abandoned land and Robinia pseudoacacia plantation on the Loess Plateau, China. *Soil Biol. Biochem.* **2019**, *134*, 1–14. [[CrossRef](#)]
34. Li, Z.L.; Liu, Z.G.; Zhang, M.; Chen, Q.; Zheng, L.; Li, Y.C.; Sun, L.L. The combined application of controlled-release urea and fulvic acid improved the soil nutrient supply and maize yield. *Arch. Agron. Soil Sci.* **2020**, *67*, 633–646. [[CrossRef](#)]
35. Lu, M.M.; Zhang, Y.B.; Su, Z.J.; Jiang, T. The NMR and spectral study on the structure of molecular size-fractionated lignite humic acid. *Resour. Environ. Sustain.* **2020**, *2*, 100004. [[CrossRef](#)]
36. Guo, J.J.; Fan, J.L.; Zhang, F.C.; Yan, S.C.; Zhang, J.; Wu, Y.; Li, J.; Wang, Y.L.; Sun, X.; Liu, X.Q.; et al. Blending urea and slow-release nitrogen fertilizer increases dryland maize yield and nitrogen use efficiency while mitigating ammonia volatilization. *Sci. Total Environ.* **2021**, *790*, 148058. [[CrossRef](#)] [[PubMed](#)]
37. Yam, K.G.; Upendra, S.; Wendie, D.B.; Job, F.J.; Joaquin, S. Mitigating N₂O and NO Emissions from Direct-Seeded Rice with Nitrification Inhibitor and Urea Deep Placement. *Rice Sci.* **2020**, *27*, 434–444. [[CrossRef](#)]
38. Liu, X.; Zhang, M.; Li, Z.W.; Zhang, C.; Wan, C.L.; Zhang, Y.; Lee, D.J. Inhibition of urease activity by humic acid extracted from sludge fermentation liquid. *Bioresour. Technol.* **2019**, *290*, 121767. [[CrossRef](#)]
39. Sun, X.; Zhong, T.; Zhang, L.; Zhang, K.S.; Wu, W.X. Reducing ammonia volatilization from paddy field with rice straw derived biochar. *Sci. Total Environ.* **2019**, *660*, 512–518. [[CrossRef](#)]
40. Yang, Y.; Liang, C.; Wang, Y.Q.; Cheng, H.; An, S.S.; Chang, S.X. Soil extracellular enzyme stoichiometry reflects the shift from P- to N-limitation of microorganisms with grassland restoration. *Soil Biol. Biochem.* **2020**, *149*, 107928. [[CrossRef](#)]
41. Zhou, L.; Monreal, C.M.; Xu, S.T.; McLaughlin, N.B.; Zhang, H.Y.; Hao, G.C.; Liu, J.H. Effect of bentonite-humic acid application on the improvement of soil structure and maize yield in a sandy soil of a semi-arid region. *Geoderma* **2019**, *338*, 269–280. [[CrossRef](#)]
42. Wu, S.S.; Liu, C.S.; Li, X.G.; Xiao, B.H.; Hu, Q.Y. Freeze-thaw controlled aggregation mechanism of humic acid-coated goethite: Implications for organic carbon preservation. *Geoderma* **2021**, *406*, 115514. [[CrossRef](#)]
43. Bhattacharyya, R.; Rabbi, S.M.F.; Zhang, Y.Q.; Young, I.M.; Jones, A.R.; Dennis, P.G.; Menzies, N.W.; Kopittke, P.M.; Dalal, R.C. Soil organic carbon is significantly associated with the pore geometry, microbial diversity and enzyme activity of the macro-aggregates under different land uses. *Sci. Total Environ.* **2021**, *778*, 146286. [[CrossRef](#)] [[PubMed](#)]
44. Sinsabaugh, R.L.; Lauber, C.L.; Weintraub, M.N.; Ahmed, B.; Zeglin, L.H. Stoichiometry of soil enzyme activity at global scale. *Ecol. Lett.* **2008**, *11*, 1252–1264. [[CrossRef](#)] [[PubMed](#)]
45. Saiya-Cork, K.R.; Sinsabaugh, R.L.; Zak, D.R. The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. *Soil Biol. Biochem.* **2002**, *34*, 1309–1315. [[CrossRef](#)]
46. Xiao, L.; Liu, G.B.; Li, P.; Li, Q.; Xue, S. Ecoenzymatic stoichiometry and microbial nutrient limitation during secondary succession of natural grassland on the Loess Plateau, China. *Soil Tillage Res.* **2020**, *200*, 104605. [[CrossRef](#)]
47. Dong, L.H.; Córdova-Kreylos, A.L.; Yang, J.S.; Yuan, H.L.; Scow, K.M. Humic acids buffer the effects of urea on soil ammonia oxidizers and potential nitrification. *Soil Biol. Biochem.* **2009**, *41*, 1612–1621. [[CrossRef](#)] [[PubMed](#)]
48. Leptin, A.; Whitehead, D.; Anderson, C.R.; Cameron, K.C.; Lehto, N.J. Increased soil nitrogen supply enhances root-derived available soil carbon leading to reduced potential nitrification activity. *Appl. Soil Ecol.* **2021**, *159*, 103842. [[CrossRef](#)]
49. Zhang, S.S.; Du, Q.; Cheng, K.; Antonietti, M.; Yang, F. Efficient Phosphorus Recycling and Heavy Metal Removal from Wastewater Sludge by a Novel Hydrothermal Humification-Technique. *Chem. Eng. J.* **2020**, *394*, 124832. [[CrossRef](#)]
50. Li, Y.; Fang, F.; Wei, J.L.; Wu, X.B.; Cui, R.Z.; Li, G.S.; Zheng, F.L.; Tan, D.S. Humic Acid Fertilizer Improved Soil Properties and Soil Microbial Diversity of Continuous Cropping Peanut: A Three-Year Experiment. *Sci. Rep.* **2019**, *9*, 12014. [[CrossRef](#)]
51. Zhang, Y.J.; Zhang, W.; Wu, M.; Liu, G.S.; Zhang, Z.J.; Yang, J.C. Effects of irrigation schedules and phosphorus fertilizer rates on grain yield and quality of upland rice and paddy rice. *Environ. Exp. Bot.* **2021**, *186*, 104465. [[CrossRef](#)]
52. Ma, Q.; Wang, M.Y.; Zheng, G.L.; Yao, Y.; Tao, R.R.; Zhu, M.; Ding, J.F.; Li, C.Y.; Guo, W.S.; Zhu, X.K. Twice-split application of controlled-release nitrogen fertilizer met the nitrogen demand of winter wheat. *Field Crops Res.* **2021**, *267*, 108163. [[CrossRef](#)]
53. Fan, H.M.; Wang, X.W.; Xia, S.; Li, Y.Y.; Sun, X.Z.; Zheng, C.S. Effects of humic acid derived from sediments on growth, photosynthesis and chloroplast ultrastructure in chrysanthemum. *Sci. Hortic.* **2014**, *177*, 118–123. [[CrossRef](#)]
54. Castro, T.A.V.T.D.; Barbara, R.L.L.; Tavares, O.C.H.T.; Mello, D.F.D.G.; Pereira, E.G.; Souza, C.D.C.B.D.; Espinosa, L.M. Humic Acids Induce a Eustress State Via Photosynthesis and Nitrogen Metabolism Leading to a Root Growth Improvement in Rice Plants. *Plant Physiol. Biochem.* **2021**, *162*, 171–184. [[CrossRef](#)] [[PubMed](#)]