



Article Herbicide Applications Reduce Gaseous N Losses: A Field Study of Three Consecutive Wheat–Maize Rotation Cycles in the North China Plain

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Abstract: Herbicide residues in farmland soils have attracted a great deal of attention in recent decades. Their accumulation potentially decreases the activity of microbes and related enzymes, as well as disturbs the nitrogen cycle in farmland soils. In previous studies, the influence of natural factors or nitrogen fertilization on the soil nitrogen cycle have frequently been examined, but the role of herbicides has been ignored. This study was conducted to examine the effects of herbicides on NH₃ volatilization- and denitrification-related nitrogen loss through three rotation cycles from 2013 to 2016. The four treatments included no urea fertilizer (CK), urea (CN), urea+acetochlor-fenoxaprop-ethyl (AC-FE), and urea+2,4D-dicamba (2,4D-DI) approaches. The results showed that the application of nitrogen fertilizer significantly increased the nitrogen losses from ammonia volatilization and denitrification in the soil. Ammonia volatilization was the main reason for the gaseous loss of urea nitrogen in a wheat-maize rotation system in the North China Plain (NCP), which was significantly higher than the denitrification loss. In the CK treatment, the cumulative nitrogen losses from ammonia volatilization and denitrification during the three crop rotation cycles were $66.64 \text{ kg N} \text{ hm}^{-2}$ and $8.07 \text{ kg N} \text{ hm}^{-2}$, respectively. Compared with CK, the nitrogen losses from ammonia volatilization and denitrification under the CN treatment increased 52.62% and 152.88%, respectively. The application of AC-FE and 2,4D-DI significantly reduced the nitrogen gas losses from the ammonia volatilization and denitrification in the soil. Ammonia volatilization reduction mainly occurred during the maize season, and the inhibition rates of AC-FE and 2,4D-DI were 7.72% and 11.80%, respectively, when compared with CN. From the perspective of the entire wheat-maize rotation cycle, the inhibition rates were 5.41% and 7.23% over three years, respectively. Denitrification reduction also mainly occurred in the maize season, with the inhibition rates of AC-FE and 2,4D-DI being 34.12% and 30.94%, respectively, when compared with CN. From the perspective of the entire wheat-maize rotation cycle, the inhibition rates were 28.39% and 28.58% over three years, respectively. Overall, this study demonstrates that herbicides could impact the nitrogen cycle of farmland soil ecosystems via the suppression of ammonia volatilization and denitrification rates, thus reducing gaseous N losses and mitigating global climate change.

Keywords: herbicides; ammonia volatilization; denitrification; North China Plain

1. Introduction

Nitrogen (N) is an essential macro element for plants and is usually an important limiting factor for plant growth and crop yield formation [1]. Intensified agricultural practices and growing food demand have resulted in high inputs of N fertilizer to farmlands. Unfortunately, this excessive investment has resulted in low nitrogen use efficiency (NUE), thus leading to significant nitrogen gas loss through denitrification [2] and ammonia volatilization [3], which has a negative impact on the environment, especially in intensive agricultural systems [4]. N₂O, a byproduct of denitrification loss, is approximately 298 times



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Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). more potent than CO_2 as a greenhouse gas [5]. Despite not being classified as a greenhouse gas, the emission and subsequent deposition of NH_3 lead to air pollution (e.g., PM2.5) [6], surface water eutrophication [7], soil acidification [8], and to indirect emissions of N_2O [9]. Therefore, reducing the gaseous loss of nitrogen fertilizers in farmlands is a crucial approach for enhancing NUE and minimizing the environmental impacts of N fertilizers.

Due to intensified agricultural practices, herbicides have been introduced into soil environments. Various herbicides with wide-ranging physicochemical properties and modes of action have been applied to protect crop yields against undesirable weeds by reducing the nutrition competition for target crops [10]. The herbicide application area reached 107 million ha in 2014 and covered 65% of the total arable land in China [11]. However, most studies have shown that herbicide application can pose potential risks to enzyme activity [12-15] and nontarget soil microorganisms [10,16,17], as well as have adverse consequences for N metabolism [18,19]. Ammonia volatilization in soil is a process in which the free NH4⁺-N in soil is converted into gaseous NH3, which then volatilizes into the air from the surface of the soil, and the main driving factor for this process is urease activity. Previous studies have shown that herbicides may reduce urease activity [17,20]. Narain Rai [21] reported that fungal, bacterial, and actinomycete populations, as well as urease activity, were reduced by 2,4-D treatment. Chen [22] studied the nonspecific maize herbicide dinitraniline, which dramatically decreased urease activity in the 0-60 cm soil layer by 30.5–33.1%. Therefore, we hypothesize that the application of herbicides can reduce ammonia volatilization by inhibiting urease activity. However, the effect of herbicides on soil ammonia volatilization has not been investigated in the laboratory or field.

Soil denitrification is considered an important component of the nitrogen cycle in agroecosystems and governs the main pathways that produce and consume N₂O [23], which is affected by microbial quantity and activity [24,25]. Thus, many studies have focused on this soil microbial process [26,27]. It has been reported that herbicides have a significant effect on reducing nitrifying bacteria. Our pervious study indicated that 50 mg kg⁻¹ of the active ingredient of 2,4D can significantly inhibit denitrifying bacteria [28]. However, although herbicide toxicity effects have been reported on soil microbial communities and denitrification processes [19,29], these studies have been based on laboratory investigations. The long-term effects of herbicides on the denitrification in the field have not yet previously been documented. We verified the hypothesis that herbicides reduce denitrification by inhibiting the activity of denitrification microoganisms through long-term field trials.

The North China Plain is the most important grain producing area in China. The wheat-maize rotation is the most dominant planting mode in the area, with the annual production of maize and wheat accounting for approximately 60% and 40% of the national total output, respectively [30]. This area accounts for 30% of the national fertilizer use [31], and excessive N inputs lead to low N use efficiency, thereby triggering large N losses. Herbicides are commonly used in these areas, but their effects on the loss of N through ammonia volatilization and denitrification in farmlands have not yet been investigated. In this study, a three-year field experiment was conducted with a wheat-maize rotation system to study the effects of several common maize and wheat herbicides on the gaseous losses that occurred due to an N fertilizer being applied. The major objective was to provide significant theoretical support for agricultural practices to improve NUE and reduce greenhouse gas emissions (Tables S1 and S2).

2. Materials and Methods

2.1. Experimental Site and Experimental Herbicides

The field plot experiment was conducted from 16 June 2013 to 24 May 2016 at Fengqiu Experimental Station of the Chinese Academy of Sciences, Xinxiang City, Henan Province. The site was flat and was 67.5 m above sea level, and the soil was classified as fluvo-aquic soil. The site was classified as a temperate continental monsoon climate with an annual temperature and precipitation of 13.9 °C and 615 mm, respectively. A wheat–maize rotation

was the most dominant planting mode in this area [32]). The physicochemical properties of the 0–15 cm soil layer of the cultivated soil were as follows: pH 8.09, organic matter (OM) 10.60 g kg⁻¹, total nitrogen (TN) 0.95 g kg⁻¹, NH₄⁺-N 1.02 mg kg⁻¹, and NO₃⁻-N 28.46 mg kg⁻¹.

The tested herbicide varieties were classified according to the herbicide action mechanism. Based on the current demand in China, herbicides are representative of the maize season (i.e., the amide acetochlor (AC) and the phenoxycarboxylic acid 2,4-dichlorophenoxyacetic acid (2,4D)) and herbicides representative of the wheat season (namely the aromatic oxyphenoxypropionic acid fenoxaprop-ethyl (FE) and the benzoic acids dicamba (DI)) were used in combined application tests, and various herbicides were applied during 3 consecutive wheat–maize rotation cycles (Table 1).

 Table 1. Variety, source, and dosage of the tested herbicides.

Herbicide Varieties	Active Ingredients (Dosage Form)	Factories	The Practical Dosage
Acetochlor	50% (EC)	Hangzhou Qingfeng Agrochemical Co., Ltd. (Hangzhou, China)	2400 g hm^{-2}
2,4-Dichlorophenoxyacetic acid	57% (EC)	Shandong Shengbang Lvye Chemical Co., Ltd. (Jinan, China)	750 mL hm^{-2}
Fenoxaprop-ethyl	69 g L ⁻¹ (EC)	Bayer CropScience (China) Co., Ltd. (Hangzhou, China)	750 mL hm^{-2}
Dicamba	180 g L ⁻¹ (EC)	Shanghai Hongbang Chemical Co., Ltd. (Shanghai, China)	$375 \text{ mL } \text{hm}^{-2}$

2.2. Experimental Design

The tested crops were the maize variety Zhengdan 958 and the wheat variety Aikang 58. The nitrogen fertilizer was urea nitrogen (46% nitrogen content, common urea, commercially available), and the phosphorus fertilizer was calcium superphosphate (12% phosphorus content, commercially available). The experimental set up consisted of 4 treatments: no N control treatment (CK), urea (CN), urea with AC-FE treatment, urea with 2,4D-DI treatment. The experiments were performed with four replicates in a completely randomized plot with an area of 4 m × 6 m for each. An isolation zone with a width of 50 cm was left in the middle of the plots, and two 20 cm ridges were adjacent to this zone, totaling 90 cm. Table 2 shows the field management records during the growing season. The annual chemical nitrogen fertilizer application rate for the maize and wheat season was 150 and 225 kg N hm⁻², respectively. All fertilizers were applied by surface broadcasting.

Table 2. Log of the field activities during the summer maize and winter wheat growing seasons.

]	ime/Year-Month-Da	Crowth Pariod and Formland Management	
	Year 2013–2014	Year 2014–2015	Year 2015–2016	Giowin renou and ranniand Management
Maize	16 June 2013	8 June 2014	7 June 2015	Sowing and apply base fertilizer (60 kg N hm ⁻²). Except for the CK and CN treatments, the other
season	17 June 2013	9 June 2014	8 June 2015	treatments were sprayed with herbicides according to the dosage in Table 1.
	26 July 2013	21 July 2014	21 July 2015	Top dressing (90 kg N hm ^{-2}).
	17 September 2013	21 September 2014	18 September 2015	Harvest
Wheat	14 October 2013	17 October 2014	17 October 2015	Sowing and apply base fertilizer (120 kg N hm ⁻²). Except for the CK and CN treatments, the other
season	17 November 2013	17 November 2014	4 11 November 2015	treatments were sprayed with herbicides according to the dosage in Table 1.
	2 March 2014	14 March 2015	10 March 2016	Top dressing (105 kg N hm ^{-2}).
	31 May 2014	25 May 2015	25 May 2016	Harvest.

2.3. Sample Test Method

2.3.1. Ammonia Measurement

NH₃ volatilization were measured via the venting method of Wang et al. [33] and Ding et al. [34]. A 15 cm diameter PVC cylinder was placed in the field and contained two pieces of phosphoglycerol-soaked sponges (diameter 15 cm); the bottom one collected NH₃ from the soil, the upper one absorbed ambient NH₃ and prevented it from entering the inside of chamber. Three cylinders were randomly placed in each plot at 8:00 a.m., and the samples were collected after 24 h (1 day). Samples were taken daily for the first week after fertilization and then every 2–3 days thereafter until no difference was observed between the N fertilizer treatment and CK. When rainfall occurred, the sampling was postponed to a time that was far removed from the rainfall event. The ammonia trapped in the bottom absorbers was extracted using 300 mL of 1.0 M KCL, and then the extracts were analyzed with a continuous-flow analyzer (Skalar, Breda, The Netherlands). The NH₃ flux was calculated according to the following equation:

NH₃ volatilization rate (kg hm⁻² d⁻¹)) = $M/(A \times D) \times 10^{-2}$,

where M is the NH_3 absorbed by sponge absorbers (mg); A is the area covered by the PVC cylinders (m²); and D is the capture time (day).

2.3.2. Denitrification Measurement

Denitrification was determined via the acetylene (C_2H_2) inhibition method on the intact soil columns in the field [35]. Briefly, two closed containers (15 cm in height and 15 cm in diameter) were used for each plot. Eight complete soil cores (15 cm in length and 3.2 cm in diameter) were randomly selected from each container and sealed. Then, one container was injected with a 10 vol% acetylene while the other was not. Subsequently, the container were buried in the soil near the experimental site, thus ensuring that the temperature in the container was consistent with the soil temperature. After incubation for 24 h, the container was taken out from the soil, and a 20 mL air sample was taken from the container using a polypropylene syringe. Sampling begun on the 3rd day after fertilization (including top dressing) to determine the loss of nitrogen through denitrification. The sampling time was based on the fertilization, irrigation, and rainfall conditions. Sampling was performed every 3 days at first, and then the sampling time interval was extended to 5–7 days. The sampling time interval during the winter was extended to once every 10–20 days. Generally, the sampling frequency had to be increased after fertilization and rainfall. When rainfall occurred, the sampling time was postponed to a time that was far removed from the rainfall event (when large changes in the soil moisture occurred, it was necessary to take samples for measurement, and the measurement interval after top-dressing was then adjusted to 3 days). The N_2O concentrations were determined using an Agilent Gas Chromatograph 7890 A (Agilent Technologies, Santa Clara, CA, USA). Due to the reduction in N_2O to N_2 having been inhibited, the denitrification rate of each sample time could be represented by the N₂O production rate of the soil treated with acetylene, and this was calculated using the following equation:

$$N_2O$$
 (kg N ha⁻¹ day⁻¹) = (C1 - C0) × 10⁻⁶ × 28/22.4 × (V1 - V2)/S/D × 10,000/1000,

where C0 and C1 is the N_2O concentration in the container before and after the incubation (24 h); V1 and V2 are the volumes of the incubation container and eight soil cores (L), respectively; S is the cross-sectional area of the eight soil cores (m²); and D is time for the containers' seal being taken off (day).

2.3.3. Inorganic N and Meteorological Parameter Measurements

At the same time as the gas sampling, soil samples were collected from a 0–15 cm soil layer and the NH_4^+ - N, NO_3^- -N, and moisture content in the soil were measured. The soil NH_4^+ -N and NO_3^- -N contents were determined via extracting 2 mol/L of KCl

at a soil/solution ratio of 1:5 (a 20 g soil sample and 100 mL KCl solution) and shaking for 60 min at 300 r/min at 25 °C with a mechanical shaker. After filtering through a filter paper, the extracts were analyzed with a continuous-flow analyzer (Skalar, Breda, The Netherlands). The soil moisture was measured in the 25 g soil samples by drying at 105 °C for 8 h. An Automatic Weather Station was used to obtained the meteorological parameters, including the soil temperature and precipitation.

2.4. Statistical Analysis

Cumulative NH_3 volatilization and denitrification loss were calculated by a linear interpolation between the dates of sampling. A one-way analysis of variance (ANOVA) with a Duncan test was used to estimate the differences in the various variables among all the treatments. Spearman ranking correlation analysis was used to calculate the correlation coefficients for the NH_3 volatilization and denitrification rates with the temperature, soil moisture, NH_4^+ -N and NO_3^- -N concentrations.

3. Results

3.1. Environmental Conditions and Temporal Variations in the Inorganic N Concentrations during the Experimental Process

The cumulative precipitation during the measurement periods was 345.6, 568.0, and 390.2 mm in the 2013–2016 growing seasons (with an average of 434.6 mm), and the soil temperature was 15.49 °C, 15.98 °C, and 15.70 °C for the same seasons (with an average of 15.72 °C). The annual average soil moisture was 13% each year (Figure 1). The variation in the NH₄⁺-N content of the CN and herbicide treatments was essentially the same. For the N fertilizer treatments, the NH₄⁺-N concentrations usually reached their peak on the first day after N application, which then subsequently declined to non-fertilized levels within 3–5 days (Figure 2). The soil NO₃⁻-N concentrations reached their peak around 15–20 days after fertilization and then gradually decreased (Figure 3). No significant differences were observed in the NH₄⁺-N and NO₃⁻-N concentrations or in the annual cumulative fluxes (p > 0.05) between the two combined herbicide treatments and the CN treatment in any of the maize and wheat seasons (Figure 3).



Figure 1. The daily precipitation and soil temperature rates at a 15 cm depth, as well as the soil moisture rates (during the denitrification experiments) across the whole experiment.



Figure 2. Temporal variations in the NH_4^+ -N concentrations in the soil. The arrows mark management actions. BF: basal fertilizer; TD: topdressing; H: herbicide; CK: no urea fertilizer; CN: urea N fertilizer; AC-FE: acetochlor-fenoxaprop-ethyl; and 2,4D-DI: 2,4D-dicamba.



Figure 3. Temporal variations in the NO₃⁻-N concentrations in the soil. The arrows mark management actions. BF: basal fertilizer; TD: topdressing; H: herbicide; CK: no urea fertilizer; CN: urea N fertilizer; AC-FE: acetochlor-fenoxaprop-ethyl; and 2,4D-DI: 2,4D-dicamba.

3.2. Temporal Variations and Cumulative NH₃ Volatilizations in the Soil

Figure 4 shows that the NH₃ volatilization strongly fluctuated with the environmental conditions and that the variations in the NH₃ volatilization in the different treatments were essentially the same. Each treatment had a significant or extremely significant positive correlation with the NH₄⁺-N concentrations (p < 0.05 or 0.01). However, the NH₃ volatilization in the CK treatment throughout the entire growth season was very low (Figure 4). Generally, the flux peaked 1–4 days after fertilization in the wheat seasons, whereas—in contrast—it peaked on the first day of the maize seasons. The NH₃ volatilization in the wheat season decreased more slowly than that in the maize season, which dropped to N-untreated levels within 10–30 days in the wheat seasons and 4 days in the maize seasons. The NH₃ volatilization rate was significantly positively correlated with the soil NH₄⁺-N concentrations (p < 0.05 or 0.01) (Table 3).

Table 4 shows the cumulative ammonia volatilization from 2013 to 2016. It was also found that the herbicide-related inhibition of the NH₃ volatilization mainly occurred in the maize season. When the nitrogen fertilizer was not applied in the maize season, the cumulative NH₃ volatilization was 30.56 kg N hm⁻². The cumulative NH₃ volatilization in the CN treatment showed a significant increase of 77.69% compared to the CK, thereby accounting for 5.28% of the applied fertilizer N. The AC and 2,4D treatments significantly reduced the NH₃ volatilization by 7.72% and 11.80% compared with the CN treatment, respectively, and there was no significant difference between the AC and 2,4D treatments. In the wheat season, there was no significant difference in the NH₃ volatilization between

each herbicide treatment and CN. From the perspective of the entire wheat–maize rotation cycle, the AC-FE and 2,4D-DI treatments significantly reduced soil NH₃ volatilization by 5.41% and 7.23%, respectively, and there was no significant difference between the AC-FE and 2,4D-DI treatments.



Figure 4. Temporal variations in the ammonia volatilization in the soil. The arrows mark management actions. BF: basal fertilizer; TD: topdressing; H: herbicide; CK: no urea fertilizer; CN: urea N fertilizer; AC-FE: acetochlor-fenoxaprop-ethyl; and 2,4D-DI: 2,4D-dicamba.

Table 3. Relationship of the NH₃ volatilization with soil moisture, soil temperature, NH_4^+ -N, and NO_3^- -N concentrations in the different treatments, respectively.

Treatment	СК	CN	AC-FE	2,4D-DI		
Soil Temperature	-0.067	0.140	0.159	0.060		
Soil Moisture	-0.006	0.150	0.083	0.088		
NH4 ⁺ -N	0.382 *	0.478 **	0.355 *	0.441 **		
NO ₃ ⁻ -N	-0.208	-0.111	0.069	-0.022		
Note: The symbols of * and ** stand for $n < 0.05$ and $n < 0.01$ significance respectively.						

Note: The symbols of * and ** stand for p < 0.05 and p < 0.01 significance, respectively.

Table 4. Cumulative ammonia volatilization and denitrification losses in the soil from 2013–2016.

		Ammonia Volatilization			Denitrification		
Crop	Treatment	Gross Loss (kg N hm ⁻²)	Nitrogen Fertilizer Loss (kg N hm ⁻²)	% of Applied Fertilizer N	Gross Loss (kg N hm ⁻²)	Nitrogen Fertilizer Loss (kg N hm ⁻²)	% of Applied Fertilizer N
	СК	$30.56 \pm 0.70 \text{ dC}$			$6.72\pm1.15~\mathrm{cC}$		
Summer maize	CN	$54.30\pm2.24~\mathrm{aA}$	23.74	5.28	$17.52\pm2.94~\mathrm{aA}$	10.80	2.40
	AC	$50.11\pm0.43~\mathrm{bB}$	19.55	4.34	$11.54\pm0.73\mathrm{bB}$	4.82	1.07
	2,4D	$\begin{array}{c c} (kg \ N \ hm^{-2}) & \begin{tabular}{lllllllllllllllllllllllllllllllllll$	3.85	$12.10\pm2.03~bB$	5.38	1.20	
	СК	$36.08\pm0.78~\text{bB}$			$1.35\pm0.19\text{bB}$		
Summer maize 	CN	$47.41 \pm 1.80~\mathrm{aA}$	11.32	1.68	$2.90\pm0.59~\mathrm{aA}$	1.71	0.25
	FE	$46.10\pm2.62~\text{aA}$	10.02	1.48	$3.08\pm0.22~aA$	1.98	0.29
	DI	$46.46\pm2.25~aA$	10.38	1.54	$2.48\pm0.59~aA$	1.17	0.17
	СК	$66.64 \pm 1.35 \text{ cC}$			$8.07\pm1.29~\mathrm{cC}$		
TA71	CN	$101.71\pm3.08~\mathrm{aA}$	35.07	3.12	$20.42\pm2.68~aA$	12.51	1.11
Wheat-maize	AC-FE	$96.21\pm2.98bAB$	29.57	2.63	$14.62\pm0.66\mathrm{bB}$	6.81	0.60
	2,4D-DI	$94.35\pm2.46bB$	27.71	2.46	$14.58\pm1.53~bB$	6.55	0.58

Data are the mean values \pm standard deviation. Different lowercase letters (and uppercase letters) in the same line indicate significant differences between different the N losses at a p < 0.05 (p < 0.01) significance.

3.3. Temporal Variations and Cumulative N Loss via Denitrification in the Soil

The rate of denitrification varied greatly, mainly in the maize season (Figure 5). The trends in the denitrification during the different treatments was essentially the same. All the treatments reached peak values on the third day after fertilization in the maize season. In the maize season, there were two distinct peaks for the denitrification after fertilizer was applied. The peak time for denitrification during the wheat season was slightly delayed, peaking 7–13 days after fertilization and then rapidly decreasing. The denitrification rate was extremely significantly positively correlated with the soil moisture and temperature (p < 0.01) (Table 5). The application of herbicides did not change the time for peak denitrification in the maize season, but it did delay the time for peak denitrification by 3–6 days in the wheat season.



Figure 5. Temporal variations in the denitrification in the soil. The arrows mark management actions. BF: basal fertilizer; TD: topdressing; H: herbicide; CK: no urea fertilizer; CN: urea N fertilizer; AC-FE: acetochlor-fenoxaprop-ethyl; and 2,4D-DI: 2,4D-dicamba.

Table 5. The respective relationships of the denitrification rates with soil moisture, soil temperature, NH_4^+ -N, and NO_3^- -N concentrations in the different treatments.

Treatment	СК	CN	AC-FE	2,4D-DI
Soil Temperature	0.675 **	0.659 **	0.653 **	0.659 **
Soil Moisture	0.441 **	0.478 **	0.489 **	0.491 **
NH4 ⁺ -N	-0.019	-0.035	-0.088	-0.049
$NO_3^{-}-N$	0.189 *	-0.081	-0.159	-0.101

Note: The symbols of * and ** stand for a p < 0.05 and p < 0.01 significance, respectively.

Generally, the cumulative denitrification in the treatments with herbicides was significantly lower than that in the treatments without herbicide addition. Although similar temporal patterns were observed in the denitrification across all treatments, significant differences in the denitrification between herbicide and CN treatments were evident during a short period after N application. The inhibitory effect of herbicides on denitrification mainly occurred in the maize season (Table 4). When nitrogen fertilizer was not applied during the maize season, the cumulative denitrification was 6.72 kg N hm⁻². With the application of nitrogen fertilizer, the cumulative denitrification significantly increased by 160.74%, accounting for 2.40% of the applied fertilizer N. The AC and 2,4D treatments significantly reduced denitrification by 34.12% and 30.94%, respectively, and there was no significant difference in the denitrification between each herbicide treatment and CN. From the perspective of the entire wheat–maize rotation cycle, the AC-FE and 2,4D-DI treatments significantly reduced denitrification by 28.39% and 28.58%, respectively, and there was no significant difference between the AC-FE and 2,4D-DI treatments. In addition, it should be pointed out that our results showed that ammonia volatilization was the main gaseous loss of urea nitrogen in a wheat–maize rotation system in the NCP, which was significantly higher than the impact of the denitrification loss (Table 4).

4. Discussion

4.1. Herbicides Reduced NH₃ Volatilization from Soil

NH₃ volatilization in soils is a process in which the free NH₄⁺-N in the soil is converted into gaseous NH₃ and then volatilized into the air through the surface of the soil. Urease catalyzes the hydrolysis of urea in soils, and it induces the formation of ammonia [36]. At present, there are no specific reports on the effect of herbicides on soil NH₃ volatilization. This research shows that herbicides can reduce soil NH₃ volatilization, and it also showed that the AC-FE and 2,4D-DI treatments mitigated NH₃ volatilization by 5.41% and 7.23%over 3 years and by 7.72% and 11.80% for the maize season, which may be due to the inhibitory effect of the herbicide on the hydrolysis of urea [37]. Baćmaga et al. [38] reported that Fuego 500 SC reduced the activity of urea by 13.39% when the herbicide was added to soil in a dose that 160-fold exceeded the recommended rate. Singh and Ghoshal [39] conducted a two-year study, whereby they indicated that the urease activity decreased consistently throughout the crop cycle for all treatments except under the butachlor+wheat straw treatment. The results of this study showed that there was no significant difference in the average NH₄⁺-N concentration between the herbicide application and CN treatment, which may be because the herbicide addition not only inhibited urease activity, but also inhibited nitrification. Our results also indicate that NH₃ volatilization is significantly, or extremely significantly, and positively correlated with NH4⁺-N concentration, which is consistent with Shan et al.'s research results [40]. This confirms that herbicide inhibits NH_3 volatilization by inhibiting urease activity, which leads to a slow release of NH_4^+-N , thereby facilitating the absorption and utilization of NH_4^+ -N by plants [41].

Different herbicides affect soil NH₃ volatilization at different times and to different extents, and this may be related to the chemical structure of the herbicides, their transformation in the soil, and the toxicity of their degradation products. The results showed that the inhibitory effect of the herbicide treatment on soil NH₃ volatilization mainly occurred within 1–2 days after fertilization in the maize season. In the wheat season, there was also an inhibitory or promoting effect for 2–3 days, but the cumulative NH₃ volatilization with herbicide treatment was not significantly different from that of the CN treatment. In this study, herbicides showed a stronger inhibitory effect during the maize season. This could be attributed to the higher temperature observed during the corn season, which enhances the activity of urease [42] and results in a more pronounced inhibitory effect of herbicide application on urease activity [14]. Generally, most NH_3 volatilization occurs in the first 5 days after N fertilizer application [9,43]. Our research showed that the peak NH₃ flux occurred one or two days after N application, and this was then followed by a sharp decline on days 4-5; as such, urea hydrolysis was found to mainly take place within a week, and the herbicides were also found to mainly affect urease activity during this period. Additionally, the results of this experiment showed that, in the maize season, the herbicide's inhibitory effect on NH₃ volatilization was greater after fertilization in the basal fertilizer period than after fertilization in the top-dressing period, which may be because the half-lives of AC, 2,4-D, FE, and DI were 5.5 d, 1.9 d, 1.8–2.4 d, and 28.3 d, respectively [44–46], in the soil. In the maize season, top-dressing fertilization was carried out approximately one month after herbicide application, which is when the herbicide was almost completely degraded. In the wheat season, the herbicide application time was approximately one month after basal fertilizer application. When the herbicide was applied, the nitrogen conversion process was almost complete, and the ammonium nitrogen content of the soil dropped to the CK level. The time for top-dressing application was more than two months after herbicide application. When the top-dressing fertilizer was applied, the herbicide was completely degraded. Therefore, there was no significant difference between the herbicide treatment and CN treatment during the entire wheat season.

4.2. Herbicides Reduced N Loss via Soil Denitrification

The AC-FE and 2,4D-DI treatments significantly reduced the denitrification in the soil, and the inhibitory effect mainly occurred after fertilization and rainfall in the maize season. The results showed that the inhibitory effect of herbicide treatment on soil denitrification mainly occurred within 1-2 days after fertilization or within 1-9 days after continuous rainfall in the maize season. Denitrification peaks tended to occur when continuous rainfall followed fertilization. Fertilization and rainfall greatly promoted denitrification in the maize season, and the effect was not noticeable in the wheat season. Low temperatures may not be conducive to the activity of the denitrification microorganisms [47]. The application of herbicides can reduce the number of denitrifying bacteria in soils [48,49]. Rahman et al. [27] revealed that the relative abundances of microbial communities, as well as the anammox and denitrification bacteria, were significantly lower in 10 of the long-term pesticides (5 insecticides, 3 fungicides, and 2 herbicides) that were applied in rice field soils than in upland soils. Su et al. [19] revealed that chlorothalonil significantly inhibits N_2O reductase activity, denitrification functional genes (narG, nirS, and norB), and potential denitrification bacteria (i.e., Pseudomonas and Streptomyces), inhibiting the denitrification process. Moreover, large differences have been observed based on herbicide dose and microorganism type. Denitrification rates were inhibited after the introduction of the fungicide deltamethrin and herbicide prosulfuron, thereby resulting in decreased denitrification potential [50]. Our test results showed that AC and 2,4D significantly inhibited soil nitrogen loss through denitrification; however, fenoxaprop-ethyl and dicamba had no significant effects on the denitrification in the wheat season. In some cases, although the changes in the microbial community were minimal when herbicides were applied, this might be an indicator of potential long-term effects. Thus, in order to clarify the changes in the soil microbial community structure and nitrogen cycle process after herbicide application, it is necessary to conduct long-term positioning studies [51].

5. Conclusions

Three years of field observation clearly showed that the gaseous N losses was mainly caused by ammonia volatilization after the application of urea nitrogen in the wheatmaize rotation system in the NCP. The application of AC-FE and 2,4D-DI did not change the average content of ammonium nitrogen and nitrate nitrogen in the soil, but it did significantly reduce the loss of N through NH₃ volatilization and denitrification in the soil. The reduction in the NH₃ volatilization- and denitrification-related nitrogen losses mainly occurred in the maize season.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14020283/s1, Table S1. Seasonal ammonia volatilization in soil during maize and wheat seasons from 2013 to 2016. Table S2. Seasonal denitrification in soil during maize and wheat seasons from 2013 to 2016.

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