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Layered-Strip Fertilization Improves Nitrogen Use Efficiency by Enhancing Absorption and Suppressing Loss of Urea Nitrogen

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Abstract: Appropriate deep application of fertilizer is the key basis for improving nitrogen use efficiency (NUE). However, the effects of different deep application methods and fertilizer types on nutrient migration, NUE and biomass in wheat season are unclear. Therefore, in this study, a barrel planting test with multilayer fertilization (¹⁵N labeled urea (U) and coated urea (CU)) was conducted in a long-term positioning trial of winter wheat in the North China Plain (NCP). We quantified the migration of fertilizer N (N_{diff}) in soil–plant–atmosphere and its effects on wheat biomass and NUE based on surface (U_{sur} , CU_{sur}), layered-strip (U_{str} , CU_{str}) and layered-mix fertilization (U_{mix} , CU_{mix}) of U and CU. Compared with surface fertilization, the concentration of mineral N in root zone (0–40 cm) was increased by U_{str} and U_{mix} (8.6–50.3%), and the concentration of ammonium N was decreased by CU_{str} and CU_{mix} (49.6–76.0%), but there was no change in the nitrate N. The biomass and total N absorption of wheat tissues (straw and root) were increased by 12.3–38.9% under U_{str} and CU_{str} . Meanwhile, the distribution of N_{diff} in the 0–10 cm soil was decreased under U_{str} and CU_{str} , but it was increased in the 10–30 cm soil, thereby promoting the absorption of N_{diff} in wheat tissues by 12.3–28.7%. The rates of absorption and loss of N_{diff} were the highest (57.6–58.5%) and the lowest (4.5%) under U_{str} and CU_{str} , respectively, compared with other treatments. Consequently, layered-strip fertilization optimized the migration and utilization of N_{diff} within the soil–plant–atmosphere system. This approach equalized distribution, enhanced absorption and minimized losses of N_{diff} , resulting in an increase in NUE by 9.6–16.7%. Under the same treatment, CU was more suitable for crop nutrient requirements than U, which was more conducive to the improvement of NUE. Our findings will provide a scientific basis for the precise directional fertilization of winter wheat in the NCP.

Keywords: deep fertilization; coated fertilizer; ¹⁵N label; nitrogen use efficiency; nitrogen cycling



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1. Introduction

Fertilizer nitrogen (N) is an essential supplementary nutrient for crop production, and N fertilizer contributes to 45–50% of food needs for the global population in the 21st century [1,2]. A global meta-analysis showed that N use efficiency (NUE) was generally maintained at 36–42%, and the loss of unused N would increase the risk of environmental pollution and threaten human health [3]. At present, the inappropriate management of fertilizer N (e.g., shallow fertilization and fast release) hinders the improvement of NUE and has gradually become the largest contributor of N sources in atmosphere and water [4]. Therefore, maximizing the NUE to achieve optimum crop growth and healthy nutrient turnover is the core objective of optimal management of fertilizer N [5]. Strategies for deep fertilization and slow-release nutrient, which could match the nutrient absorption rules in

crop and balance the nutrient distribution in soil, have become the key breakthrough fields to improve the NUE.

Selecting a suitable fertilization method is one of the key steps to achieve successful crop production and high NUE. Less than 2% of the inorganic N in soil is available for direct use by plants, prompting farmers to apply fertilizer N to meet plant N requirements [6,7]. Traditional fertilization patterns (single base fertilizer or a combination of base and top-dressing) generally lead to the nutrients being concentrated in the topsoil and can hardly match the nutrient demands of crops in the whole growth period (especially for deep-rooted crops) [8,9]. In addition, shallow fertilization results in the loss of fertilizer nutrients, such as the loss of more than 30% of the N from nitrate and amide nitrogenous fertilizers during crop growth [10]. Topdressing increases labor and time costs, deteriorates soil physical structure and hinders the normal growth of crops (damage root structure), thus affecting the nutrient absorption and final yield [11]. In recent years, an increasing number of studies have paid attention to the technology of deep fertilization (>10 cm) and preliminarily prove that deep fertilization has the advantages of balancing soil nutrients distribution, reducing nutrient loss, matching crop nutrient requirements and improving NUE [12–14]. However, full-chain quantitative studies on the migration of fertilizer N in soil–crop–atmosphere after the balanced application of fertilizers to different soil layers are still insufficient.

Selecting the type of fertilizer that matches the rule of crop nutrient absorption is a prerequisite for further promoting the NUE and reducing the loss of fertilizer N. Urea is commonly used in intensively seeded crops (e.g., wheat) because of its high N concentration, low process cost and wide application range [15,16]. Under traditional shallow fertilization, about 50–60% urea N is lost from soil through leaching (NO_3), volatilization (NH_3) and denitrification (N_2 and N_2O) because the rule of nutrient release from fertilizer does not match the rule of nutrient absorption of the crop [17,18]. Previous studies have shown that the NUE of the wheat season in China is 30–35%, much lower than the world average rate of 40–60% [19]. The application of new techniques such as slow and controlled release in fertilizer has proved to be an effective strategy for wheat production. Other studies have found that slow or controlled release technology can improve NUE and reduce volatilization or leaching losses by regulating the mode and period of nutrient release from fertilizer, and has an obvious yield promotion effect [20,21]. However, some studies have found that the application of slow or controlled release fertilizer did not achieve the expected effect, especially in winter wheat-growing areas with a large seasonal span. The slow-release fertilizer did not improve wheat yield and NUE, although N loss was reduced [22]. The combination of slow-release fertilizer and deep fertilization has the comprehensive advantages of balanced soil nutrient distribution, matching crop nutrient demands and reducing nutrient loss, which is in line with the development trend of 4R nutrient stewardship [23]. Therefore, it is vital to investigate the migration path and utilization efficiency of fertilizer N under the deep application of slow-release fertilizer.

The North China Plain (NCP) is an important wheat production base in China, accounting for 50% of the national planting area and nearly 70% of the total national production [24]. The growth and productivity of wheat are closely dependent on the application of N fertilizer. Studies have shown that wheat yield has increased at an annual rate of nearly 90 kg ha^{-1} in the past 50 years in the NCP [25]. Unfortunately, this rapid growth pattern is dependent on excess input of N fertilizer. For example, the annual habitual N application by farmers is more than 300 kg ha^{-1} , far exceeding the annual N demand of $150\text{--}180 \text{ kg ha}^{-1}$ in the wheat season [26]. In addition, fertilizer N will be quickly released and concentrated in the shallow layer (<10 cm) or even on the soil surface under shallow fertilization or topdressing, which is dislocated in time and space with crop nutrient requirements. These factors have led to obvious loss of N through leaching and volatilization (23% and 18%), as well as the low rate of NUE (27–35%) [26,27]. As a result, wheat system in the NCP is considered environmentally unfriendly and unsustainable. Moreover, the government has put forward the plan of zero increase in fertilizer from 2015, which has prompted researchers to develop new fertilization strategies to reduce fertilizer losses and improve the NUE.

Therefore, layered deep fertilization combined with slow-release technology is theoretically an effective means of nutrient optimization management in the NCP. Quantitative research on residue, absorption and loss of fertilizer N under layered deep fertilization combined with slow-release technology has an important meaning for precise nutrient management and is also of interest to us. Therefore, this study relied on a long-term positioning trial for typical winter wheat in the NCP to (a) quantify the migration and storage of fertilizer N in the soil–crop–atmosphere system under layered fertilization, and (b) reveal the effects of layered application of quick-release and slow-release fertilizers on NUE and yield. This study will provide technical support for the optimal management of deep fertilization and the matching supply of nutrients in the NCP or other similar agro-ecosystems.

2. Materials and Methods

2.1. Site Description and Material Preparation

The barrel planting experiment of wheat was conducted from October 2018 to June 2019 in Luancheng Agricultural Experimental Station of Chinese Academy of Sciences, China (37°53' N, 114°41' E). The station is located in the NCP, a typical warm temperate monsoon climate, with annual average temperature and rainfall of 12.3 °C and 482 mm, respectively. Long-term positioning fertilization experiments based on continuous cropping patterns of maize and wheat were carried out in the station from 1997. The soil at this station is the alluvial fan fluvo-aquic soil type with sandy loam texture. About 2 tons of 0–30 cm topsoil were collected and thoroughly mixed after removing gravel and plant and animal residues. The pre-treated soil samples with an organic carbon concentration of 15 g kg⁻¹, total N (TN) concentration of 1 g kg⁻¹, pH of 8.1, and sand, silt and clay composition of 53%, 34% and 13%, respectively. The uniform soil was filled into the same specification of uncapped tin buckets (40 cm in diameter and 50 cm high), and the height and compactness of the soil column were maintained at the same level (height 40 cm, bulk weight 1.2 g cm⁻³).

2.2. Experimental Design

A total of six fertilization treatments were set up in this study, with four replicates per treatment, including two kinds of urea and three methods of fertilization. The pure N concentration and atom % ¹⁵N were 46.30% and 13.15% in the labeled U, and were 43.97% and 13.15% in labeled CU. The U and CU were provided by Shanghai Engineering Research Center for Stable Isotopes and Beijing Academy of Agriculture and Forestry Sciences, respectively. The fertilization treatments were as follows: (1) U was applied to 0–10 cm soil in strip (U_{sur}); (2) U was applied to 0–30 cm soil in three strips (U_{str}); (3) U was evenly mixed and applied to 0–30 cm soil (U_{mix}); (4) CU was applied to 0–10 cm soil in strip (CU_{sur}); (5) CU was applied to 0–30 cm soil in three strips (CU_{str}); (6) CU was evenly mixed and applied to 0–30 cm soil (CU_{mix}). Meanwhile, the soil without adding any fertilizer was set as the blank control (CK). Details regarding the amount and depth of urea (U) application are provided in Table 1. The wheat seeds (KeNong 2011) were artificially sown in each bucket at the rate of 300 plants m⁻² after fertilization. The addition amount and frequency of other fertilizers (phosphate and potash fertilizers) and irrigation water were consistent. All the buckets were buried in the field in a random arrangement, and the soil inside and outside of the buckets was kept at the same height.

Table 1. Treatments of fertilization at different soil depths (unit: kg N ha⁻¹).

Depth	CK	U _{sur}	U _{str}	U _{mix}	CU _{sur}	CU _{str}	CU _{mix}
0–10 cm	-	160	53	53	160	53	53
10–20 cm	-	-	53	53	-	53	53
20–30 cm	-	-	53	53	-	53	53
30–40 cm	-	-	-	-	-	-	-

CK, blank soil; U_{sur}, surface application of urea; U_{str}, layered-strip application of urea; U_{mix}, layered-mix application of urea; CU_{sur}, surface application of coated urea; CU_{str}, layered-strip application of coated urea; CU_{mix}, layered-mix application of coated urea.

2.3. Sampling and Analyses

The wheat was harvested on 12 June 2019. The wheat roots, straw and grains in each bucket were collected, rinsed and killed at 100 °C for 35 min, and then dried at 70 °C to a constant weight. Field biomass of wheat was evaluated by actual dry weight of different wheat tissues in each bucket. Soil samples at different depths (0–10, 10–20, 20–30 and 30–40 cm) were collected in each bucket and air-dried naturally after removing any visible animal and plant residues. Representative subsamples from wheat tissues and soil samples of each replicate were ground into powder with a ball mill (MM2000, Retsch, Haan, Germany) and then passed through a 0.15 mm sieve for the determination of TN and ¹⁵N atom % using an Isotope Ratio Mass Spectrometer (IsoPrime100, Elementar, Hanau, Germany). Soil ammonium N (NH₄⁺-N) and nitrate N (NO₃⁻-N) were extracted by potassium chloride (soil–liquid ratio 1:5) and determined by Continuous Flow Analytical System (AA3, Seal Analytical GmbH, Hamburg, Germany). The bulk density of soil layers at different depths was determined by cutting ring-drying method.

2.4. Distribution of Urea-N

The absorption, residue and loss of urea-N under different fertilization treatments were analyzed according to the characteristics of ¹⁵N atom % of wheat tissues and soil samples. The absorption-N (N_{dff-Abs}), residual-N (N_{dff-Res}), loss-N (N_{dff-Los}) and NUE derived from urea were calculated by Equations (1)–(4), respectively:

$$N_{dff-Abs} = N_{dff-wheat} / N_{fer} \times 100\% \quad (1)$$

$$N_{dff-Res} = N_{dff-soil} / N_{fer} \times 100\% \quad (2)$$

$$N_{dff-Los} = (N_{fer} - N_{dff-wheat} - N_{dff-soil}) / N_{fer} \times 100\% \quad (3)$$

$$NUE = N_{dff-Abs} / N_{fer} \times 100\% \quad (4)$$

where N_{dff-wheat} is the amount of urea-N absorbed by wheat (grain, straw and root) (kg N ha⁻¹), N_{dff-soil} is the amount of urea-N remained in the soil layer (kg N ha⁻¹), N_{fer} is the amount of urea-N input (kg N ha⁻¹), and N_{dff-wheat} and N_{dff-soil} are calculated according to Equations (5) and (6), respectively:

$$N_{dff-wheat} = M_{wheat} \times N_{wheat} \times (\delta^{15}N_{wheat} - \delta^{15}N_o) / (\delta^{15}N_{fer} - \delta^{15}N_o) \quad (5)$$

$$N_{dff-soil} = M_{soil} \times N_{soil} \times (\delta^{15}N_{soil} - \delta^{15}N_o) / (\delta^{15}N_{fer} - \delta^{15}N_o) \quad (6)$$

where M_{wheat} is the dry weight of wheat tissues (grain, straw and root) (kg ha⁻¹), N_{wheat} is the concentration of TN in the tissues of wheat (kg ha⁻¹), ¹⁵N_{wheat} is the ¹⁵N atom of wheat tissues (%), ¹⁵N_o is a natural ¹⁵N atom % (0.336%), ¹⁵N_{fer} is the ¹⁵N atom of labeled fertilizer (%), M_{soil} is the weight of a layer of soil per 10 cm depth (kg ha⁻¹), N_{soil} is the concentration of TN of a layer of soil per 10 cm depth (kg ha⁻¹) and ¹⁵N_{soil} is the ¹⁵N atom of different soil layers (%).

2.5. Statistical Analysis

The preliminary collation and in-depth calculation of all test data were completed with Excel 2010 (Microsoft, Redmond, WA, USA). The statistical differences of wheat biomass, nitrogen absorbed in wheat, NO_3^- -N, NH_4^+ -N, TN, ^{15}N atom %, $\text{N}_{\text{dff-Abs}}$, $\text{N}_{\text{dff-Res}}$, $\text{N}_{\text{dff-Los}}$ and NUE under different fertilization treatments and soil layers were identified by analysis of variance and Duncan's multiple comparisons ($p < 0.05$) in SPSS 20 (IBM, Chicago, IL, USA). All figures were prepared in SigmaPlot version 12.5 (Systat Software, Chicago, IL, USA).

3. Results

3.1. N Distribution in Soil and N Absorption in Wheat

Compared with U_{sur} , U_{str} and U_{mix} increased the NO_3^- -N concentration in 0–40 cm soil by 42.3% and 50.3%, respectively, and U_{mix} increased the NH_4^+ -N concentration in 0–40 cm soil by 13.8% (Figure 1b,d). CU_{str} and CU_{mix} reduced the NH_4^+ -N concentration by 49.6% and 70.0%, respectively, while there was no change in NO_3^- -N concentration (compared to CU_{sur}). Layered fertilization had a tendency to decrease the TN concentration of 0–10 cm soil and increase the TN concentration of 10–30 cm soil (Figure S1). Compared with CK, U_{str} and CU_{str} increased the biomass of straw by 19.1% and 15.6%, respectively, while there was no change in the biomass of grain or root among the different treatments (except CU_{sur} , Figure 2a). Compared with U_{sur} , U_{str} and U_{mix} increased the N absorption (N-Abs) of straw by 32.2% and 25.3%, respectively, and U_{str} increased the N-Abs of grain by 18.7%, but there was no increase in the N-Abs of root. Compared with CU_{sur} , CU_{str} and CU_{mix} increased the N-Abs of straw by 33.6% and 29.5%, respectively, CU_{str} increased the N-Abs of grain by 12.3%, and CU_{str} and CU_{mix} increased the N-Abs of root by 28.9% and 29.1%, respectively (Figure 2b).

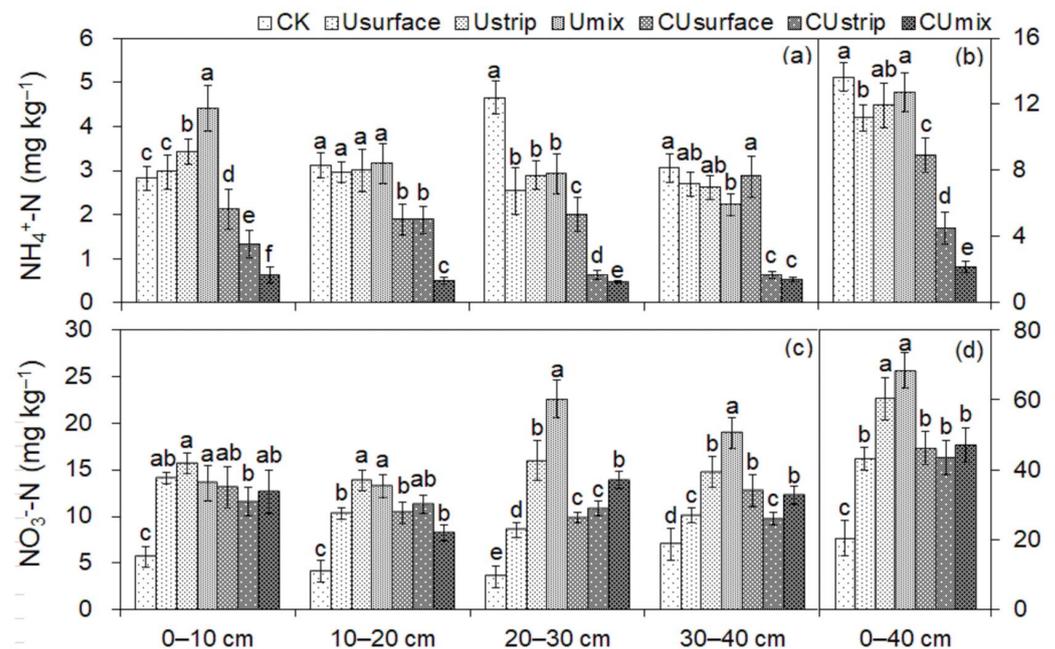


Figure 1. The concentration and distribution of ammonium nitrogen (a,b) and nitrate nitrogen (c,d) in the root zone (0–40 cm) under different fertilization treatments. Data in the figure are expressed as the mean of four replicates ($n = 4$). The vertical lines represent the standard error, and the different lowercase letters indicate significant differences among different treatments ($p < 0.05$). CK, blank soil; U_{sur} , surface application of urea; U_{str} , layered-strip application of urea; U_{mix} , layered-mix application of urea; CU_{sur} , surface application of coated urea; CU_{str} , layered-strip application of coated urea; CU_{mix} , layered-mix application of coated urea.

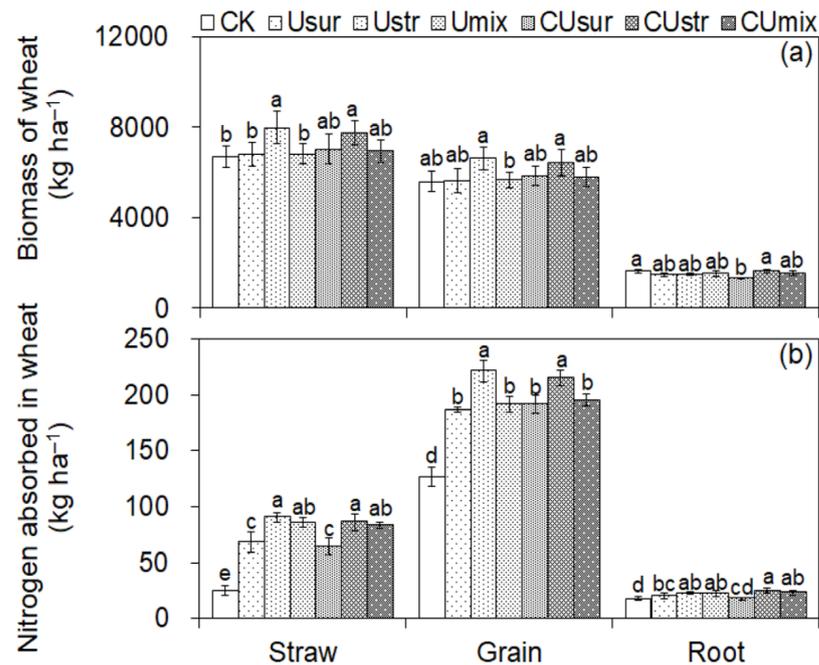


Figure 2. Biomass (a) and nitrogen absorption (b) of wheat tissues (straw, grain and root) under different fertilization treatments. Data are expressed as the mean of four replicates ($n = 4$). The vertical lines represent the standard error, and the different lowercase letters indicate significant differences among different treatments ($p < 0.05$). CK, blank soil; U_{sur} , surface application of urea; U_{str} , layered-strip application of urea; U_{mix} , layered-mix application of urea; CU_{sur} , surface application of coated urea; CU_{str} , layered-strip application of coated urea; CU_{mix} , layered-mix application of coated urea.

3.2. Urea-N Residue and Absorption

The ¹⁵N atom % of 0–10 cm soil under U_{sur} and CU_{sur} were higher than that under U_{str} , U_{mix} , CU_{str} and CU_{mix} , while the opposite was true for the 10–30 cm layers ($p < 0.05$, Figure 3). The ¹⁵N atom % of 30–40 cm soil was increased only under U_{str} and U_{mix} (compared with U_{sur}). The $N_{dff-Res}$ of 0–10 cm soil under U_{sur} and CU_{sur} were higher than that under U_{str} , U_{mix} , CU_{str} and CU_{mix} ($p < 0.05$). The $N_{dff-Res}$ of 10–30 cm soil under U_{str} and U_{mix} increased by 114.9–178.2% compared to that under U_{sur} , while the $N_{dff-Res}$ of 10–30 cm soil under CU_{str} and CU_{mix} increased by 60.2–124.5% compared with that under CU_{sur} ($p < 0.05$). There were no changes in $N_{dff-Res}$ of 30–40 cm soil among U_{sur} , U_{str} and U_{mix} or CU_{sur} , CU_{str} and CU_{mix} .

Compared with U_{sur} , U_{str} increased the $N_{dff-Abs}$ of straw by 24.9% and $N_{dff-Abs}$ of grain by 13.9%, but there was no change in $N_{dff-Abs}$ of root among U_{sur} , U_{str} and U_{mix} ($p < 0.05$, Figure 4). Compared with CU_{sur} , CU_{str} and CU_{mix} increased the $N_{dff-Abs}$ of straw by 28.1% and 20.7%, respectively, CU_{mix} reduced the $N_{dff-Abs}$ of grain by 6.4%, CU_{str} had no significant effect on the $N_{dff-Abs}$ of grain, and CU_{str} and CU_{mix} increased the $N_{dff-Abs}$ of root by 28.7% and 34.3%, respectively ($p < 0.05$, Figure 4).

3.3. Nitrogen Use Efficiency (NUE)

Compared to the surface application of U or CU (U_{sur} and CU_{sur}), layered-strip fertilization (U_{str} and CU_{str}) significantly improved the NUE (Figure 5). Compared with U_{sur} , U_{str} increased the NUE by 16.6%, while U_{mix} had no obvious effect on the NUE. Compared to CU_{sur} , CU_{str} increased the NUE by 9.6%, while CU_{mix} had no obvious effect on the NUE. The NUE under CU_{sur} increased by 8.1% more than that under U_{sur} , and the NUE under CU_{mix} increased by 11.0% more than that under U_{mix} . The highest NUE were found in U_{str} and CU_{str} (57.6% and 58.5%, respectively), and there was no significant difference between them.

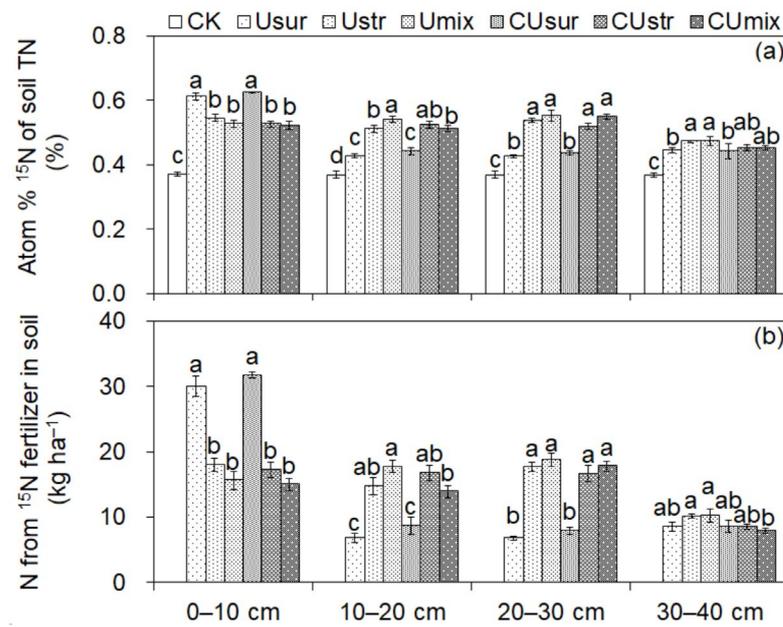


Figure 3. The atom % of ¹⁵N (a) and the distribution of nitrogen derived from fertilizer (b) in each soil layer (0–40 cm) under different fertilization treatments. Data are expressed as the mean of four replicates (*n* = 4). The vertical lines represent the standard error, and the different lowercase letters indicate significant differences among different treatments (*p* < 0.05). CK, blank soil; U_{sur}, surface application of urea; U_{str}, layered-strip application of urea; U_{mix}, layered-mix application of urea; CU_{sur}, surface application of coated urea; CU_{str}, layered-strip application of coated urea; CU_{mix}, layered-mix application of coated urea.

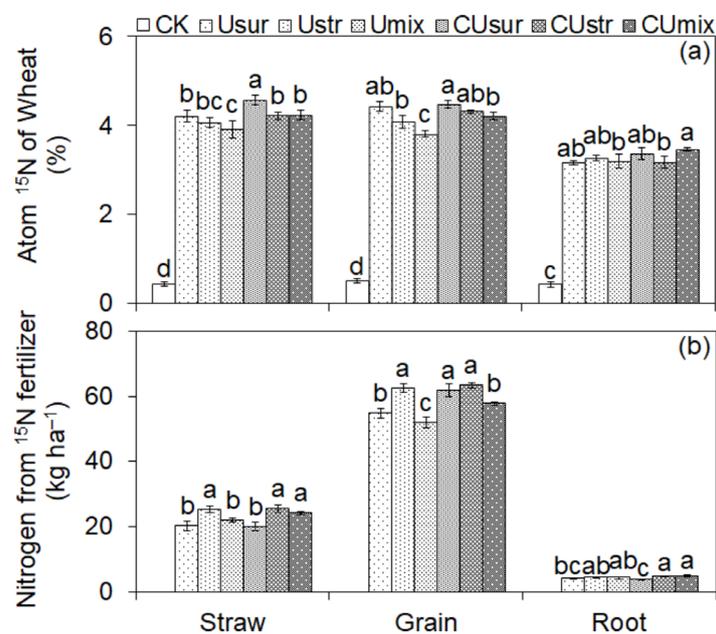


Figure 4. The atom % of ¹⁵N (a) and the distribution of nitrogen derived from fertilizer (b) in all tissues of wheat under different fertilization treatments. Data are expressed as the mean of four replicates (*n* = 4). The vertical lines represent the standard error, and the different lowercase letters indicate significant differences among different treatments (*p* < 0.05). CK, blank soil; U_{sur}, surface application of urea; U_{str}, layered-strip application of urea; U_{mix}, layered-mix application of urea; CU_{sur}, surface application of coated urea; CU_{str}, layered-strip application of coated urea; CU_{mix}, layered-mix application of coated urea.

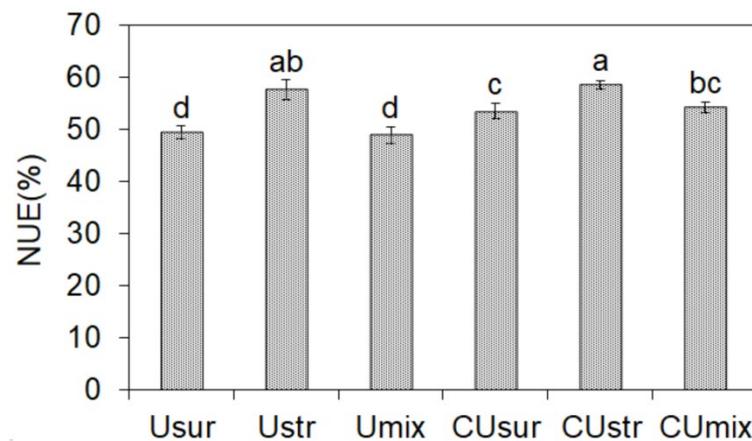


Figure 5. The nitrogen use efficiency (NUE) under different fertilization treatments. The vertical lines represent the standard error ($n = 4$), and the different lowercase letters indicate significant differences among different treatments ($p < 0.05$). CK, blank soil; U_{sur}, surface application of urea; U_{str}, layered-strip application of urea; U_{mix}, layered-mix application of urea; CU_{sur}, surface application of coated urea; CU_{str}, layered-strip application of coated urea; CU_{mix}, layered-mix application of coated urea.

3.4. Balance of Urea-N

The absorption, residue and loss of N derived from U or CU are shown in Table 2. Compared with U_{sur}, U_{str} increased the N_{diff}-Abs by 16.6%, but U_{mix} had no significant effect on the N_{diff}-Abs. Compared with CU_{sur}, CU_{str} increased the N_{diff}-Abs by 9.6%, but CU_{mix} had no significant effect on the N_{diff}-Abs. U_{str} and U_{mix} increased the N_{diff}-Res by 16.1% and 19.6% compared to that under U_{sur}, respectively. There was no change in N_{diff}-Res among CU_{sur}, CU_{str} and CU_{mix}. Compared with U_{sur}, U_{str} and U_{mix} reduced the N_{diff}-Los by 74.9% and 32.9%, respectively. Compared with CU_{sur}, U_{str} reduced the N_{diff}-Los by 59.4%, while CU_{mix} had no significant effect on N_{diff}-Los. The N_{diff}-Abs of CU_{sur} and CU_{mix} were higher than that of U_{sur} and U_{mix}, respectively. Among all the treatments, the N_{diff}-Abs under U_{str} and CU_{str} were the highest (57.6% and 58.5%, respectively), and the N_{diff}-Los was the lowest (4.5% and 4.5%, respectively) (Figure 6). Compared with the surface fertilization, layered fertilization reduced the N_{diff}-Res in the 0–10 cm soil layer from 18.8–19.8% to 9.4–11.3%, and increased the N_{diff}-Res in the 10–30 m soil layers from 4.2–5.4% to 8.7–11.8%.

Table 2. The distribution of nitrogen derived from fertilizers after the complete growth cycle of wheat.

Treatment	N _{diff} -Absorption (kg ha ⁻¹)	N _{diff} -Residue (kg ha ⁻¹)	N _{diff} -Loss (kg ha ⁻¹)
U _{sur}	79.01 ± 2.75 d	52.21 ± 0.54 b	28.79 ± 2.96 a
U _{strip}	92.15 ± 1.15 ab	60.61 ± 1.54 a	7.24 ± 0.69 c
U _{mix}	78.24 ± 2.38 d	62.46 ± 4.16 a	19.31 ± 4.98 b
CU _{sur}	85.40 ± 3.00 c	56.97 ± 2.51 ab	17.63 ± 1.41 b
CU _{str}	93.57 ± 1.55 a	59.27 ± 2.32 ab	7.16 ± 3.16 c
CU _{mix}	86.82 ± 0.70 bc	54.63 ± 2.14 ab	18.56 ± 2.25 b

Values presented are mean ± standard error ($n = 4$). Different lowercase letters after the number indicate significant differences among treatments ($p < 0.05$). N_{diff}-Absorption, absorption of nitrogen derived from fertilizer in wheat; N_{diff}-Residue, residue of nitrogen derived from fertilizer in soil; N_{diff}-Loss, loss of nitrogen derived from fertilizer in atmosphere; U_{sur}, surface application of urea; U_{str}, layered-strip application of urea; U_{mix}, layered-mix application of urea; CU_{sur}, surface application of coated urea; CU_{str}, layered-strip application of coated urea; CU_{mix}, layered-mix application of coated urea.

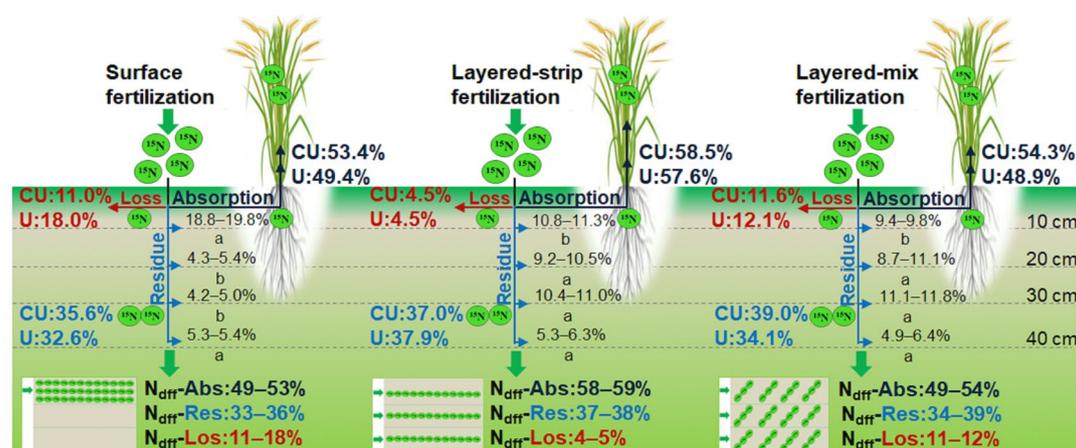


Figure 6. The rates of absorption, residue and loss of nitrogen derived from fertilizer in soil–plant–atmosphere of the winter wheat system. Different lowercase letters (horizontal row) indicate significant differences among different treatments ($p < 0.05$). U, urea; CU, coated urea; N_{dff}-Abs, absorption of nitrogen derived from fertilizer in wheat; N_{dff}-Res, residue of nitrogen derived from fertilizer in soil; N_{dff}-Los, loss of nitrogen derived from fertilizer in atmosphere.

4. Discussion

4.1. N Distribution in Soil and N Absorption in Wheat

Layered deep fertilization increased the concentration of mineral N in the soil root zone (especially in 10–30 cm layer), and layered application of CU was beneficial in reducing the leaching intensity of mineral N. N fertilizer applied to cropland is often hydrolyzed into mineral forms that can be directly absorbed and utilized by crop roots, such as NH_4^+ -N and NO_3^- -N. As previously reported, the concentration and distribution of soil mineral N are closely related to crop production [10,28]. In this study, layered application of U (U_{str} or U_{mix}) obviously increased the concentration of NH_4^+ -N (39.9–58.8%) and NO_3^- -N (13.8%) in 0–40 cm soil compared to U_{sur} (Figure 1b,d). Our results were basically consistent with the findings of Chen et al. (2023) [29], which showed that the concentration of NO_3^- -N and NH_4^+ -N in the root zone (0–40 cm) increased by 6.0–36.3% under the deep fertilization (15 cm). Layered deep fertilization might reduce the decomposition rate of U and reduce volatilization loss of mineral N to gaseous N (N_2O , NH_3) by increasing physical protection [10]. By contrast, layered application of CU did not increase the mineral N in 0–40 cm soil; even the concentration of NH_4^+ -N decreased by 49.6% and 76.0% under CU_{str} and CU_{mix} , respectively. This might be due to the slow-release characteristics of N derived from CU, and most of it was directly absorbed by roots in the form of NH_4^+ -N, which reduced the accumulation intensity of NH_4^+ -N. In addition, the conversion rate of NH_4^+ -N to NO_3^- -N was slowed down in the oxygen-deprived deep soil, thus reducing excess accumulation of NO_3^- -N [21]. The lowest concentration of NH_4^+ -N under CU_{mix} may be attributed to the fact that positively charged NH_4^+ binds to negatively charged soil colloids or coating materials, which reduced the concentration of free NH_4^+ . Moreover, microbial metabolism throughout the root zone also consumed a portion of NH_4^+ [11]. In addition, compared to U_{sur} or CU_{sur} , U_{str} and U_{mix} effectively increased the NO_3^- -N concentration in deep soil (20–40 cm), while the effect of CU_{str} and CU_{mix} were relatively weak. For example, U_{str} or U_{mix} increased the NO_3^- -N concentration in the 20–30 cm soil layer by 86.8–163.9%, while it was 41.0% under CU_{mix} , and there was no increase in 30–40 cm soil (Figure 1c). On the one hand, layered fertilization increased the mineral N in deep soil; on the other hand, CU reduced the leaching intensity of NO_3^- -N. Therefore, layered application of U enriched the mineral N in the root zone (0–40 cm), and the layered application of CU may be more suitable for crop nutrient absorption.

Crop growth and nutrient absorption are directly related to soil nutrient status. Our study has demonstrated that layered fertilization and fertilizer type could change the

concentration and distribution of mineral N in the 0–40 cm root zone. In this study, layered-strip application of U or CU increased the biomass of straw and root (only CU) compared with surface fertilization, and the biomass of grain also showed increased potential under layered fertilization. Our findings are similar to those of Chen et al. (2023) and Cheng et al. (2020) [29,30]. This might be because layered fertilization increased the distribution of mineral N in root-zone soil, and the N released from CU could better match the absorption of root. Compared with surface fertilization, the biomass of straw, grain or root was not effectively increased by layered-mix fertilization (Figure 2a). We considered that this might be because the full contact between fertilizer and soil produced an extensive priming effect and microbial utilization, which resulted in volatilization loss and microbial fixation [31]. To reduce the adverse effects on wheat growth and fertilizer utilization, the mixed application of fertilizer in deep layers should be avoided in wheat season in the NCP.

Layered fertilization altered soil nutrient status, which affected N-Abs by roots and then the transfer among roots and stems, leaves and grains [32]. N-Abs of straw was increased under the layered fertilization, N-Abs of grain was increased only under U_{str} and CU_{str} , while N-Abs of root was increased only under CU_{str} . Hence, layered-strip application of CU could promote the N-Abs of wheat tissues, which further indirectly verified that the N release rule of CU was more closely matched with the N-Abs of roots, thus promoting the N-Abs of roots and the transfer among other tissues. The maximum N-Abs of wheat was achieved under layered-strip fertilization (Figure 2b). On the one hand, this might be because the biomass of wheat tissues under layered-strip fertilization was the highest. On the other hand, layered-strip fertilization was more conducive to the stable preservation of nutrients, avoiding excessive concentration and excessive dispersion distribution patterns, thereby minimizing the nutrient loss and fixation [33].

4.2. Urea-N Distribution in Soil and Urea-N Absorption in Wheat

The depth and type of fertilizer application affect the distribution and absorption of fertilizer N in soil and crop. Whether due to the ecological environment, human health or the national plan of zero increase in fertilizer use, the key breakthrough aim of researchers is to improve the absorption of fertilizer N while taking into account soil conservation by optimizing the fertilization strategy [2,34]. In our study, the ^{15}N isotope labeling technique was used to quantify the migration and distribution of N derived from U and CU between soil and wheat tissues. The soil ^{15}N atom % under surface fertilization of U or CU was higher than that under layered fertilization in 0–10 cm soil, while the soil ^{15}N atom % under layered fertilization in the 10–20, 20–30 or 30–40 cm soil layer was higher than that under surface fertilization (except for CU in 30–40 cm, Figure 3, $p < 0.05$). This was obviously influenced by the layered fertilization. Most of the unused N and ^{15}N from U or CU concentrated in the 0–10 cm soil under surface fertilization, which resulted in a higher ^{15}N atom % of 0–10 cm soil than that of 10–40 cm soil. However, the U and CU were evenly distributed in 0–30 cm soil under layered fertilization, and unused fertilizer ^{15}N caused a higher ^{15}N atom % of deep soil (10–20, 20–30 or 30–40 cm) than that of surface soil (0–10 cm). The distribution characteristics of $N_{diff-Res}$ in the whole 0–40 cm soil were similar to that of soil ^{15}N atom %. The surface fertilization resulted in the accumulation of fertilizer N in the surface soil, and the layered fertilization increased the distribution of fertilizer N in the deep soil. Therefore, layered fertilization is an effective way to balance N distribution in the soil profile, thus reducing the risk of microbial extravagant N consumption and volatilization loss caused by excessive concentration of fertilizer N in the surface layer [35,36].

Compared with the surface fertilization, the ^{15}N atom % of wheat tissues (straw, grain and root) were not obviously increased by layered fertilization, and the ^{15}N atom % of straw and root were even decreased (Figure 4). This is possibly because both the biomass and N-Abs of wheat tissues were increased under layered fertilization, which weakened the variation in ^{15}N atom %. Wan et al. (2021) also found that when the variation range of plant biomass was much larger than that of the ^{15}N atom %, no change in ^{15}N atom % of plants was observed [37]. However, $N_{diff-Abs}$ in wheat tissues varied obviously

under different fertilization methods. Compared with surface fertilization, U_{str} and CU_{str} effectively increased the $N_{diff-Abs}$ in wheat tissues (except roots under U_{str} , Figure 4b), but the promotion effect of U_{mix} and CU_{mix} was relatively weak. The promotion effect of layered-strip fertilization on $N_{diff-Abs}$ was better than that of layered-mix fertilization. This is possibly due to the formation of multilayer nutrient patches in strip deep fertilization, and the increase in layers and depth will provide a relatively stable transformation environment for fertilizer N, thereby avoiding excessive volatilization loss caused by the concentration of N in the surface layer. Meanwhile, the strip nutrient patches also matched the fertilizer displacement characteristics and nutrient absorption regularity of wheat roots under strip planting [33,38]. Layered-mix fertilization appeared to create a nutrient-homogeneous plough layer, but it would increase the intensity of adsorption and fixation of fertilizer N by soil colloid, chemical materials and microorganisms, as well as the priming effect [31,39]. In addition, layered application of CU had a more obvious promoting effect on $N_{diff-Abs}$ of roots compared with layered application of U (Figure 4b). The results of this study are in accordance with the findings of Shen et al. (2022) [21], and the suitability of CU to the nutrient requirements of roots was verified again.

4.3. NUE and Balance of Urea-N

NUE, which directly reflects the ability of crop N assimilation, is the core index to evaluate whether certain fertilization strategies match crop production systems [40]. In this study, U_{str} or CU_{str} increased the NUE in wheat season by 16.6% and 9.6%, respectively, compared with U_{sur} or CU_{sur} , while there was no change under other treatments (Figure 5). Qiang et al. (2021) and Shen et al. (2022) also found that deep application of U or CU could effectively improve the NUE [21,35]. These researchers believed that deep fertilization (15 cm) was an effective way to regulate the synchronous matching of crop N uptake and soil N supply, thus improving the ability of crops to assimilate fertilizer N. Previous reports have proved that improving the compatibility between soil nutrient supply and crop nutrient demand is an important breakthrough to improve fertilizer nutrient utilization [41]. On the basis of deep fertilization, nutrients were divided into multi-layer patches, which could not only provide an environment to ensure the stable conversion of nutrients but also match the nutrient requirements of crop roots, so as to show the optimal NUE. This was consistent with the previous study in which layered-strip fertilization increased the $N_{diff-Abs}$ of wheat tissues and mineral N in root zone (0–40 cm) (Figure 2). In addition, the NUE under CU_{sur} or CU_{mix} was higher than that under U_{sur} or U_{mix} , which was mainly due to the fact that the slow-release technique reduced the release rate of nutrients and avoided the leaching and volatilization losses caused by rapid decomposition [10].

Layered-strip fertilization showed a good advantage in improving the NUE, and the migration of fertilizer N in the complete system of soil–plant–atmosphere under this field practice was also worth exploring. For this purpose, we quantified the specific pathways of absorption (i.e., NUE), residue and loss of fertilizer N under surface, layered-strip and layered-mixed fertilization (Figure 6). The $N_{diff-Abs}$ was the highest (58–59%) and the $N_{diff-Los}$ was the lowest (4–5%) under layered-strip fertilization compared with other fertilization methods. As mentioned above, layered-strip fertilization improved NUE and reduced N loss through physical protection of fertilizer N and matching nutrient requirements of roots, while the layered-mix fertilization might lead to stronger fixation and priming effect of fertilizer N, thus limiting the increase in NUE (in Section 4.2). The overall $N_{diff-Res}$ in 0–40 cm root zone did not change among different fertilization methods, but the $N_{diff-Res}$ at different soil depths varied significantly. Compared with surface fertilization, layered fertilization decreased the $N_{diff-Res}$ in 0–10 cm soil but increased the $N_{diff-Res}$ in 10–20 and 20–30 cm soil. This further verified that layered fertilization could effectively balance the distribution of fertilizer N in the root-zone soil, so as to avoid the hot-spot effect caused by excessive accumulation of nutrients, such as microbial extravagant N consumption and gaseous conversion loss (N_2O/NH_3) [34,42]. The balanced and stable distribution of nutrients in the root zone not only was able to match the rule of nutrient

requirements of wheat with deep roots but also prolonged the availability of fertilizer N and mineral N in soil [29]. This was also supported by layered fertilization to increase the concentration of mineral N in the whole root-zone soil (0–40 cm) (Figure 1b,d).

Our results indicated that layered-strip fertilization (0–30 cm) could effectively improve the biomass of wheat tissues and the NUE by matching absorption (58–59%) and reducing loss (4–5%) of fertilizer N in the winter-wheat system in the NCP. The slow-release characteristics of N derived from CU could better match the nutrient requirements of crops, but it is necessary to avoid deep mixed application. These findings will provide theoretical guidance for nutrient optimization management of winter wheat in the NCP. In order to optimize the layered fertilization strategy, further studies are needed to determine the fertilizer proportion in different soil layers, and the combination of active and slow fertilizers, while taking variations in climate, soil type and crop type into account. The interaction effect, the soil nitrogen capacity and the recovery rate of ^{15}N should be considered more in the calculation of NUE using atom % ^{15}N .

5. Conclusions

In this study, the ^{15}N isotope labeling technique was used to quantify the migration path of fertilizer (U and CU) N in a soil–plant–atmosphere system under layered fertilization, and to identify an appropriate method that can effectively increase NUE. Compared with surface fertilization, layered fertilization equalized nutrient distribution in the root zone, increased mineral N concentration in the deep soil (10–40 cm), promoted wheat tissue biomass and increased TN absorption. Layered-strip fertilization was observed to have the best effect on the promotion of the concentration of mineral N and fertilizer N in the root zone, wheat biomass and NUE. The heterogeneous nutrient distribution formed by strip deep fertilization was more conducive to soil conservation and crop absorption, and minimized fertilizer nutrient loss. Because of its slow-release characteristics regarding nutrients, and combined with layered fertilization to achieve deep optimization distribution, CU could appropriately match the temporal and spatial rules of crop nutrient requirements, thus promoting the NUE. Although the distribution of mineral N and fertilizer N in the deep soil was increased by layered-mixed application, the loss of fertilizer N was still very high, which resulted in no improvement in NUE. Therefore, as far as possible, strip fertilization rather than mixed fertilization is used in the field. Our results will help policymakers and producers to formulate appropriate fertilization strategies to promote the NUE of winter wheat in the NCP.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy13092428/s1>. Figure S1: The concentration and distribution of total nitrogen in soil layer (0–40 cm) under different fertilization treatments.

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