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Quantifying the Flows of Nitrogen Fertilizer under Different Application Rates in a Soil–Forage Triticale–Dairy Cow System

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Abstract: Nitrogen (N) can enhance the biomass and feeding quality of forage crops and advance the growth of the herbivorous livestock industry. Investigating the N fertilizer dynamics in the soil–crop–livestock system is important for resource-use efficiency and environmental safety. By using the ^{15}N-labeled technology and the in vitro incubation technique, an experiment was conducted in the North China Plain (NCP) in 2015–2016 to quantify the migration and distribution of N fertilizer in the soil–forage triticale (*X Triticosecale* Wittmack)–dairy cow system. The results showed that 34.1–37.3% of the applied N fertilizer was absorbed by forage triticale, in which 35.9–39.6% N accumulated in the stems and 60.4–64.1% accumulated in the leaves. In addition, 36.3–39.1% of the applied N fertilizer remained in the 0–100 cm soil layer, in which 81.8–91.3% was distributed in the 0–40 cm soil layer. The remaining 24.6–26.8% of the applied N fertilizer was lost in various ways and 28.1–31.3% of the N fertilizer could be utilized by dairy cows. When N fertilizer was applied between 0–225 kg N ha^{-1}, the increased application of N fertilizer improved the biomass yield from 14.0 to 17.5 t ha^{-1} and enhanced the N content of the forage triticale from 1.3% to 1.4%; however, it did not significantly affect the distribution rate of N fertilizer in the soil–forage triticale–dairy cow system. The optimum N fertilizer application rate for forage triticale is less than 225 kg N ha^{-1} to maintain high-efficient N use in the soil–crop–livestock system and reduce the environmental risks in the NCP. Our results quantified the N fertilizer dynamics in the soil–forage triticale–dairy cow system and provided a significant reference for guiding rational strategies of forage triticale cultivation.

Keywords: ^{15}N-labeled technology; soil–crop–livestock system; forage triticale; migration and distribution; nitrogen use efficiency

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1. Introduction

Nitrogen (N) is one of the most crucial macro-elements for plant growth [1]. N fertilizer has produced tremendous contributions to improve crop yield, alleviating poverty and hunger worldwide [2]. From 1961 to 2014, grain production in the world increased by 3.2 times and the amount of N fertilizer use increased by 9–fold. During the same period, China's grain yield and N fertilizer consumption increased by 2.1 and 3.5 times, respectively [3,4]. With the increase in N fertilizer application, the problem of low nitrogen-use efficiency (NUE) has become prominent. According to Ladha et al. [5], the N-recovery efficiency of rice, wheat, and maize in the world is 46%, 57%, and 65%, respectively. In recent years, the NUE of China's three major grain crops has greatly increased. Zhang et al. [6] documented that the NUE of rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), and corn (*Zea mays* L.) in different regions of China were 28.3%, 28.2%, and

26.1%, respectively. According to 2020 statistics from the Ministry of Agriculture and Rural Affairs of the People's Republic of China, the NUE of rice, wheat, and corn has risen to 40.2%, on average. However, the average degree of over-fertilization was close to 60%. Consequently, excessive application of N fertilizer has caused a large amount of N loss and many environmental problems, mainly consisting of soil acidification and greenhouse gases emissions [7–9].

N is also an important feed nutrient for animal husbandry, as it can ensure the growth and development of livestock and stabilize production. Bai et al. [10] discovered that the NUE in China's dairy cow industry was 24.0%, which was significantly lower than that in Euro–American countries [11]. Most of the unused N was excreted with the manure and urine of the livestock. The return rate of manure and urine nutrients from livestock in China is only 30–50% [12], and large amounts of N are lost to the environment, which further exacerbates environmental pollution. Revealing the quantitative assessment of N-fertilizer migration and distribution within the soil–crop–livestock system is, thus, an urgent necessity to enhance NUE and mitigate environmental pollution arising from N loss.

Many studies investigated the N fertilizer flows in soil–crop, crop–livestock, and soil–microbe–plant systems [13–15], especially including the soil–cereal–rye system [16], the forage–cow system [17,18] and the forage–lamb system [19]. However, these studies mainly focused on either the soil–crop system or the crop–livestock system, and research reported on the migration and distribution of N fertilizer across the entire soil–crop–livestock system is still underrepresented.

Triticale (×Triticosecale Wittmack) is an artificially created plant species resulting from the interspecific hybrid between wheat and rye (*Secale cereale* L.). It has gradually gained recognition as a valuable forage crop, utilized for hay or silage production, as well as for direct grazing [20,21]. Forage triticale has been planted in more than 30 countries around the world, including the United States, Australia, and China [22]. As a new type of cool-season annual forage, forage triticale can provide forage for herbivorous livestock, such as dairy cows, during early winter and spring. This effectively solves the problem of forage shortages for herbivorous livestock in early winter and spring and has strong prospects for utilization. Some studies have estimated the spatial distribution of N fertilizer in the soil–forage triticale system. For instance, Gibson et al. [23] studied the NUE of winter triticale harvested as forage in Iowa, USA; they found that the absorption of N by winter triticale increased with the increase in N application and mainly occurred before the flowering stage. Using the ^{15}N tracer method, Ferchaud et al. [24] determined the fertilizer NUE of triticale when harvested as an energy crop in the Esmons region of northern France and showed that 32.3–46.0% of the N fertilizer was absorbed by triticale. In northern Kazakhstan, Zavalin et al. [25] quantified the fertilizer NUE of spring triticale harvested as grain and found that the fertilizer NUE of the whole plant biomass was 19–42%, the recovery rate in the soil was 26–45%, and the loss rate was 14–50%. As a kind of forage crop, the N fertilizer absorbed by forage triticale will be utilized by livestock and then, eventually, be transformed into animal products. However, the migration and distribution of N fertilizer within the entire soil–forage triticale–dairy cow system have been scarcely documented, which greatly reflects our knowledge on the N-fertilizer flows in the soil–plant–dairy system and the high-efficient use of N resources in agroecosystems.

The migration and distribution of N fertilizer in the soil–forage triticale–dairy cow system, which greatly determines the productivity, profitability, nutrient utilization, and environmental protection of the system, are still not clear. Thus, the objectives of this study were (1) to understand the response of forage triticale biomass yield to N-fertilizer application, (2) to clarify the N-fertilizer dynamics in the soil–forage triticale–dairy cow system in the NCP, and (3) to quantify the distribution proportion of N fertilizer in the soil–forage triticale–dairy cow system.

2. Materials and Methods

2.1. Experimental Site

The experiment was conducted from October 2015 to May 2016 at the Agricultural Experimental Station of Dryland Farming and Water–saving of the Hebei Academy of Agricultural and Forestry Sciences (37°53′ N, 115°42′ E, 20 m elevation above sea level), located in Hengshui, Hebei province, China. The study site is in the NCP and has a temperate semi–humid monsoon climate. The long–term mean annual precipitation is 498.2 mm (1981 to 2010)—65% occurs in July and August. Rainfall during the forage triticale season (October to May) is 130.3 mm. The mean annual air temperature is 13.2 °C, and the duration of the frost-free period spans 206 days. The precipitation was 128.5 mm from October 2015 to May 2016, and the maximum, minimum, and mean of the air temperature were good representatives of the long-term average (1981 to 2010), effectively representing the climatic characteristics of the forage triticale growing season in the NCP. The soil texture at the experimental site is classified as silt loam. Soil samples were collected on 1 October 2015 using a soil auger at depths of 0–20 cm and 0–40 cm in order to determine the primary chemical and physical properties, respectively [26]. The initial soil properties in the 0–40 cm soil depth are presented in Table 1.

Table 1. Nutrient content and pH value of the 0–40 cm soil layer at the beginning of this study in October 2015.

Soil Layer (cm)	Sand (%)	Silt (%)	Clay (%)	Total N g kg^{-1}	Total P g kg^{-1}	Total K g kg^{-1}	Organic Matter g kg^{-1}	Available N mg kg^{-1}	Available P mg kg^{-1}	Available K mg kg^{-1}	pH
0–20	35.2	44.7	20.1	1.1	1.3	21.2	18.0	68.7	22.3	142.3	8.5
20–40	33.7	42.8	23.5	0.9	0.8	21.5	12.2	46.6	6.3	120.8	8.5

2.2. Experimental Design

2.2.1. Fertilization Experiment

Six treatments of N fertilizer were conducted, using a randomized complete-block design with 3 replications. Each of the 18 plots had an area of 7.2 m^2 (3.0 m × 2.4 m). These plots were encircled by a buffer strip measuring approximately 1.5 m in width. Subsequently, experimental plots were set up in all the plots with areas of 0.4 m^2 (1.0 m × 0.4 m). The experimental plots were separated from the adjoining plots by an iron frame (length × width × height = 1.0 m × 0.4 m × 1.05 m, respectively; there was no back cover), which was installed at a depth of 100 cm below the soil surface and positioned 0.05 m above the ground.

The treatments included (1) treatment N_0, applying 0 kg N ha^{-1} and 180 kg P_2O_5 ha^{-1}; (2) treatment N_{75}, applying 75 kg N ha^{-1} and 180 kg P_2O_5 ha^{-1}; (3) treatment N_{150}, applying 150 kg N ha^{-1} and 180 kg P_2O_5 ha^{-1}; (4) treatment N_{225}, applying 225 kg N ha^{-1} and 180 kg P_2O_5 ha^{-1}; (5) treatment N_{300}, applying 300 kg N ha^{-1} and 180 kg P_2O_5 ha^{-1}; and (6) control (CK), without N fertilizer and P_2O_5 fertilizer application. All the P_2O_5 fertilizer and 50% of the N fertilizer were applied as basal fertilizer before sowing. The other 50% of the N fertilizer was applied as a top application at the elongation stage. The amount and method of fertilization were as described by You et al. [27]. The available K content in this experimental site is relatively high (Table 1). In order to eliminate the impact of phosphorus deficiency on the experiment, phosphorus fertilizer was added in all six N-fertilizer treatments. The N_0 and CK treatments were implemented to investigate the impact of phosphorus fertilizer application on forage triticale yield in the absence of N fertilizer.

The ^{15}N–labeled urea (with a ^{15}N abundance of 10.16%) was applied within the experimental plots, while ordinary urea (with an N content of 46.4%) was applied outside the experimental plots, excluding the buffer strips. The production of the ^{15}N–labeled urea was carried out by the Shanghai Research Institute of Chemical Industry. The phosphate fertilizer used was granular superphosphate (P_2O_5 of 18%).

Forage triticale zhongsi No.1048 was sown in strips with a seeding density of 2,000,000 plants ha^{-1} on 16 October 2015, and irrigation was conducted before sowing, with irrigation amounts of 600 m^3 ha^{-1}. Fifteen rows (2.4 m long for each) were planted in every plot, and the row spacing was 20 cm. Four rows of forage triticale were sown in the experimental plots with 20 plants in each row. No pesticides were used during the growth period of forage triticale, and weeds were removed manually. Forage triticale was harvested in the stage of milk maturity on 25 May 2016.

2.2.2. In-Vitro Incubation Experiment

Three Holstein cows, aged 18 months with an average body weight of 650 ± 14 kg (mean ± SD), were utilized to assess the ruminal degradability of forage triticale labeled with ^{15}N. The cows were equipped with permanent rumen fistula at the mid-to-late lactation stage. The animals were provided with the same feed three times a day (at 7:30, 14:30, and 18:30), while water was available ad libitum. The composition of the total mixed rations is shown in Table S1. All experimental animals in this study were ratified by the Animal Protection Committee of China Agricultural University (approval No. AW201102002–6–2). The procedures of the experiment employed in this study adhered to the university's guidelines for animal research, aligning with the standards of academic and scientific rigor. All efforts were aimed at minimizing animal suffering and reducing the number of animals used. Before the experiment, ^{15}N-labeled forage triticale samples were subjected to forced-air drying at 65 °C for 48 h. Subsequently, the dried plant material was finely ground to pass through a 2.5 mm sieve. Approximately 4 g of forage triticale was placed into numbered nylon bags measuring 6 cm × 10 cm with a mesh pore size of 40 µm. A polyester mesh bag (32 cm × 45 cm, fixed to the intubation with a 50 cm-long rope) was used to fix the bag in the rumen. The nylon bags containing the forage triticale samples were put into the rumen fistula of the cows one hour before morning feeding, and placed in the rumen for 48 h. Immediately after retrieval, all the bags were immersed in cold tap water to prevent microbial fermentation and, subsequently, they underwent five rounds of manual washing with cold tap water until the effluent became clear [28,29]. The residual contents were then dried at 65 °C for 48 h to determine the degradation rate.

2.3. Measurement Indices and Methods

2.3.1. The Yield and Nutrient Contents of Forage Triticale

The yields of forage triticale were assessed during the stage of milk maturity on 25 May 2016. The plant heights of ten randomly selected forage triticale plants were measured prior to harvest in each experimental plot. All forage triticale plants in each experimental plot were cut along the ground to determine the fresh yield, and then the stems and leaves (including ears) were separated. The stems and leaves were subjected to a drying process at 65 °C for 48 h in order to determine their dry weight, and the dry weights per plant were calculated by dividing the sum of the stems' and leaves' dry weights in the experimental plot by the number of plants. The biomass yield per hectare was calculated from the dry weight per plant and the planting density. The samples of the stem and leaf of forage triticale were finely ground to pass through a 2.5 mm sieve and the nutrient contents were determined. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined following the protocols established by Van Soest et al. [30]. The starch and water-soluble carbohydrate (WSC) contents were quantified using the anthrone colorimetric method [31].

2.3.2. The Distribution of N Fertilizer in Forage Triticale

A K–05 automatic N analyzer (Shanghai Shengsheng Automatic Analysis Instrument Co., Ltd., Shanghai, China) and an isotope ratio mass spectrometer (Thermo Fisher Delta V Advantage IRMS, Boston, MA, USA) were employed for the determination of total N concentration and ^{15}N abundance. The following Equations (1)–(5)were referred to by Zhang et al. [32].

The N absorbed from the N fertilizer in a given sample (N_{dff}) was calculated as follows:

$$N_{dff} = (A_S - A_0)/(A_L - A_0) \times N_C \tag{1}$$

where N_C is the total N in a given sample, A_S is the ^{15}N atom% in the labeled sample, A_0 is the ^{15}N atom% in the control sample (which was not provided with ^{15}N–labeled urea), and A_L is the ^{15}N atom% in the labeled fertilizer. N_C was calculated as follows:

$$N_C = W \times N\% \times 1000 \tag{2}$$

where W is the dry weight of a given sample and $N\%$ is the total N content in a given sample. The fertilizer NUE by forage triticale (F_{NUE}) was calculated as follows:

$$F_{NUE}(\%) = N_{dff} / N_F \times 100 \tag{3}$$

where N_{dff} is the amount of N absorbed from the fertilizer in a given crop part and N_F is the total N fertilizer applied.

2.3.3. The Distribution of N Fertilizer in the Soil

Following the harvest of forage triticale on 25 May 2016, soil samples were collected from all plots across five layers within the uppermost 100 cm of soil (0–20 cm, 20–40 cm, 40–60 cm, 60–80 cm, and 80–100 cm). Every soil sample was a mixture of three soil segments from the same depth from each experimental plot. The dried soils samples were finely ground to pass through a 2 mm sieve, and the ^{15}N abundance was analyzed. The ^{15}N abundance of all soil samples was determined using an isotope ratio mass spectrometer (Thermo Fisher Delta V Advantage IRMS). The soil bulk densities were measured before the start of the experiment in October 2015 (1.44, 1.54, 1.48, 1.49, and 1.48 g cm^{-3} for depths of 0–20 cm, 20–40 cm, 40–60 cm, 60–80 cm, and 80–100 cm, respectively) [32]. The N_{dff} in the soil samples was calculated using Equations (1) and (2). The N-fertilizer recovery efficiency in the 0–100 cm soil layer (F_{NRE}) was calculated as follows:

$$F_{NRE}(\%) = \frac{N_{\mathrm{dff}} \text{ in 0–100 cm soil}}{N_F} \times 100 \tag{4}$$

where 'N_{dff} in 0–100 cm soil' is the quantity of N fertilizer recovered in the 0–100 cm soil layer and N_F is the total N fertilizer applied. The N-fertilizer loss efficiency (F_{NLE}) was calculated as follows:

$$F_{NLE}(\%) = 1 - F_{NUE}(\%) - F_{NRE}(\%) \tag{5}$$

where F_{NUE} is the fertilizer NUE by forage triticale and F_{NRE} is the N fertilizer recovery efficiency in the 0–100 cm soil layer.

2.3.4. The Distribution of N Fertilizer in the Livestock

The ^{15}N abundance in the residual contents of the forage triticale samples was determined using an isotope ratio mass spectrometer (Thermo Fisher Delta V Advantage IRMS). The N_{dff} in the residue was calculated using Equations (1) and (2). The N-fertilizer digestion efficiency of the dairy cow (F_{NDE}) was calculated as follows [33]:

$$F_{NDE}(\%) = \frac{N_{dff}\ in\ sample - N_{dff}\ in\ residue}{N_{dff}\ in\ sample} \times 100 \tag{6}$$

where 'N_{dff} in sample' is the initial amount of N fertilizer in a given sample placed in the rumen and 'N_{dff} in residue' is the final amount of N fertilizer in the residue after the rumen incubation. The fertilizer NUE of the dairy cow (F_{NDC}) was calculated as follows [33]:

$$F_{NDC}\ (\%) = \frac{N_{dff}\ in\ total\ forage\ triticale \times F_{NDE}}{N_F} \times 100 \tag{7}$$

where 'N_{dff} in total forage triticale' is the amount of N fertilizer in the total forage triticale of all experimental plots, F_{NDE} is the N fertilizer digestion efficiency of the dairy cow, and N_F is the total N fertilizer applied.

2.4. Statistical Analysis

The recovery of N fertilizer in the soil–forage triticale–dairy cow system was determined through mass balance calculations, considering all measurements on an oven-dried basis. Additionally, N recovery calculations were conducted for forage triticale, soil samples, and residual contents of forage triticale after in vitro rumen incubation. Statistical analysis was performed using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA), with ANOVA tests and Duncan's post hoc test used to assess differences between treatments at a significance level of 0.05 ($p < 0.05$).

3. Results

3.1. The Growth and Development of Forage Triticale

The dry weight of the forage triticale organs (stem, leaf, and whole plant) exhibited variations in response to different levels of applied N fertilizer (Table 2). With the increase in N fertilizer application, the dry weight first increased and then decreased. The dry weights of the stem, leaf, and whole plant were the lowest in the CK treatment. However, the dry weights in the CK treatment were not significantly different from those in the N_0 treatment ($p > 0.05$). The biomass yield of forage triticale was the highest (17.5 t ha^{-1}) under the N_{225} treatment, which was 8.7–33.6% higher than that of the other treatments.

Table 2. The effect of N fertilizer on the growth and development of forage triticale. N_0 represents fertilizer applications of 0 kg N ha^{-1} and 180 kg P_2O_5 ha^{-1}; N_{75}, N_{150}, N_{225}, and N_{300} represent fertilizer applications of N 75, 150, 225, and 300 kg ha^{-1} and fertilizer applications of P_2O_5 180, 180, 180, and 180 kg ha^{-1}; CK represents applications without N fertilizer and P_2O_5 fertilizer. Different lowercase letters in the same column represent significant differences at the 0.05 level, and the same lowercase letter in the same column represents no significance at the 0.05 level.

Treatment	Phenological Stage (Month–Day)		Plant Height (cm)	Stem-to-Leaf Ratio	Dry Weight (g Plant^{-1})			Biomass Yield Per Hectare (t ha^{-1})
	Heading	Flowering			Stem	Leaf	Whole Plant	
N_0	May–3	May–10	156.8 a	1.5 a	4.2 cd	2.8 bc	7.0 cd	14.0 c
N_{75}	May–3	May–10	155.0 a	1.5 a	4.2 cd	2.9 bc	7.1 cd	14.2 bc
N_{150}	May–3	May–10	157.4 a	1.5 a	4.5 bc	3.0 ab	7.5 bc	15.1 bc
N_{225}	May–4	May–11	160.9 a	1.6 a	5.3 a	3.4 a	8.7 a	17.5 a
N_{300}	May–4	May–11	157.7 a	1.5 a	4.9 b	3.2 ab	8.1 ab	16.1 ab
CK	May–3	May–10	163.2 a	1.6 a	4.0 d	2.6 c	6.5 d	13.1 c

3.2. Nutritive Quality of Forage Triticale

The impacts of different N-fertilizer treatments on the nutritional contents of forage triticale are presented in Table 3. The N content in the whole plant exhibited a significant increase in response to N-fertilizer application treatments compared to the CK treatment ($p < 0.05$). Among these treatments, the highest N content (1.45%) was observed under the N_{300} treatment, representing a 9.0% increase over the N_0 treatment. Moreover, the NDF content in the whole plant significantly increased under the N_{225}, N_{300}, and CK treatments, compared to other treatments ($p < 0.05$).

Table 3. The effect of N fertilizer on the N content, water-soluble carbohydrate (WSC), starch, neutral detergent fiber (NDF), acid detergent fiber (ADF) contents of forage triticale at milk maturity stage. N_0 represent fertilizer applications of 0 kg N ha^{-1} and 180 kg P_2O_5 ha^{-1}, N_{75}, N_{150}, N_{225} and N_{300} represents fertilizer applications of N 75, 150, 225, and 300 kg ha^{-1} and fertilizer applications of P_2O_5 180, 180, 180, and 180 kg ha^{-1}; CK represents applications without N fertilizer and P_2O_5 fertilizer. Different lowercase letters in the same column represent significant differences at the 0.05 level, and the same lowercase letter in the same column represents no significance at the 0.05 level.

Treatment	N Content (%)	WSC (%)	Starch (%)	NDF (%)	ADF (%)
N_0	1.3 bc	24.8 ab	6.8 ab	50.1 b	31.6 a
N_{75}	1.4 ab	24.8 ab	7.7 a	49.8 b	31.0 a
N_{150}	1.4 ab	25.5 a	6.0 b	50.4 b	31.5 a
N_{225}	1.4 ab	25.2 ab	6.0 b	56.6 a	32.8 a
N_{300}	1.5 a	24.3 ab	6.8 ab	55.2 a	32.3 a
CK	1.3 c	22.8 b	7.5 a	55.4 a	32.7 a

3.3. The Migration of N Fertilizer in the Crop

With increasing concentration of the N fertilizer application, the accumulation of N in the forage triticale organs (stem, leaf, and whole plant) first increased and then decreased (Table 4). When the forage triticale plants were harvested in the stage of milk maturity, the accumulation of N in the stem, leaves, and whole plant was highest under the N_{225} treatment and was significantly higher than under all the other treatments except N_{300} ($p < 0.05$). The N accumulation in the whole plant was significantly higher in N_0 than in CK ($p < 0.05$).

Table 4. Total N, N_{dff}, and F_{NUE} in forage triticale plants under different fertilizer N treatments. 'N_{dff} in forage triticale' represents the amount of fertilizer N in forage triticale, F_{NUE} represents fertilizer NUE by forage triticale. N_0 represents fertilizer applications of 0 kg N ha^{-1} and 180 kg P_2O_5 ha^{-1}, N_{75}, N_{150}, N_{225} and N_{300} represent fertilizer applications of N 75, 150, 225, and 300 kg ha^{-1} and fertilizer applications of P_2O_5 180, 180, 180, and 180 kg ha^{-1}; CK represents applications without N fertilizer and P_2O_5 fertilizer. Different lowercase letters in the same column represent significant differences at the 0.05 level, and the same lowercase letter in the same column represents no significance at the 0.05 level. "--" represents no value, because no fertilizer N was applied in N_0 and CK treatments.

Treatment	Total N (mg $Plant^{-1}$)			N_{dff} in Forage Triticale (mg $Plant^{-1}$)			The Proportion of N_{dff} in Total N of Forage Triticale (%)			F_{NUE} (%)		
	Stem	Leaf	Whole Plant	Stem	Leaf	Whole Plant	Stem	Leaf	Whole Plant	Stem	Leaf	Whole Plant
N_0	35.3 cd	57.7 cd	93.0 b	--	--	--	--	--	--	--	--	--
N_{75}	36.2 cd	63.8 bc	100.0 b	5.0 d	8.9 d	13.8 d	13.8 d	14.0 d	13.9 d	13.3 a	23.6 a	36.9 a
N_{150}	40.1 bc	63.1 bc	103.2 b	10.7 c	17.3 c	28.0 c	26.6 c	27.4 c	27.1 c	14.2 a	23.1 a	37.3 a
N_{225}	48.0 a	72.9 a	120.9 a	16.6 b	25.3 b	42.0 b	34.6 b	34.7 b	34.7 b	14.8 a	22.5 a	37.3 a
N_{300}	45.4 ab	71.2 ab	116.6 a	19.8 a	31.4 a	51.2 a	43.7 a	44.1 a	43.9 a	13.2 a	20.9 a	34.1 a
CK	30.7 d	51.1 d	81.8 c	--	--	--	--	--	--	--	--	--

The N_{dff} in the forage triticale organs (stem, leaf, and whole plant) gradually increased with the increase in the N-fertilizer application. The greatest N_{dff} was observed in the N_{300} treatment, and this value was significantly higher than the value in the other treatments ($p < 0.05$). When the N application increased from N_{75} to N_{300}, the proportion of N_{dff} in total N of stem, leaf, and whole plant significantly increased, from 13.8% to 43.7%, from 14.0% to 44.1%, and from 13.9% to 43.9%, respectively ($p < 0.05$). It was found that 35.9–39.6% of the N_{dff} accumulated in the stems and 60.4–64.1% accumulated in the leaves. The F_{NUE} in the whole plant ranged from 34.1% to 37.3% (Table 4), with the highest F_{NUE} under the N_{225} treatment and the lowest F_{NUE} under the N_{300} treatment.

3.4. The Distribution of N Fertilizer in the Soil

The amount of N fertilizer recovered in the 0–100 cm soil layer under different N fertilizer treatments was 2.7–11.7 g m^{-2}. The amount of N fertilizer recovered increased with the increase in the N-fertilizer application rate ($p < 0.05$) (Table 5) and was 36.1–333.3% higher under the N_{300} treatment than under the other treatments. The recovered N fertilizer was mainly distributed in the surface soil layers (0–20 cm and 20–40 cm); 55.7–66.8% was distributed in the 0–20 cm soil layer and 22.7–27.0% was distributed in the 20–40 cm soil layer. The 0–40 cm soil layer accounted for 81.8–91.3% of the recovered N fertilizer.

Table 5. The N_{dff} and F_{NRE} in the different soil layers and the F_{NLE} after harvesting forage triticale plants in the stage of milk maturity. 'N_{dff} in the different soil layers' represents the amount of N fertilizer recovered in the different soil layers. 'F_{NUE} in the different soil layers' represents N fertilizer recovery efficiency in the different soil layers. F_{NLE} represents the N fertilizer loss efficiency. N_{75}, N_{150}, N_{225}, and N_{300} represent fertilizer applications of N 75, 150, 225, and 300 kg ha^{-1} and fertilizer applications of P_2O_5 180, 180, 180, and 180 kg ha^{-1}. Different lowercase letters in the same column represent significant differences at the 0.05 level, and the same lowercase letter in the same column represents no significance at the 0.05 level.

Treatment	N_{dff} (g m^{-2}) in Different Soil Layers (cm)						F_{NRE} (%) in Different Soil Layers (cm)						F_{NLE} (%)
	0–20	20–40	40–60	60–80	80–100	0–100	0–20	20–40	40–60	60–80	80–100	0–100	
N_{75}	1.5 d	0.7 d	0.2 c	0.2 b	0.1 b	2.7 d	20.2 b	9.5 b	3.1 a	2.3 a	1.3 a	36.3 b	26.8 a
N_{150}	3.7 c	1.3 c	0.3 bc	0.2 b	0.2 ab	5.7 c	24.6 a	8.6 c	1.9 b	1.3 b	1.3 a	37.8 ab	25.0 a
N_{225}	5.7 b	2.1 b	0.4 b	0.2 b	0.2 ab	8.6 b	25.4 a	9.3 b	1.7 b	0.8 c	0.8 a	38.1 a	24.6 a
N_{300}	7.3 a	3.2 a	0.7 a	0.3 a	0.3 a	11.7 a	24.5 a	10.6 a	2.2 b	0.9 c	1.0 a	39.1 a	26.8 a

The F_{NRE} under different N-fertilizer treatments ranged from 36.3% to 39.1% and increased with the increase in the N-fertilizer application rate. The F_{NRE} was significantly higher under the N_{225} and N_{300} treatments than under the N_{75} treatment ($p < 0.05$). No significant difference was observed for the F_{NLE} among the various N-fertilizer treatments ($p > 0.05$).

3.5. The Utilization of N Fertilizer in the Livestock

^{15}N–labeled forage triticale was used for the in vitro dairy cow rumen incubation to determine the F_{NDE} in the dairy cows (Figure 1). The F_{NDE} was 81.9–85.0% in the dairy cows; thus, 15.0–18.1% of the N was not digested and utilized by the cow. Nevertheless, there were no significant differences in F_{NDE} among treatments ($p > 0.05$). The F_{NDC} exhibited by the dairy cow was calculated using the N_{dff} in total forage triticale, F_{NDE}, and N_F. The results showed that the F_{NDC} of the dairy cow in the different N-fertilizer treatments was 28.1–31.3%, with no significant differences among treatments ($p > 0.05$).

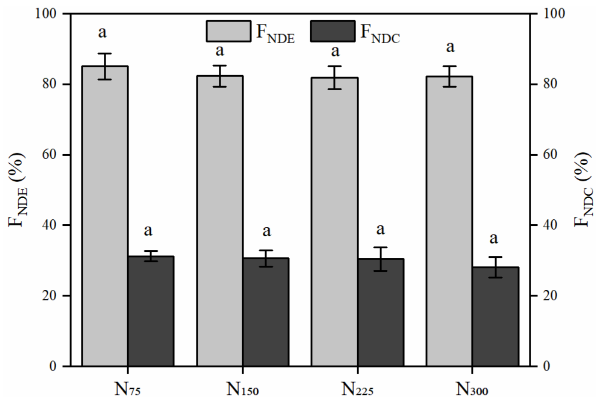

Figure 1. The F_{NDE} and F_{NDC} in dairy cows under different N-fertilizer treatments. F_{NDE} represents the N-fertilizer digestion efficiency of the dairy cows and F_{NDC} represents the fertilizer NUE of the

dairy cows. N_{75}, N_{150}, N_{225}, and N_{300} represent fertilizer applications of N 75, 150, 225, and 300 kg ha^{-1}, respectively, and fertilizer applications of $P_2O_5$180, 180, 180, and 180 kg ha^{-1}, respectively. Different lowercase letters in the different treatments represent significant differences at the 0.05 level, and the same lowercase letter in the in the different treatments represents no significance at the 0.05 level.

3.6. The Distribution of N Fertilizer in the Soil–Forage Triticale–Dairy Cow System

Overall, 34.1–37.3% of the N fertilizer was absorbed by forage triticale: 13.2–14.8% accumulated in the stems, accounting for 35.9–39.6% of the total, and 20.9–23.6% accumulated in the leaves, accounting for 60.4–64.1% of the total. Furthermore, 36.3–39.1% of the N fertilizer was recovered in the 0–100 cm soil layer; 29.7–35.2% was distributed in the 0–40 cm soil layer, accounting for 81.8–91.3% of the recovery in the 0–100 cm soil layer (Figure 2); the rest (24.6–26.8% of the N fertilizer) was lost in various ways. In addition, 28.1–31.3% of the N fertilizer was digested by the dairy cows and 15.0–18.2% of the N fertilizer in the forage triticale was not digested by the dairy cows, which accounted for 5.6–6.9% of the N fertilizer (Figure 3).

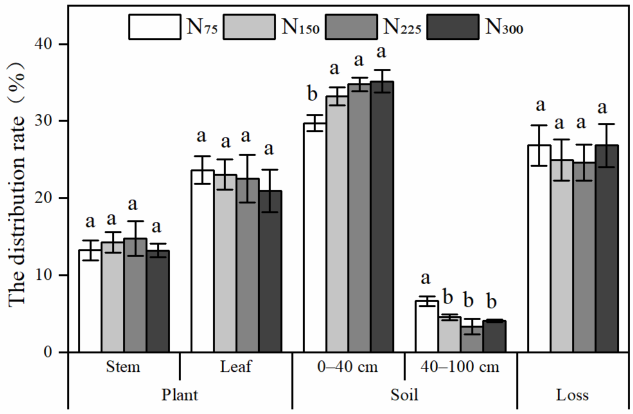

Figure 2. The distribution rate of N fertilizer in the soil–forage triticale system, including the stem and leaf of forage triticale, 0–40 cm and 40–100 cm soil layers, and loss in various ways. N_{75}, N_{150}, N_{225}, and N_{300} represent fertilizer applications of N 75, 150, 225, and 300 kg ha^{-1}, respectively, and fertilizer applications of $P_2O_5$180, 180, 180, and 180 kg ha^{-1}, respectively. Different lowercase letters in the different treatments represent significant differences at the 0.05 level, and the same lowercase letter in the in the different treatments represents no significance at the 0.05 level.

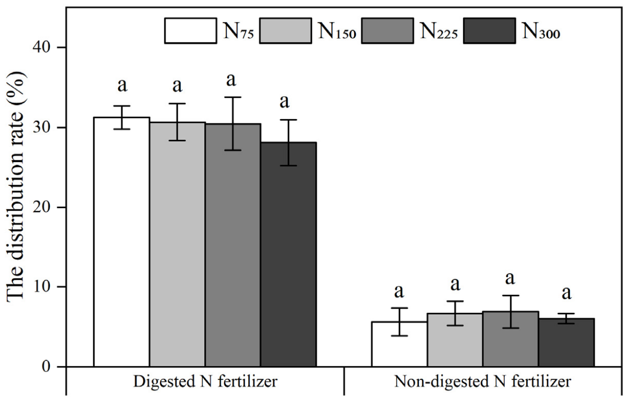

Figure 3. The distribution rate of N fertilizer in the forage triticale–dairy cow system. N_{75}, N_{150}, N_{225}, and N_{300} represent fertilizer applications of N 75, 150, 225, and 300 kg ha^{-1}, respectively, and

fertilizer applications of $P_2O_5$180, 180, 180, and 180 kg ha^{-1}, respectively. Different lowercase letters in the different treatments represent significant differences at the 0.05 level, and the same lowercase letter in the in the different treatments represents no significance at the 0.05 level. Different lowercase letters in the different treatments represent significant differences at the 0.05 level, and the same lowercase letter in the in the different treatments represents no significance at the 0.05 level.

4. Discussion

4.1. Effects of N-Fertilizer Application on Growth of Forage Triticale

The impact of N fertilizer on crop biomass is influenced by soil fertility, agronomic practices, and management strategies for fertilizer applications [34]. He et al. [35] emphasized that the impact of N fertilizer on biomass yield and the N content of forage triticale was influenced not only by the quantity of applied fertilizer, but also by prevailing weather conditions. Rainfall and temperature affected the uptake of N by crops, which in turn influenced the biomass yield and N content of forage triticale. In our study, the biomass yield of forage triticale tended to increase with increasing N fertilizer rates and gained the highest biomass yield (17.5 t ha^{-1}) when the N-fertilizer application rate was 225 kg N ha^{-1}, while there was no significant difference in the N content of forage triticale between different N application rates ($p > 0.05$). However, Gibson et al. [23] showed that the maximum biomass yield of forage triticale was highest (9.2 t ha^{-1}) under the treatment of 33 kg N ha^{-1} at Ames, while the maximum biomass yield was highest (10.3 t ha^{-1}) under the treatment of no N application at Lewis. In another experiment, conducted by You et al. [27], the result indicated that there was no significant difference in the two-year average biomass yield of forage triticale (15.5 t ha^{-1}) between the N-fertilized treatments (60–300 kg N ha^{-1}) and CK (no N fertilizer applied). In addition, the crude protein content was significantly higher in the N–applied treatments than that in CK. The studies showed inconsistent results on the effects of N-fertilizer application on the biomass and N content of forage triticale. In addition to the inconsistencies in the years and locations of the studies, another important reason may be the initial soil N content. In our research, the initial soil N contents in 0–20 and 20–40 cm soil layers were higher (up to 68.7 mg·kg^{-1} and 46.6 mg·kg^{-1}, respectively), while the initial soil N content in 0–20 cm soil layers was 62.8 mg·kg^{-1} in the study of You et al. [27]. Gibson et al. [23] did not provide the initial soil N content in 0–20 cm at the Ames and Lewis sites, which may be the reason why the biomass yield of forage triticale in our study was higher than that of Gibson et al. [23] and You et al. [27].

4.2. N-Fertilizer Recovery and Allocation by Forage Triticale

Regarding the migration and distribution patterns of N fertilizer, the absorption kinetics of N fertilizer by crops is particularly imperative for optimizing the efficient utilization of this nutrient [36]. In this study, ^{15}N–labeled urea was applied to forage triticale in order to investigate the migration and distribution of N fertilizer within the soil–forage triticale–dairy cow system. The results showed that the forage triticale absorbed 34.1–37.3% of the N fertilizer when harvested in the stage of milk maturity. This result is consistent with the fertilizer NUE (32.3–46.0%) observed for forage triticale by Ferchaud et al. [24]. There was no significance observed in the fertilizer NUE of forage triticale with the increasing application rate in N fertilizer. The absorption of soil N by the forage triticale represented 56–86% of the total N absorbed, which was higher than the absorption of N fertilizer (Table 4), and it decreased gradually with the increase in N application. The results agreed well with the findings of Rimski-Korsakov et al. [14]. In our study, the maximum, minimum, and mean of the air temperature and precipitation during the experiment were good representatives of the long-term average of NCP (1981 to 2010), well representing the climatic characteristics of the forage triticale growing season in the NCP. Therefore, the results of this study can accurately express the N recovery and distribution of forage triticale in the NCP. Jenkinson et al. [37] and Rao et al. [38] proposed that the application of N fertilizer to soil could enhance the uptake of native soil N (i.e., the added–N interaction ANI). However, due to preferential fixation, ^{15}N was found to be more tightly bound in the soil compared to ^{14}N, resulting in limited availability of ^{15}N for crop uptake and, consequently, reducing the recovery of N fertilizer through ^{15}N–labeled technology. In our study, the absorption of soil N by the forage triticale reduced with increasing the N-fertilizer application rates, and no ANI were observed (Table 4). Quan et al. [13] proposed that the abundance of available N in soil inhibits the 'pool substitution' between N fertilizer and soil N, resulting in reduced uptake of soil N by maize under N treatment, compared to the control. Therefore, our study employed ^{15}N–labeled technology to accurately quantify N-fertilizer recovery and allocation, ensuring reasonable and reliable results.

In this study, the fertilizer NUE of forage triticale was low, except for the factors of variety and weather conditions. This may also be attributed to the high initial soil N content (Table 1). Based on the total N uptake by forage triticale (Table 4) and its seeding density, forage triticale absorbed between 186–233 kg N ha^{-1} throughout the growing season, with a majority of the absorbed N (56–86%) originating from soil N. Soils with high N content are common throughout the world, which was one of the reasons why we continued to study the migration and distribution of N fertilizer with such high initial soil N content. However, in order to maintain soil N levels at an optimal range for ensuring sustainable agricultural development, the application of N-based fertilizers assumes a pivotal role in preserving soil N equilibrium while, concurrently, enhancing soil fertility and productivity. Another reason was the fertilization period under the same application rate, which was also verified in wheat. The fertilizer NUE of wheat at the tillering stage was found to be significantly lower than that at the elongation stage [39]. In the present experiment, 50% of the N fertilizer was applied as seed fertilizer during sowing, while the remaining 50% was applied at the elongation stage. The forage triticale was harvested at the milk maturity stage, which occurred approximately one month earlier than the grain stage. The high growth rates and the consequent shortened growth period may contribute to the low fertilizer NUE. In the present study, estimates of N fertilizer recovery were based solely on above-ground biomass, without considering nitrogen accumulation in roots. This is another reason explaining the low fertilizer NUE of forage triticale.

In general, during the vegetative stage, the distribution of N in each organ of the plant prioritizes meeting the growth center and young plant tissue requirements before fulfilling the reproductive organs' growth needs [40]. Evans [41] proposed that the N distribution in the leaves prior to flowering primarily caters to plant material synthesis demands. In this study, when harvesting forage triticale at the milk stage, N-accumulation was found to be higher in the leaves than in the stems. Statistically, 35.88–39.62% of absorbed N fertilizer accumulated in stems, while 60.38–64.12% accumulated in the leaves. This result was in accordance with the findings of Liu [42], but it was incompatible with the findings of Zhang et al. [43]. When measuring N accumulation in the different organs at the heading stage of forage triticale, Zhang et al. [43] divided the plants into roots, stems, and leaves, but did not make clear whether the ears were counted with the stems or the leaves. In the present study, the ears were grouped with the leaves, and this was probably the reason for the differences between our research results and those of Zhang et al. [43].

4.3. N-Fertilizer Residue in Soil

When fertilizer N was applied to forage triticale at 75–300 kg N ha^{-1}, the recovery rate of N fertilizer in the 0–60 cm soil was between 32.8 and 37.3% (Table 5). This recovery rate was higher than the rate of 12.9–24.4% found by Ferchaud et al. [24], who applied N fertilizer at a rate of 60–120 kg N ha^{-1} and took samples at a soil depth of 58 cm. The N-fertilizer application rate used by Ferchaud et al. [24] was, thus, lower than those used in our study, which may explain the lower recovery rate of N fertilizer in the soil. Meanwhile, Ma et al. [44] showed that the recovery rate of N fertilizer in wheat was 15.8–34.3% when 168 kg N ha^{-1} and 240 kg N ha^{-1} of N fertilizer were applied, which was consistent with the results of the present study.

Ferchaud et al. [24], Li et al. [45], and Macdonald et al. [46] showed that the N fertilizer was mainly distributed in the surface soil (0–20 cm). In the present study, 55.7–66.8% of the residual ^{15}N was distributed in the 0–20 cm soil layer, and the percentage was lower than the results of Ferchaud et al. [24] (83%), Li et al. [45] (78%), and Macdonald et al. [46] (85%). Ferchaud et al. [24] considered that part of the added ^{15}N may have downward movement in the soil, but it was not measured in their study. In our study, 81.8–91.3% of the N fertilizer was distributed in soil depths of 0–40 cm, suggesting that the N fertilizer did not move downward in the soil. The N fertilizer generally moves downward with water flows in the soil; however, the average annual rainfall provided to the forage triticale growing in the study area was 130.3 mm during the growth period, so the possibility of leaching was low. The rate of recovery of N fertilizer in the different soil layers when the forage triticale plants were harvested also showed that the possibility of leaching in the study area was low.

In the present study, the N distribution was divided into plant absorption (stems and leaves) and soil recovery, and the rest was calculated according to the loss after clarifying the migration and distribution of the N fertilizer. Plant litter and roots also absorb a large amount of N fertilizer, and this was not counted as plant absorption or soil recovery. These crop residues (litter and roots) remain in the soil and are converted into soil N via the action of microorganisms [46]. Therefore, the N-fertilizer loss rate calculated in the present study was higher than the actual loss. The results of this study showed that the F_{NLE} under different N-fertilizer treatments was 24.6–26.8%. However, the loss route was not partitioned between volatilization and leaching. Further limitations of this study were that the existing form of N fertilizer recovered in the soil, and the absorption and utilization of N fertilizer recovered in the soil by the following crops, were not investigated.

4.4. N-Fertilizer Flows in Soil–Forage Triticale–Dairy Cow System

Quan et al. [13] investigated the dynamics of N fertilizer in the soil–maize system in China and found that maize at harvest took up 52.0% of the N fertilizer; 25% of the N fertilizer remained in the soil and 23% was lost. Thomsen and Christensen [47] studied the recovery of N fertilizer in winter wheat, spring barley (*Hordeum vulgare* L.), and soil in different tillage systems. They reported that 74–75% of N fertilizer was recovered in the crops and sand soil and 86–92% was recovered in the crops and loamy sand soil. Regarding the crop–livestock systems, there have been many reports on the migration and distribution of N fertilizer from crops to livestock. Powell et al. [33] used four ^{15}N–labeled fodders to feed cows and found that the fertilizer NUE by the cow was significantly different ($p < 0.05$). Barros et al. [17] reported that 61% of ^{15}N was recovered into the milk, urine, and feces of lactating dairy cows. In the soil–plant–cow system, Huang et al. [48] used ^{15}N–labeled urea to determine the fertilizer NUE of eight types of forage, used eight types of ^{15}N–labeled forage to feed mice, and measured the fertilizer NUE of the mice. However, in their study, the mice did not have functioning rumens and, thus, cannot reflect the digestion and utilization of N fertilizer within the forage. Therefore, as far as we know, our study could be the first to use the ^{15}N–labeled technology to quantify the migration and distribution of N fertilizer in the soil–forage triticale–dairy cow system. Considering the high price of ^{15}N–labeled urea, we adopted the method of in situ rumen incubation. Despite the fact that this method is different from the actual feeding effects, it can basically quantify the migration and distribution of N fertilizer in the soil–forage triticale–dairy cow system. In this study, the CP digested in the rumen was regarded as the N fertilizer utilized and recovered by animals. However, the CP digested by the small intestine and the N fertilizer excreted in the manure and urine still need further quantification. In addition, feeding cows with feed containing nitrates in quantities exceeding the maximum permissible concentration can be harmful to the health of the animals [49]. In rumen, nitrates are converted into nitrites faster than nitrites are converted into ammonia, so they accumulate and, after absorption into the blood, convert hemoglobin into methemoglobin, which leads to nutritional anemia of animals, and their productivity is significantly reduced [50]. We did not check the nitrate concentration in the forage triticale sample and the relations between the plant nitrate contents and the N application rates need further exploration in the future.

5. Conclusions

Our study used the ^{15}N-labeled technology and the in situ rumen incubation technique to quantify the migration and distribution of N fertilizer in the soil–forage triticale–dairy cow system. The results indicated that under the N-fertilizer application rate of 75–300 kg N ha^{-1}, 34.1–37.3% of the N fertilizer was absorbed by forage triticale, 36.3–39.1% was recovered in the 0–100 cm soil layer, and 24.6–26.8% was lost in various ways. The in situ rumen incubation technique revealed that 28.1–31.3% of the N fertilizer was digested and utilized by the dairy cows. Within the forage triticale plants, 35.9–39.6% of the N fertilizer accumulated in the stems and 60.4–64.1% accumulated in the leaves. The recovered N fertilizer in the soil was mainly distributed in the surface soil (0–40 cm), accounting for 81.8–91.3% of the recovered N fertilizer. Overall, 81.9–85.0% of the N fertilizer absorbed by the forage triticale was digested by the dairy cows, and 15.0–18.1% was lost. The amount of N fertilizer absorbed by the forage triticale, stored in the soil, and the losses to the environment increased with the increase in the N-fertilizer application rate; however, the distribution of N fertilizer in the soil–forage triticale-dairy cow system had no significant effect. Although increased N-fertilizer application improved the biomass yield and N content of forage triticale, it also increased N storage in the soil and losses to the environment. Therefore, no more than 225 kg N ha^{-1} is recommended as the optimal application rate to maintain high-efficient N use in the soil–forage triticale–dairy cow system, while reducing the environmental risks in the NCP.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13123073/s1. Table S1: Basic diets composition and nutrition levels of mid-late lactation cows [51].

Author Contributions: Y.Y.: conceptualization, methodology, writing—original draft and editing; G.L.: supervision, funding acquisition; X.Y.: investigation, formal analysis; Z.W.: software, resources; Y.L.: data curation, formal analysis; X.L.: data curation, software, editing; Y.S.: project administration, funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare that they have no conflict of interest.

References

1. Quan, Z.; Li, S.; Zhang, X.; Zhu, F.; Li, P.P.; Sheng, R.; Chen, X.; Zhang, L.M.; He, J.Z.; Wei, W.X.; et al. Fertilizer nitrogen use efficiency and fates in maize cropping systems across China: Field ^{15}N tracer studies. *Soil Tillage Res.* **2020**, *197*, 104498. [CrossRef]
2. Tilman, D.; Balzer, C.; Hill, J.; Befort, B.L. Global food demand and the sustainable intensification of agriculture. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 20260–20264. [CrossRef] [PubMed]
3. Yu, C.Q.; Huang, X.; Chen, H.; Godfray, H.C.J.; Wright, J.S.; Hall, J.W.; Gong, P.; Ni, S.Q.; Qiao, S.C.; Huang, G.R.; et al. Managing nitrogen to restore water quality in China. *Nature* **2019**, *567*, 516–520. [CrossRef]
4. Li, Z.; Cui, S.; Zhang, Q.P.; Xu, G.; Feng, Q.S.; Chen, C.; Li, Y. Optimizing wheat yield, water, and nitrogen use efficiency with water and nitrogen inputs in China: A synthesis and life cycle assessment. *Front. Plant Sci.* **2022**, *13*, 930484. [CrossRef] [PubMed]
5. Ladha, J.K.; Pathak, H.; Krupnik, T.J.; Six, J.; Kessel, C.V. Efficiency of fertilizer nitrogen in cereal production: Retrospects and prospects. *Adv. Agron.* **2005**, *87*, 85–156.
6. Zhang, F.S.; Wang, J.Q.; Zhang, W.F.; Cui, Z.L.; Ma, W.Q.; Chen, X.P.; Jiang, R.F. Nutrient use efficiencies of major cereal crops in China and measures for improvement. *Acta Pedol. Sin.* **2008**, *45*, 915–924. (In Chinese)
7. Dragon, K. Groundwater nitrate pollution in the recharge zone of a regional Quaternary flow system (Wielkopolska region, Poland). *Environ. Earth Sci.* **2013**, *68*, 2099–2109. [CrossRef]
8. Zhang, Y.; Dore, A.J.; Ma, L.; Liu, X.J.; Ma, W.Q.; Cape, J.N.; Zhang, F.S. Agricultural ammonia emissions inventory and spatial distribution in the North China Plain. *Environ. Pollut.* **2010**, *158*, 490–501. [CrossRef]

9. Zheng, J.S.; Qu, Y.; Kilasara, M.M.; Mmari, W.N.; Funakawa, S. Nitrate leaching from the critical root zone of maize in two tropical highlands of Tanzania: Effects of fertilizer-nitrogen rate and straw incorporation. *Soil Tillage Res.* **2019**, *194*, 104295. [CrossRef]
10. Bai, Z.H.; Ma, L.; Oenema, O.; Chen, Q.; Zhang, F.S. Nitrogen and phosphorus use efficiencies in dairy production in China. *J. Environ. Qual.* **2013**, *42*, 990–1001. [CrossRef]
11. Powell, J.M.; Gourley, C.J.P.; Rotz, C.A.; Weaver, D.M. Nitrogen use efficiency: A potential performance indicator and policy tool for dairy farms. *Environ. Sci. Policy* **2010**, *13*, 217–228. [CrossRef]
12. Bai, Z.H.; Ma, L.; Qin, W.; Chen, Q.; Oenema, O.; Zhang, F.S. Changes in pig production in China and their effects on nitrogen and phosphorus use and losses. *Environ. Sci. Technol.* **2014**, *48*, 12742–12749. [CrossRef] [PubMed]
13. Quan, Z.; Li, S.L.; Zhang, X.; Zhu, F.F.; Li, P.P.; Sheng, R.; Chen, X.; Zhang, L.M.; He, J.Z.; Wei, W.X.; et al. Fates of ^{15}N-labeled fertilizer in a black soil-maize system and the response to straw incorporation in Northeast China. *J. Soils Sediments* **2018**, *18*, 1441–1452. [CrossRef]
14. Rimski-Korsakov, H.; Rubio, G.; Lavado, R.S. Fate of the nitrogen from fertilizers in field-grown maize. *Nutr. Cycl. Agroecosystems* **2012**, *93*, 253–263. [CrossRef]
15. Ma, L.N.; Gao, X.L.; Liu, G.F.; Xu, X.F.; Lü, X.; Xin, X.P.; Lü, Y.X.; Zhang, C.X.; Zhang, L.H.; Wang, R.Z. The retention dynamics of N input within the soil–microbe–plant system in a temperate grassland. *Geoderma* **2020**, *368*, 114290. [CrossRef]
16. Roth, R.T.; Lacey, C.G.; Camberato, J.J.; Armstrong, S.D. Quantifying the fate of nitrogen from cereal rye root and shoot biomass using ^{15}N. *Nutr. Cycl. Agroecosystems* **2023**, *125*, 219–234. [CrossRef]
17. Barros, T.; Powell, J.M.; Danes, M.A.C.; Aguerre, M.J.; Wattiaux, M.A. Relative partitioning of N from alfalfa silage, corn silage, corn grain and soybean meal into milk, urine, and feces, using stable ^{15}N isotope. *Anim. Feed Sci. Technol.* **2017**, *229*, 91–96. [CrossRef]
18. Chen, Y.T.; McNamara, J.P.; Ma, G.L.; Harrison, J.H.; Block, E. Milk ^{13}C and ^{15}N discriminations as biomarkers of feed efficiency and energy status in early lactation cows. *Anim. Feed Sci. Technol.* **2020**, *269*, 114638. [CrossRef]
19. Gao, W.; Gao, X.; Chen, A.; Zhang, F.; Chen, D.; Liu, C. Effect of dietary dry matter intake on endogenous nitrogen flows in growing lambs. *J. Anim. Physiol. Anim. Nutr.* **2017**, *101*, 383–393. [CrossRef]
20. Ayalew, H.; Kumssa, T.T.; Butler, T.J.; Ma, X. Triticale improvement for forage and cover crop uses in the southern great plains of the United States. *Front. Plant Sci.* **2018**, *9*, 1130. [CrossRef]
21. Villalobos, L.; Brummer, J.E. Yield and nutritive value of cool-season annual forages and mixtures seeded into pearl millet stubble. *Agron. J.* **2017**, *109*, 432–441. [CrossRef]
22. Mcgoverin, C.M.; Snyders, F.; Muller, N.; Botes, W.; Fox, G.; Manley, M. A review of triticale uses and the effect of growth environment on grain quality. *J. Sci. Food Agric.* **2011**, *91*, 1155–1165. [CrossRef] [PubMed]
23. Gibson, L.R.; Nance, C.D.; Karlen, D.L. Winter triticale response to nitrogen fertilization when grown after corn or soybean. *Agron. J.* **2007**, *99*, 49–58. [CrossRef]
24. Ferchaud, F.; Vitte, G.; Machet, J.M.; Beaudoin, N.; Catterou, M.; Mary, B. The fate of cumulative applications of ^{15}N-labelled fertilizer in perennial and annual bioenergy crops. *Agric. Ecosyst. Environ.* **2016**, *223*, 76–86. [CrossRef]
25. Zavalin, A.A.; Kurishbayev, A.K.; Ramazanova, R.K.; Tursinbaeva, A.E.; Kassipkhan, A. Fertilizer nitrogen use by spring triticale and spring wheat on dark-chestnut soil of the dry steppe zone of Kazakhstan. *Russ. Agric. Sci.* **2018**, *44*, 153–156. [CrossRef]
26. Jia, S.L.; Wang, X.B.; Yang, Y.M.; Dai, K.A.; Meng, C.X.; Zhao, Q.S.; Zhang, X.M.; Zhang, D.C.; Feng, Z.H.; Sun, Y.M.; et al. Fate of labeled urea-^{15}N as basal and topdressing applications in an irrigated wheat–maize rotation system in North China Plain: I winter wheat. *Nutr. Cycl. Agroecosystems* **2011**, *90*, 331–346. [CrossRef]
27. You, Y.L.; Li, Y.; Zhao, H.M.; Wu, R.X.; Liu, G.B. Effects of nitrogen and phosphate fertilizer application on yield and forage quality of forage triticale on the Haihe Plain. *Acta Prataculturae Sin.* **2020**, *29*, 137–146. (In Chinese)
28. Monteiro, H.F.; Paula, E.M.; Muck, R.E.; Broderick, G.A.; Faciola, A.P. Effects of lactic acid bacteria in a silage inoculant on ruminal nutrient digestibility, N metabolism, and lactation performance of high-producing dairy cows. *J. Dairy Sci.* **2021**, *104*, 8826–8834. [CrossRef]
29. Zhang, X.M.; Gruninger, R.J.; Alemu, A.W.; Wang, M.; Tan, Z.L.; Kindermann, M.; Beauchemin, K.A. 3-nitrooxypropanol supplementation had little effect on fiber degradation and microbial colonization of forage particles when evaluated using the in situ ruminal incubation technique. *J. Dairy Sci.* **2020**, *103*, 8986–8997. [CrossRef]
30. Van Soest, P.J.; Sniffen, C.J.; Mertens, D.R.; Fox, D.G.; Robinson, P.H.; Krishnamoorthy, U. A net protein system for cattle: The rumen submodel for nitrogen. In *Protein Requirements for Cattle (MP109-P)*; Oklahoma State University: Stillwater, OH, USA, 1981; Volume 256.
31. Murphy, R. A method for the extraction of plant samples and the determination of total soluble carbohydrates. *J. Sci. Food Agric.* **1958**, *9*, 714–717. [CrossRef]
32. Zhang, C.; Rees, R.M.; Ju, X.T. Fate of ^{15}N-labelled urea when applied to long-term fertilized soils of varying fertility. *Nutr. Cycl. Agroecosystems* **2021**, *121*, 151–165. [CrossRef]
33. Powell, J.M.; Barros, T.; Danes, M.; Aguerre, M.; Wattiaux, M.; Reed, K. Nitrogen use efficiencies to grow, feed, and recycle manure from the major diet components fed to dairy cows in the USA. *Agric. Ecosyst. Environ.* **2017**, *239*, 274–282. [CrossRef]
34. Blesh, J.; Drinkwater, L.E. Retention of ^{15}N-labeled fertilizer in an Illinois prairie soil with winter rye. *Soil Sci. Soc. Am. J.* **2014**, *78*, 496–508. [CrossRef]

35. He, J.F.; Goyal, R.; Laroche, A.; Zhao, M.L.; Lu, Z.X. Water stress during grain development affects starch synthesis, composition and physicochemical properties in triticale. *J. Cereal Sci.* **2012**, *56*, 552–560. [CrossRef]
36. Chen, X.P.; Cui, Z.L.; Fan, M.S.; Vitousek, P.; Zhao, M.; Ma, W.Q.; Wang, Z.L.; Zhang, W.J.; Yan, X.Y.; Yang, J.C.; et al. Producing more grain with lower environmental costs. *Nature* **2014**, *514*, 486–489. [CrossRef]
37. Jenkinson, D.S.; Fox, R.H.; Rayner, J.H. Interactions between fertilizer nitrogen and soil nitrogen–the so–called 'priming' effect. *J. Soil Sci.* **1985**, *36*, 425–444. [CrossRef]
38. Rao, A.C.S.; Smith, J.L.; Papendick, R.I.; Parr, J.F. Influence of added nitrogen interactions in estimating recovery efficiency of labeled nitrogen. *Soil Sci. Soc. Am. J.* **1991**, *55*, 1616–1621. [CrossRef]
39. Recous, S.; Machet, J.M.; Mary, B. The fate of labelled ^{15}N urea and ammonium nitrate applied to a winter wheat crop: II. Plant uptake and N efficiency. *Plant Soil* **1988**, *112*, 215–224. [CrossRef]
40. Han, C.; Zhang, J.H.; Zhang, S.C.; Zhou, G.Z.; Li, D.P. The varieties between the fertilizer and rice leaf nitrogen concentration based on^{15}N tracer. *Chin. Agric. Sci. Bull.* **2010**, *26*, 111–116. (In Chinese)
41. Evans, J.R. Nitrogen and photosynthesis in the flag leaf of wheat (*Triticum aestivum* L.). *Plant Physiol.* **1983**, *72*, 297–302. [CrossRef]
42. Liu, J. Studies on the Photosynthetic Performance, Nitrogen Use Efficiency, Forage Productivity, and Adaptability of Forage. Ph.D. Thesis, Gansu Agricultural University, Lanzhou, China, 2019.
43. Zhang, X.Z.; Kuang, Y.; Li, T.X. Effect of nitrogen application rate on biomass and nitrogen accumulation of triticale. *J. Triticeae Crops* **2013**, *33*, 1237–1242. (In Chinese)
44. Ma, X.H.; Yu, Z.W.; Liang, X.F.; Yan, H.; Shi, G.P. Effects of nitrogen application rate and ratio of base and topdressing on nitrogen utilization and soil NO_3-N content in winter wheat. *J. Soil Water Conserv.* **2006**, *20*, 95–98.
45. Li, P.F.; Li, X.K.; Hou, W.F.; Ren, T.; Cong, R.H.; Du, C.W.; Xing, L.H.; Wang, S.H.; Lu, J.W. Studying the fate and recovery efficiency of controlled release urea in paddy soil using ^{15}N tracer technique. *Sci. Agric. Sin.* **2018**, *51*, 3961–3971. (In Chinese)
46. Macdonald, A.J.; Poulton, P.R.; Stockdale, E.A.; Powlson, D.S.; Jenkinson, D.S. The fate of residual ^{15}N-labelled fertilizer in arable soils: Its availability to subsequent crops and retention in soil. *Plant Soil* **2002**, *246*, 123–137. [CrossRef]
47. Thomsen, I.K.; Christensen, M.B.T. Fertilizer ^{15}N recovery in cereal crops and soil under shallow tillage. *Soil Tillage Res.* **2007**, *97*, 117–121. [CrossRef]
48. Huang, X.S.; Zhong, Z.M.; Huang, Q.L.; Feng, D.Q.; Chen, Z.D.; Wang, M.G. Fertilizer-N uptake and conversion efficiency in 8 Species of gramineous pastures by using^{15}N-tracing technique. *J. Nucl. Agric. Sci.* **2014**, *28*, 1677–1684. (In Chinese)
49. Costagliola, A.; Roperto, F.; Benedetto, D.; Anastasio, A.; Marrone, R.; Perillo, A.; Russo, V.; Papparella, S.; Paciello, O. Outbreak of fatal nitrate toxicosis associated with consumption of fennels (*Foeniculum vulgare*) in cattle farmed in Campania region (southern Italy). *Environ. Sci. Pollut. Res.* **2014**, *21*, 6252–6257. [CrossRef] [PubMed]
50. Reynolds, M.B.; Drewnoski, M.E. Is it time to rethink our one-size-fits-all approach to nitrate toxicity thresholds in forages? *Transl. Anim. Sci.* **2022**, *6*, txac023. [CrossRef]
51. *NY/T 815–2004*; Feeding Standard of Beef Cattle. MOA (Ministry of Agriculture of China): Beijing, China, 2004.