Impacts of Fertilization Optimization on Ammonia Volatilization, Soil Nitrification, Denitrification Intensity From Wheat Fields and Nitrogen Utilization Under Water-saving Irrigation

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Abstract

Scholars have proposed the practice of split N fertilizer application (SNFA), which has proven to be an effective approach for enhancing N use efficiency. However, the effect of SNFA on NH_3 volatilization, nitrification and denitrification in soil, remain largely unknown. As such, the current study assessed soil NH_3 volatilization, nitrification and denitrification intensities, abundance of nitrogen cycle-related functional genes, and invertase activity for different treatments. We applied a rate of $240 \text{ kg} \cdot \text{ha}^{-1}$ of N, and the following fertilizer ratios of the percent base to that of topdressing under water-saving irrigation: N1 (basal/dressing, 100%/0%), N2 (basal/dressing, 70%/30%), N3 (basal/dressing, 50%/50%), N4 (basal/dressing, 30%/70%), and N5 (basal/dressing, 0%/100%). N3 treatment resulted in a significant decrease in rate of NH_3 volatilization. This treatment also significantly reduced nitrification and denitrification intensities, primarily owing to the reduced functional genes abundance involved in the nitrogen cycle (AmoA-AOB, nirK and nirS) and reduced invertase activity (urease, nitrate reductase, nitrite reductase) in wheat-land soil. ^{15}N tracer studies further demonstrated that N3 treatments significantly increased the grain nitrogen accumulation by 9.50-28.27% compared with that under other treatments. This increase was primarily due to an increase in the amount of N absorbed by wheat from soil and fertilizers, which was caused by an enhancement in total N uptake (7.2-21.81%). Collectively, these results suggest that the N3 treatment (basal/dressing, 50%/50%) improves N uptake by wheat, reduces the soil NH_3 volatilization rate, and has the potential to reduce the amount of N_2O generated by nitrification and denitrification.

1 Introduction

NH_3 volatilization is the major pathway by which nitrogen is lost from wheat cultivation systems (Abdo, 2021). Moreover, the proportion of total nitrogen lost as NH_3 from nitrogen fertilizers varies from 9 to 40% (Fu et al., 2020). One of the major anthropogenic sources of NH_3 release into the atmosphere is agricultural fields, primarily owing to nitrogen based fertilization and the associated management of this practice. Meanwhile, the primary air pollutants now include atmospheric NH_3 , which is rapidly deposited on the earth's surface within 4 to 5 km of its sources (Behera, 2013). It is, therefore, crucial to manage nitrogen fertilizers in a manner that minimizes its effects on the environment. The simultaneous measurement of NH_3 emissions has the potential to provide valuable information on the processes responsible for their formation, as well as their contribution to both environmental and air pollution.

Soil denitrification and nitrification are vital processes in the global nitrogen cycle (Jetten, 2008; Harter et al., 2014), as well as in the generation of environmental pollution, and plant metabolism (Kuypers et al., 2018; Li et al., 2018). Previous research has shown that high rates of nitrification and denitrification can result in reduced efficiency of nitrogen use by crops and a corresponding increase in loss of nitrogen to the atmosphere (e.g., as nitrogen oxides) or leaching into surface or ground waters (e.g., as NO_3^- or NO_2^-) (He et al., 2018; Herrera et al., 2016; Yang et al., 2017). Ammonia (NH_4^+) is oxidized to nitrate (NO_3^-) by nitrite (NO_2^-) during nitrification with nitrite as an intermediary step. These processes are catalyzed separately by nitrite oxidoreductase (nxrA) and ammonia monooxygenase (amoA), respectively. Meanwhile, denitrification is a sequential reduction by which NO_3^- is reduced to N_2O and/or dinitrogen gas (N_2) via NO_2^- and nitric oxide (NO), which are catalyzed by a series of enzymes that include copper-containing nitrite reductase (nirK) and cytochrome cd1-containing nitrite reductase (nirS). Hence, the functional genes for these enzymes have been used as molecular markers to study the dynamics of communities of denitrifying and nitrifying bacteria in an array of environments that include field soils (Morales et al., 2010; Chon et al., 2011; Petersen et al., 2012; Francis et al., 2013; Levy-Booth et al., 2014). However, soil nitrification and denitrification are highly vulnerable to the environment in the external soil (Neal et al., 2017; Wang et al., 2018). In addition, the effects of nitrogen fertilization on these processes can be modulated by the physiochemical properties of soil, including inorganic nitrogen (Fraser et al., 2017) and N cycle gene abundance and the critical enzyme activity (Neal et al., 2017). Thus, knowledge of the effects of soil physicochemical factors on soil nitrification and denitrification is vital to promote the efficient utilization of N in agriculture through the application of various strategies, including the amendment of soils and rational fertilization.

Soil moisture is an important element that affects NH_3 emissions and soil nitrification and denitrification. Our previous research proposed a water-saving irrigation technology (WCT), which is based on measuring soil moisture at the key stage of wheat growth (man et al., 2014). Compared with traditional quantitative irrigation, this technology not only reduces water use, but also improves wheat growth and yield. Meanwhile, other studies have shown that improving irrigation management practices may significantly reduce NH_3 emissions in Southeastern Queensland, Australia (Scheer et al., 2012). However, few studies have sought to understand the effects of split nitrogen fertilizer method under WCT condition on nitrogen loss.

The most important crop used to model cultivation on the North China Plain (NCP) is winter wheat. In an attempt to obtain high yields of grain, excessive amounts of nitrogen (N) fertilizer are often used. The rate applied annually can be as high as 325 kg N ha^{-1} , which poses a problem as fertilization with nitrogen serves as the major source of reactive N (Sun et al., 2020). Although this nutrient is indispensable for wheat ecosystems, an inadvertent effect can include high N loss and low fertilizer efficiency. In fact, the efficiency of N use (NUE) in wheat crops commonly ranges from 30–40% following fertilization (Wu et al., 2019). However, the excessive use of N fertilizer contributes to rapid losses of N through volatilization in the form of ammonia (NH_3), nitrification, denitrification and surface runoff (Peng et al., 2006). Although previous research examined the individual effects of nitrogen fertilizer management on wheat plant nitrogen use efficiency, NH_3 emission flux, as well as the rates of nitrification and denitrification in different ecosystems (Chen et al., 2012; Kou et al., 2015; Qiu et al., 2018), few experiments have investigated their combined effects (Qiu et al., 2018). In this experiment, ^{15}N isotope tracer technology was used to compare nitrogen uptake and utilization in different split nitrogen fertilizer treatment schemes. To explore the potential effects of split nitrogen fertilizer on NH_3 emission flux and the rates of denitrification and nitrification, as well as the

utilization to plant nitrogen, a field experiment was performed in a crop of winter wheat. The goals of this study were as follows (1) determine the primary changes in ammonia volatilization in soil amended with split nitrogen fertilizer (2) define the impact of these amendments on nitrification and denitrification intensity, as well as the relative abundance of genes for the N cycle in wheat-land soil; (3) characterize the primary properties of soil chemicals for that influence soil nitrification and denitrification intensity, and the relative abundance of genes involved in the N cycle ; (4) elucidate the manner by which changes in the N cycle and fertility of the soil affect the availability of mineral nitrogen to be taken up by wheat plants.

2 Materials And Methods

2.1 Experimental site description

The field experiments took place in Xiaomeng town, Jining City, Shandong Province, China, during the growing seasons of winter wheat from 2016 to 2017 and 2017 to 2018 (Figure 1). This site is situated in a typical warm continental climate. The average annual climate factors include an average annual temperature of 15 °C, average annual precipitation of 600 mm. Summer maize-winter wheat is the major crop rotation regime in this region. According to the FAO classification, the wheat-land soil is loam. The composition was as follows in the layer of soil tillage (0-20 cm): concentration of organic matter, 14.20 g·kg⁻¹; available phosphorus, 38.11 mg·kg⁻¹; available potassium, 129.44 mg·kg⁻¹; available nitrogen, 122.60 mg·kg⁻¹; PH, 7.6 and total nitrogen, 1.13 g·kg⁻¹. The data for the mean monthly precipitation are shown in Figure 2.

2.2 Split nitrogen fertilizer treatment and water-saving irrigation

The cultivar of winter wheat was ‘Jimai 22’, which was used in this study. The wheat was seeded on October 12th, October 24th and harvested on June 8th, June 7th in 2016-2017 and 2017-2018. And density was 1.8 million·ha⁻¹.

The field experiment consisted of five different split nitrogen fertilizer applications at an application rate of 240 kg·ha⁻¹ (basal/dressing, 100%/0%, 70% /30%, 50%/50%, 30%/70%, 100%/0%; hereafter referred to as N1, N2, N3, N4 and N5, respectively) with a randomized plot design (Table 1). Each application was performed in triplicate, resulting in a total of 15 plots (plot area 20 m²). The rate of fertilizer application that was selected was 240 kg·ha⁻¹, as it is commonly used by the local farmers. Single superphosphate (P₂O₅ 12%) and potassium chloride (K₂O 60%) were applied to provide P (P₂O₅ 150 kg·ha⁻¹) and K (112.5 K₂O kg·ha⁻¹), respectively. The basal fertilizer was comprised of P and K, while the nitrogen was applied in two split applications. All potash and phosphate fertilizers, as well as the basal nitrogen fertilizer were spread over the soil surface before the wheat was sown. A rotary cultivar was used to immediately mix the soil to a depth of 20 cm. During the jointing stage, nitrogen fertilizer was applied to create furrows that were immediately covered.

The soil moisture was measured to manage this parameter based on a WCT. The relative water content in the 0~40 cm soil layer was supplemented to 70% at the jointing and anthesis. The amounts of irrigation was calculated using the method of Man (Man et al., 2014). All irrigation processes involved the use of a hose, and the amount of water used to irrigated each event was determined manually and recorded with a water meter. The detailed apply nitrogen fertilizer and irrigation regimes are shown in Table 1. The fields were managed according to the local practices of farming with standard applications of herbicides and pesticides.

Table 1
Agronomic managements under different split nitrogen fertilizer treatments.

Treatments	Fertilizer regimes		Irrigation regimes	
	Seeding stage		Jointing stage	Anthesis stage
N1	240 N kg·ha ⁻¹ as urea	150 P ₂ O ₅ kg·ha ⁻¹ as superphosphate and 112.5 K ₂ O kg·ha ⁻¹ as potassium chloride	0 N kg·ha ⁻¹ as urea	Relative water content in 0~40 cm soil layer is supplemented to 70% according to soil moisture content in 0~40 cm soil layer.
N2	168 N kg·ha ⁻¹ as urea		72 N kg·ha ⁻¹ as urea	
N3	120 N kg·ha ⁻¹ as urea		120 N kg·ha ⁻¹ as urea	
N4	72 N kg·ha ⁻¹ as urea		168 N kg·ha ⁻¹ as urea	
N5	0 N kg·ha ⁻¹ as urea		240 N kg·ha ⁻¹ as urea	

2.3 NH₃ volatilization measurement

NH₃ was collected from October 2016 to June 2018 by the ventilation method. A polyvinyl chloride (PVC) cylinder (25 cm height, 15 cm diameter) was inserted 70 mm into the soil. The cylinder had a sponge soaked with phosphoglycerol (5%, V/V, phosphoric acid and 4%, V/V, glycerol) near its top to absorb ambient NH₃ and one close to the bottom to collect NH₃ from the soil (Dong et al., 2019). The sponges were collected each day for one week after each application of fertilizer and at each growth stage. The NH₃-N that the sponges had trapped was extracted with 300 mL of 1 mol·L⁻¹ KCL. The solution of NH₄⁺-N that had been extracted was analyzed with an AA3 continuous flow analyzer (Bran+Luebbe company, Germany). The flux of NH₃ was calculated using the formula described by Yang et al (2020). The cumulative volatilization of NH₃ was calculated as the integral sum of the gas emissions from the stages that were sampled. The NH₃ volatilization factor and yield-scaled volatilization rate were calculated using the following formulas (Yang et al., 2020):

$$\text{NH}_3 \text{ volatilization factor (\%)} = (N_{\text{fertilizer}} - N_{\text{control}}) / (F_n)$$

$$\text{Yield-scale NH}_3 \text{ volatilization (kg N t}^{-1} \text{ grain)} = N_{\text{fertilizer}} / G$$

Where $N_{\text{fertilizer}}$ and N_{control} are the total cumulative NH₃ that had volatilized during the entire wheat growing season under the treatment with nitrogen fertilizer and the control, respectively. F_n is the total nitrogen that had been applied (240 kg·ha⁻¹). G is the grain yield.

2.4 ¹⁵N measurement

We conducted isotopic labeling microzone experiments with ¹⁵N urea during the wheat growing season in 2017 and 2018. The micrograph (area size of 45 cm× 15 cm, depth, 30 cm) was created using an iron sheet (Figure 3) and processed in the same manner as the field map. In A, ¹⁵N-urea and urea were applied during the base dressing and jointing stages, respectively, and in B, urea and ¹⁵N-urea were applied during the base dressing and jointing, respectively, using the appropriate rates according to the treatment (Chen et al., 2019). In both wheat growing seasons, the plants were sampled from each micro-zone during the maturity stage. Each sample was first rinsed with running water and transported to the laboratory where they were dried in an oven at 105 °C for 30 min and subsequently at 70 °C to a constant weight. In the final step, the sample is ground using a ball mill. Stable isotope tests were performed using an element analyzer (Flash 2000HT, Thermo Fisher Scientific, Waltham, MA, USA) and an isotope mass spectrometer (Delta V advantage, Thermo Fisher Scientific) to determine the ¹⁵N atomic percentage of the sample. Since the results of the two study growing seasons are similar, only 2017-2018 data are included here. All indexes were calculated using the formulas (Chen et al., 2019):

- $\text{NDF}(\%) = [(AT\%^{15}\text{N}_1 - 0.3663) \times 100] / (AT\%^{15}\text{N}_2 - 0.3663)$ (1)
- $\text{NDF}(\text{mg} \cdot \text{stem}^{-1}) = \text{NDF} \times \text{nitrogen content in plant components}$ (2)
- $\text{NDFS} = \text{N uptake} - \text{NDF}$ (3)
- $\text{NAAG} = \text{Grain weight} \times \text{grain nitrogen content}$ (4)
- $\text{TNAA} = \text{plant weight} \times \text{plant nitrogen content}$ (5)
- $\text{SN} = \text{NO}_3^- \text{ accumulation amount} + \text{NH}_4^+ \text{ accumulation amount}$ (6)

Where NDF represents the percentage of nitrogen obtained from nitrogen fertilizer, $AT\%^{15}\text{N}_1$ represents the atomic percentage of ¹⁵N, and $AT\%^{15}\text{N}_2$ represents the atomic percentage of ¹⁵N in the fertilizer. 0.3668 is the standard value of natural ¹⁵N abundance and N absorption is the total N in aboveground biomass. NDF and NDF calculated microplots A (base fertilizer application ¹⁵N) and B (¹⁵N applied at jointing) .

2.5 Soil sample collection and preparation

The soil from each treatment was sampled at the stages of wheat heading, anthesis and maturity (Meng et al., 2020). Each soil sample was a mixture of 5 randomly selected locations in a given plot. The soil samples were then passed through a 1 mm sieve for division into three fractions, mixed thoroughly, and stored at 4 °C for subsequent microbial biomass carbon or nitrogen (MBC or MBN), and inorganic N content (NH₄⁺ and NO₃⁻). An aliquot was air dried and urease activity and ¹⁵N abundance were determined through a 1-mm sieve. The last sample was freeze-dried and stored at -80 °C for subsequent DNA extraction and real-time PCR analysis.

2.6 Total soil DNA extraction, q-PCR, and cloning of bacterial genes

Soil DNA was extracted from each samples using a FastDNA Spin Kit for Soil (MP Biomedicals, LLC., Solon, OH, USA) according to the manufacturer's instructions. The DNA was then stored at -80°C and analyzed within 3 days. A Nanodrop®ND-2000 UV-vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) was used to quantify the DNA and examine its purity. To quantify the abundance of AmoA-AOA, AmoA-AOB, nirS and nirK genes, quantitative poly-merase-chain-reaction (qPCR) assays were performed in triplicate using real-time PCR with a LightCycler 480 (Roche Applied Science). The conditions and primers are given in Table 2. The standard curves for real-time PCR were prepared as previously described (Li et al., 2017). A plasmid that contained 102–109copiesμL⁻¹ was obtained by serial 10× dilutions. A q-PCR assay was then performed in triplicate to

provide an external standard curve for determine the numbers of unknown gene copies. The efficiency for amplification of target genes in the assays ranged from 92.3 to 105.2% and the R values were from 0.996 to 0.999.

Table 2
Primers used for quantitative PCR.

Targer group	Primer	Sequence(5-3)	Length of amplicon	Thermal profile	Reference
Amoa-AOA	Arch-amoA26F/Arch-amoA417R	GACTACATMTTCTAYACWGAYTGGGC/GGKGTCATRTATGGWGGYAAYGTTGG	415	5 min at 95 °C followed by 40 cycles of 15 s at 95 °C and 30 s at 60 °C	(Park et al., 2008)
Amoa-AOB	amoA-F/amoA-R	GGGGTTTCTACTGGTGGT/CCCCTCKGSAAAGCCTTCTTC	491	5 min at 95 °C followed by 40 cycles of 15 s at 95 °C and 30 s at 60 °C	(Rotthauwe et al., 1997)
nirK	nirK-1479yF	ATCGGCGGYRAAGGCCGA	164	95 °C for 5 min, 40 cycles at 95 °C for 5 s, 62 °C for 30 s, 95 °C for 10 s (+0.3)	(Usyskin-Tonne et al., 2020)
nirS	nirS128 43F	CTGCTCGGTCTGGCAGTT	1622	95 °C for 5 min, 40 cycles at 95 °C for 5 s, 62 °C for 30 s, 95 °C for 10 s (+0.3)	(Usyskin-Tonne et al., 2020)

2.7 Potential nitrification rates (PNRs) and potential denitrification rates (PDRs)

We conducted an in situ experiment on N mineralization during the growing season of winter wheat in 2017 and 2018. Since most mineralization takes place in the top 2.5-5 cm of soil (Norton et al., 2004; Schimel and Parton, 1986), we focused on the potential nitrification rates (PNRs) and potential denitrification rates (PDRs) in the top 10 cm soil layer.

The PNRs was measured by shaking mud method (Yao et al., 2011). The PDRs were measured via by acetylene suppression technique (Liu et al., 2014). And the specific operation steps are determined according to Meng’s method (Meng et al., 2020).

2.8 Soil nitrogen invertase activit, NO₃⁻-N and NH₄⁺-N, microbial biomass carbon and nitrogen

At the same time as each NH₃ volatilization sampling, three soil samples from each plot were collected from the top 10 cm of the soil, which was situated near the gas chambers, to determine the concentrations of NO₃⁻-N and NH₄⁺-Nand soil moisture. Soil moisture was determined gravimetric method by drying the soil at 105°C for 24 h, and the results were expressed in grams of dry soil. The concentrations of NH₄⁺-N and NO₃⁻-N were measured using a continuous flow analytical system (Futura Continuous Flow Analytical System, Alliance Instruments, Eragny-Sur-Oise, France) after the samples had been extracted with 1 mol·L⁻¹ KCL. The activity of urease was determined using indophenol blue. Two grams of soil (1-mm sieved and air-dried soil) were pre incubated with 1 mL of toluene for 15 min and 20 mL of PH 6.7 citrate buffer at 37 °C for 3 h. The cumulative NH₄⁺ content was measured on a spectrophotometer at 690 nm using salicylic acid colorimetry. The activity of urease was expressed asµg NH₄⁺-N g⁻¹ dry

soil d⁻¹. The activities of nitrate reductase and nitrite reductase activities were assayed as described by Dominchin (2021). The MBC and MBN were measured by chloroform fumigation-extraction. The MBC was calculated as the difference in DOC levels between the samples that had been fumigated and a control thta had not with an efficiency factor of 0.45. The MBN is calculated as the difference of total extractable nitrogen contents between fumigated soil and unfumigated soil, and the efficiency coefficient is 0.54.

2.9 Statistical analyses

The effects of different split nitrogen fertilizer treatments on gene expression and biochemical parameters of key enzymes in nitrogen cycle were analyzed by SPSS v. 18.0 (IBM Corp., Armonk, NY, USA)

3 Results

3.1 Ammonia volatilization

The peak of daily NH₃ was detected 2 days after each application of nitrogen fertilizer and then decreased to a relatively low levels 6-7 days after each application of nitrogen fertilizer (Figure 4). During this period, the NH₃ fluxes ranged from 0.2 to 3.0 kg N ha⁻¹ d⁻¹ and increased with increasing basal/topdressing nitrogen fertilizer ratio. Furthermore, the NH₃ flux increased with increasing basal nitrogen fertilizer proportion between the sowing stage and jointing stage, as well as with increasing topdressing nitrogen fertilizer proportion from the jointing stage to the end of the experiment. This indicates that different treatments had significantly effect on NH₃ volatilization losses from soil.

Table 3 presents the cumulative volatilization of NH₃ under different treatments with split nitrogen during the 2017 and 2018 seasons of wheat growing. The cumulative losses of NH₃ volatilization across all nitrogen treatments varied between 0.2 and 3.0 kg N ha⁻¹ d⁻¹ (15.97–19.23%) in both growing seasons. The percent cumulative volatilization of NH₃ under the N3 treatment was 5.56–13.42% and 5.22–10.83%, lower than those under N1 or N2 and N4 or N5 treatments, respectively. Moreover, the largest decreases in the volatilization factor for NH₃ and yield-scaled volatilization of NH₃ were observed under the N3 treatment. N3 treatment decreased the volatilization factor for NH₃ by 9.43–21.36% and the yield-scaled volatilization by 9.20–18.16% in both growing seasons compared with that under the N1 or N2 and N4 or N5 treatments, respectively. Similarly, under the N3 treatment, the yield-scaled NH₃ volatilization decreased by 10.76–22.27% and 9.91–22.78% in both growing seasons, respectively.

Table 3
Effect of split nitrogen fertilizer on cumulative NH₃ volatilization, NH₃ volatilization factor and yield-scaled NH₃ volatilization.

Year	Treatments	Cumulative NH ₃ volatilization			NH ₃ volatilization factor (%)	Yield-scaled NH ₃ volatilization
		(kg N ha ⁻¹)				
		BF	TF	Total		
2016-	N1	11.7a	3.64e	19.23a	5.01a	2.56a
2017	N2	8.82b	5.62d	18.08b	4.53b	2.28b
	N3	6.81c	6.26c	16.65c	3.94d	1.99c
	N4	5.68d	8.14b	17.63b	4.35c	2.23b
	N5	3.07e	10.92a	17.95b	4.48b	2.49a
2017-	N1	10.13a	3.91e	17.91a	4.46a	2.59a
2018	N2	7.81b	5.36d	16.87b	4.03b	2.22c
	N3	6.13c	6.14c	15.97c	3.65c	2.00d
	N4	5.38d	7.52b	16.85b	4.02b	2.27c
	N5	3.04e	9.73a	16.88b	4.06b	2.42b
BF, basal nitrogen fertilizer, TF, topdressing nitrogen fertilizer.						

3.2 Effects of split nitrogen fertilizer on soil nitrification intensity, denitrification intensity, abundance of nitrogen cycle functional genes and biochemical index

3.2.1 Soil nitrification intensity and denitrification intensity

Fertilization strategy significantly affected soil nitrogen conversion (Figure 5). At the jointing stage, the nitrification and denitrification intensities increased with increasing topdressing nitrogen fertilizer proportion. At anthesis stage, the nitrification and denitrification intensities under the N3

treatment were significantly lower than those under the N4 or N5 treatments, while the N1, N2, and N3 treatments did not differ significantly. At the maturity stage, the split nitrogen treatments did not result in any significant differences in nitrification intensity. Additionally, the denitrification intensity under the N3 treatment was significantly lower than that under N4 or N5 treatment, while that under N1, N2, and N3 treatments did not differ significantly.

3.2.2 Abundance of nitrogen cycle functional genes

Q-PCR based on the 16S rRNA gene was used to estimate the abundances of nitrogen cycle functional genes in soil under different split nitrogen fertilizer treatments (Figure 6). During the entire sampling process, changes in the *Amoa-AOA*, *Amoa-AOB*, *nirK*, and *nirS* counts in each group were similar, showing gradual decreases. The fertilization strategies did not significantly affect the *Amoa-AOA* counts. In all treatments, the *Amoa-AOB*, *nirK*, and *nirS* counts at each stage increased with increasing proportion of topdressing nitrogen fertilizer at the jointing stage. During the anthesis and maturity stages, the *Amoa-AOB*, *nirK*, and *nirS* counts were significantly lower under the N3 treatment than those under the N4 or N5 treatments, and the N1, N2, and N3 treatments did not differ significantly. Hence, the one-time addition of excess nitrogen fertilization can increase the abundance of key genes for nitrification and denitrification. Moreover, a reasonable ratio of basal to topdressing nitrogen fertilizer (N3) can ensure that the numbers of these genes remain at a low level.

3.2.3 Soil nitrogen invertase activity

Differences were observed in soil nitrogen invertase activity among the split nitrogen fertilizer treatments (Figure 7). At the jointing stage, the urease content, nitrate reductase activity, nitrite reductase activity, and protease activity increased with increasing topdressing nitrogen fertilizer proportion. At the anthesis stage, the urease and protease activities under the N3 treatment were significantly higher than those under the N1 treatment, whereas the N2, N3, N4, and N5 treatments did not differ significantly. Moreover, the activities of nitrate reductase and nitrite reductase under the N3 treatment were significantly lower than those under the N4 or N5 treatments, however, no significant differences were observed among the N1, N2, and N3 treatments. The changes in soil nitrogen invertase activity during the maturity stage and the soil nitrogen invertase activity in the anthesis stage showed similar trends. These results demonstrate that N3 treatment leads to decreased activity of nitrate reductase and nitrite reductase in the soil, causing low intensity denitrification.

3.2.4 Soil nitrate accumulation, ammonium nitrogen accumulation, soil microbial biomass N and C

At the jointing stage, the soil MBN, MBC, nitrate accumulation, and ammonium nitrogen accumulation increased with increasing topdressing nitrogen fertilizer proportion (Figure 8). Meanwhile, during the anthesis stage, the soil MBN under the N3 treatment was significantly higher than those under the other treatments. The soil MBC under N3 treatment was significantly higher than that under N1 or N2 treatment. However, no significant difference was observed among the N3, N4, and N5 treatments. Furthermore, the split nitrogen fertilizer treatments did not significantly affect nitrate or ammonium nitrogen accumulation. At the maturity stage, the soil MBN and MBC under the N3 treatment were higher than those of other treatments. Changes in the accumulation of nitrate and ammonium nitrogen showed similar trends.

3.2.5 Relationship between nitrification intensity, denitrification intensity and soil properties

As revealed by Pearson correlation analysis (Figure 9), nitrification intensity positively correlated with the ammonia monooxygenase gene in the ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB), as well as with the Cu nitrate reductase gene, *cd1* nitrate reductase gene, nitrate reductase activity, ammonium nitrogen accumulation, and soil MBN and MBCC, however, negatively correlated with protease activity and nitrate accumulation. The copies of nitrification intensity and denitrification intensity showed similar correlations with soil properties.

[IMAGE-C:\Workspace\ACDC\ImageHandler\9a

1: Ammonia monooxygenase gene in AOA populations; 2: Ammonia monooxygenase gene in AOB populations; 3: Cu nitrate reductase gene; 4: *cd1* nitrate reductase gene; 5: Urease content; 6: Nitrate reductase activity; 7: Nitrite reductase activity; 8: Protease activity; 9: Nitrate accumulation; 10: Ammonium nitrogen accumulation; 11: Soil microbial biomass N; 12: Soil microbial biomass C.

Figure 9. The relationship between soil properties and nitrification intensity, denitrification intensity. *: $P \leq 0.05$; **: $P \leq 0.01$.

3.3 Wheat nitrogen utilization by ^{15}N tracer technique

3.3.1 Nitrogen absorption, utilization and residues in wheat-soil system

As shown in Figure 10A, split nitrogen fertilizer treatment significantly affected nitrogen accumulation in wheat plant and grain. Compared with those under N1, N2, N4, or N5 treatment, the total plant nitrogen accumulation amount (TNAA) under N3 treatment increased by 19.89%, 7.20%, 10.74%, and 21.81%, respectively; while NAAG increased by 27.36%, 9.50%, 13.16%, and 28.27%, respectively.

In addition to the nitrogen obtained from other crops that fix nitrogen, the nitrogen in winter wheat primarily originates from the soil and nitrogen fertilizer. The plants treated with N3 absorbed the greatest amount of nitrogen from fertilizers and soil, followed by plants treated with N2 and N4, whereas the plants treated with N1 or N5 had the lowest levels. Moreover, the amount of nitrogen absorbed from basal/topdressing nitrogen fertilizer increased with increasing basal/topdressing nitrogen ratio. These results demonstrate that N3 treatment was more conducive to nitrogen nutrient uptake by wheat plants, resulting in high-yield and high-efficiency.

3.3.2 ¹⁵N fertilizer in wheat plant-soil system

We compared and analyzed the ratios of nitrogen fertilizer residue, plant recovery, and potential loss to nitrogen fertilizer application under the five treatments with split nitrogen (Figure 11). No significant difference was observed in the different ratios of nitrogen fertilizer residue to nitrogen fertilizer application. The ratio of plant recovery to nitrogen fertilizer application in N3 increased by 5.16–28.59% compared with those under N1, N2, N4, or N5 treatment. Similarly, under N3 treatment, the ratio of potential loss to nitrogen fertilizer application decreased by 12.81–24.73% compared with those under N1, N4, or N5 treatment. Increases in potential loss indicate that the significant loss of nitrogen to the atmosphere occurs through other channels, resulting in environmental issues.

4 Discussion

4.1 The effect of split nitrogen fertilizer on ammonia volatilization

Traditional methods of applying nitrogen fertilizer in winter wheat planting are often inefficient (Nkebiwe et al., 2016), whereas split nitrogen fertilization may be a promising method to improve grain yield while reducing nitrogen loss via NH₃ volatilization (Afroz et al., 2014; Huda et al., 2016; Nkebiwe et al., 2016). Our results show that application of nitrogen fertilization had a significant influence on cumulative NH₃ losses, which is consistent with the findings of Mkhabela et al. (2009). Cumulative NH₃ loss from the different treatments accounted for 15.97~19.23% of the total nitrogen inputs over the two years. In contrast, it was evident that N3 treatments has the ability to reduce NH₃ losses by 5.22~13.42% of the cumulative NH₃ volatilization, with significant reductions of 9.20~21.36% and 9.91~22.78% in both the volatilization of NH₃ and that of yield-scaled NH₃, respectively, under the N3 treatment over the two years. Hence, an appropriate split nitrogen fertilization scheme may decrease the rate of urea hydrolysis, which could contribute to decreases in NH₃ volatilization.

It is evident that nitrogen loss via NH₃ volatilization increases in conjunction with increasing proportions of basal or topdressing nitrogen (Li et al., 2018). Indeed, the current study was purposefully conducted in this region to demonstrate that NH₃ volatilization from basal nitrogen fertilizer is higher than that from topdressing nitrogen fertilizer (Zhong et al., 2021). This may be due to immediate irrigation following topdressing nitrogen application causing infiltration of fertilizer into the deep soil, with a subsequent reduction in the amount of ammoniacal nitrogen in the top soil layer. This would consequently lower the losses from the volatilization of NH₃, which supports the findings from previous studies (Holcomb et al., 2011). In our study, the peak of daily NH₃ fluxes was identified 2~3 days after the application of N fertilizer, and they subsequently decreased to relatively low levels 6~7 days after application. These results suggest that the loss of NH₃ primarily occurs during the early period following nitrogen application. In general, the duration of our gas sampling measurements following nitrogen fertilizer application could effectively capture most of the NH₃ volatilization induced by fertilizer application.

4.2 The effect of split nitrogen fertilizer on nitrification intensity

The nitrification intensity is a metric with the goal of determining the maximum capacity of nitrifiers in the transformation of ammonium (Li et al., 2018). Nitrogen fertilizer addition significantly alters the nitrification intensity at the stages of wheat jointing, anthesis and maturity, suggesting that nitrogen fertilizer affects the current season's wheat. The nitrification intensity at heading is significantly and negatively correlated with N uptake by wheat, showed that nitrification is an critical factor in the growth period of wheat (Yang et al., 2017).

AOA and AOB are two critical groups that participate in nitrification. The findings of our study indicate that nitrification activity was stimulated by large topdressing nitrogen fertilizer proportions and was accompanied by a significant increase in the abundance of AOB. AOB can frequently outcompete AOA for the inorganic nitrogen fertilizer (Hink et al., 2017). This competition can include the inhibition of AOA functions and growth, which prefer to use native soil N in contrast to an exogenous N source as a substrate (Fisk et al., 2015). The increase in soil nitrification could result in loss of nitrogen from agricultural systems and subsequent pollution of groundwater owing to nitrate leaching and denitrification. Our results also show that larger topdressing nitrogen fertilizer proportions produce a higher average accumulation of nitrate and ammonium in soil than other split nitrogen fertilizer ratios.

We demonstrated the inhibitory effect of the optimum split nitrogen fertilizer ratio on nitrification intensity from another perspective. The consequences of split nitrogen fertilizer on the microbial community structure in soil nitrification merit further study. Generally, the primary action of excess topdressing nitrogen fertilizer is to improve urea hydrolysis (Cantarella et al., 2018), and the results of this study suggest that the activity of urease also increases with the increasing topdressing nitrogen proportion.

4.3 The effect of split nitrogen fertilizer on denitrification intensity

A substantial amount of previous research has shown that total N_2O emissions positively correlated with soil denitrification intensity (Wang et al., 1991), which is closely related to the size of the pool labile N forms, such as $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and MBN. In this study, N3 treatment resulted in a significant decrease in the concentrations of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$, suggesting that this split nitrogen fertilizer ratio reduces urea degradation and that such treatment improves the absorption of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ ions and soluble organic N compounds (Lu et al., 2014; Liu et al., 2017; Li et al., 2018b). This relationship was confirmed by Harter et al. Who found that the emissions of N_2O from the soil are indirectly reduced by a decrease in the concentrations of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$.

In our field trial, the N3 treatment significantly decreased the intensity of soil denitrification and the activities of soil nitrate reductase and nitrite reductase. These findings indicate that nitrate reductase and nitrite reductase in the soil play important roles in the amount of N_2O that is emitted from the soil (Ding et al., 2011; Fan et al., 2018). The processes of soil denitrification are the primary sources of emissions by soil N_2O (Cayuela et al., 2013; Wu et al., 2017a). Moreover, split nitrogen fertilizer can alter the rates of soil denitrification by altering the soil microbial and chemical properties of the soil (Nguyen et al., 2017; Li et al., 2018b). For example, managing nitrogen fertilizer appropriately can result in a reduction in the gross rates of soil denitrification by altering the community structure of soil AOB (Dempster et al., 2012; Li et al., 2018b).

Zhang et al. (2010b) also reported that *nirK* and *nirS* are the predominant genes in soil denitrification, the abundance of which was significantly reduced in our study following application of an optimal split nitrogen fertilizer ratio. A possible explanation for this observation could be that N3 treatment maintains the contents of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the soil at a low level, and thus, denitrifying bacteria only have a low level of activity. Along with the increased soil denitrification rates following split nitrogen fertilizer application, these results suggest that N3 treatment decreases soil N_2O emission by reducing nitrification and denitrification rates. Based on these data, we conclude that the optimal split nitrogen fertilizer application ratio decreases soil N losses by decreasing the concentrations of labile N, the activities of N-cycling enzymes, and the abundance of N-cycling key genes, as well as the rates of denitrification in wheat-land.

4.4 The effect of split nitrogen fertilizer on wheat nitrogen utilization by ^{15}N tracer technique

In the ^{15}N tracer experiment, separate applications of basal fertilizer ^{15}N and topdressing fertilizer ^{15}N were used to overcome an issue with the utilization of traditional fertilizers, which only examine the utilization of the total nitrogen fertilizer during the process of wheat growing. Based on measurements in soil samples and wheat plants, we estimated the applications of basal and topdressing nitrogen fertilizers in the wheat-soil system and the accumulation of nitrogen from basal/topdressing fertilizer in the system of wheat and soil. The results indicated that the N3 treatment resulted in a higher TNAA of wheat to fertilizer ^{15}N than that under other split nitrogen fertilizer treatments. The TNAA in wheat was increased by 7.20-21.81% relative to that from fertilizer ^{15}N , and NAAG (9.50-28.27%) was the highest under N3 treatment. In addition, under N3 treatment, the nitrogen accumulation from soil increased by 7.20-27.45%, compared with that under other split nitrogen fertilizer treatments. These results indicate that the N3 treatment contributes to a high accumulation of nitrogen by improving the absorption and utilization of soil and fertilizer nitrogen by wheat (Shi et al., 2012). In fact, a single application of excess fertilization resulted in a soil nitrogen surplus due to a difference in the supply of N supply and demand of the crop (Fageria et al., 2005).

Previous studies have shown that $\text{NO}_3\text{-N}$ leaching will lose excess N and pollute the environment (Oborn et al., 2003; Sieling et al., 2006). Our results indicate that the plants accumulate higher amounts of nitrogen when it is applied as topdressing rather than basal fertilizer. Thus, applications of basal N and high-level topdressing with N lead to a surplus of soil N and possibly loss via the leaching of $\text{NO}_3\text{-N}$, the loss of basal N results in a loss of N throughout the entire growing season due to the poor synchrony between the supply of N and the demand of crops. Further evidence suggests that altering the type of N fertilizer and applying it at the optimal rates for fertilization can meet the dual goals of sustaining the accumulation of nitrogen in crops and mitigating the volatilization of NH_3 and greenhouse gases in winter wheat systems.

5 Conclusion

Compared with current conventional strategies, a cleaner nitrogen fertilization strategy for winter wheat production should decrease wheat-land soil NH_3 volatilization while increasing grain yields and nitrogen accumulation to achieve sustainable agricultural development. In this study, N3 treatment significantly decreased soil NH_3 volatilization as well as nitrification and denitrification intensities, while increasing nitrogen accumulation in grains in the winter wheat cropping system, resulting in a lower overall environmental burden. Thus, appropriately splitting nitrogen fertilizer applications under water-saving irrigation conditions is an effective fertilization strategy with benefits for both agronomy and the environment.

Declarations

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Data availability: The datasets used or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

Figure 1
Map showing the study site.

Figure 2
Effective precipitation and temperature during wheat growth period.

Figure 3

Map showing the micro-plots.

Figure 4

Effect of split nitrogen fertilizer on NH_3 flux.

Figure 5

The effects of split nitrogen fertilizer on the nitrification intensity and denitrification intensity.

Figure 6

The effects of split nitrogen fertilizer on the abundance of Amoa-AOA: ammonia monooxygenase gene in AOA populations; Amoa-AOB: ammonia monooxygenase gene in AOB populations; nirK: Cu nitrate reductase gene; nirS: cd1 nitrate reductase gene.

Figure 7

The effects of split nitrogen fertilizer on the soil nitrogen invertase activity.

Figure 8

The effects of split nitrogen fertilizer on the nitrate accumulation, ammonium nitrogen accumulation, soil microbial biomass N and C.

Figure 9

The relationship between soil properties and nitrification intensity, denitrification intensity. *: $P \leq 0.05$; **: $P \leq 0.01$.

Figure 10

Effect of split nitrogen fertilizer on the plant nitrogen accumulation form fertilizer or soil. NAAG, nitrogen accumulation amount in grains; TNAA, Total plant nitrogen accumulation amount; SN, soil inorganic nitrogen content.

Figure 11

Effect of split nitrogen fertilizer on the fate of ^{15}N fertilizer in plant-soil system.