agronomy

Article

Performance of Nitrogen Fertilization and Nitrification Inhibitors in the Irrigated Wheat Fields

Shahram Torabian [1,2], Salar Farhangi-Abriz [3], Ruijun Qin [1,*], Christos Noulas [4,*] and Guojie Wang [5]

1 Hermiston Agricultural Research and Extension Center, Oregon State University, Hermiston, OR 97838, USA
2 Agricultural Research Station, Virginia State University, Petersburg, VA 23806, USA
3 Department of Plant Eco-Physiology, Faculty of Agriculture, University of Tabriz, Tabriz 5166616471, Iran
4 Institute of Industrial and Forage Crops, Hellenic Agricultural Organization "Demeter", 41335 Larissa, Greece
5 Department of Plant Science, The Pennsylvania State University, University Park, PA 16802, USA
* Correspondence: ruijun.qin@oregonstate.edu (R.Q.); noulaschristos@gmail.com (C.N.)

Abstract: Effective nitrogen (N) management practices are critical to sustain crop production and minimize nitrate (NO_3^-) leaching loss from irrigated fields in the Columbia Basin (U.S.), but studies on the applied practices are limited. Therefore, from 2014 to 2016, two separate field studies were conducted in sandy loam soils in the region to evaluate the performance of various N fertilizers in spring and winter wheat. The treatments consisted of two nitrification inhibitors (NIs) (Instinct® II and Agrotain® Ultra) in combination with two N fertilizers (urea and urea ammonium nitrate [UAN]) under two application methods (single vs. split-application) and two rates (100% vs. 85% of growers' standard). The results from these field trials demonstrated that N fertilizer treatments did not affect wheat grain yield (GY) and grain protein (GP). In the spring wheat trial, higher NH_4^+-N content but lower NO_3^--N content was observed in the UAN treatments (0–30 cm). However, the application of NIs had no considerable effect on soil N content. In the winter wheat trial, the split N application generally reduced NO_3^--N and total mineral nitrogen (TMN) content, especially at 30–60 cm, in comparison to a single application. The use of Instinct® II tended to reduce NO_3^--N and TMN contents, while Agrotain® Ultra was not effective in inhibiting nitrification. Our findings suggest that more studies on the effectiveness of NIs and N applications would enable growers to optimize N use efficiency and crop production in the region.

Keywords: nitrification inhibitors; nitrogen management; soil ammonium; soil nitrate; grain yield

Citation: Torabian, S.; Farhangi-Abriz, S.; Qin, R.; Noulas, C.; Wang, G. Performance of Nitrogen Fertilization and Nitrification Inhibitors in the Irrigated Wheat Fields. *Agronomy* **2023**, *13*, 366. https://doi.org/10.3390/agronomy13020366

Academic Editor: Wei Wu

Received: 23 December 2022
Revised: 21 January 2023
Accepted: 24 January 2023
Published: 27 January 2023

1. Introduction

Nitrogen (N) supply is highly relevant in wheat production, affecting yield and yield components. However, insufficient N in the rhizosphere is one of the most yield-limiting practices in intensive agricultural systems [1,2]. Balanced N management is key to sustainable wheat production and a cost-effective strategy to increase crop yields and improve long-term product quality [3]. While increased N fertilizer applications in intensive agriculture enhance yields, they increase the risk of N release into the environment (gaseous N loss, erosion, leaching) [4]. To maximize crop returns, farmers repeatedly apply N fertilizers in various forms (i.e., urea—$CO(NH_2)_2$, ammonium nitrate—NH_4NO_3, ammonium sulfate—$(NH_4)_2SO_4$, etc.), although these applications are not accompanied by proportional increases in N use efficiency (NUE) [5]. High NUE is paramount to reducing environmental pollution and guaranteeing acceptable yield while minimizing unnecessary fertilizer waste. Soil erosion, surface runoff, ammonia (NH_3) volatilization, nitrate (NO_3^-) leaching, and denitrification make N unavailable to plants. These processes are widely dependent on the cropping system, the form of fertilizer, and the method of application [6].

Ammonium (NH_4^+) and NO_3^- are the two N forms available for plants, and they have an important effect on crop growth and quality. More than 90% of soil N is in the organic form [7]. Physiologically, NH_4^+ uptake by plants from the soil is faster than NO_3^- [8].

Salsac et al. [9] found that assimilation of NH_4^+ requires 5 ATP mol^{-1} NH_4^+, while NO_3^- assimilation needs about 20 ATP mol^{-1} NO_3^-. Moreover, absorption of 1 mol NH_4^+ by plant roots consumes about 0.31 mol O_2, but 1 mol of NO_3^- absorption requires 1.5 mol O_2 [10]. Hence, NO_3^- absorption requires about five times more energy compared to NH_4^+ absorption. In addition, NH_4^+ can be directly used by plants to produce amino acids, but NO_3^- must be converted to NO_2^- and then to NH_4^+. Thus NO_3^- metabolism requires more energy than NH_4^+ metabolism.

Maintaining N in the NH_4^+ form in the soil would prevent its loss to nitrification and denitrification. In agricultural soils, NO_3^- originates from fertilizers, animal manure, atmospheric deposition, and nitrification of NH_4^+. During nitrification, NH_4^+ is converted by specific nitrifying microorganisms to NO_3^-, which is highly mobile and can leach after heavy rainfall or extensive irrigation management events [11]. NO_3^- readily moves with water from the root zone to deeper soil layers, depleting the plant-available N supply and causing environmental pollution [12]. The potential for nitrate-N to leach depends on soil type, N fertilizer source, and farm management strategies.

Management practices and the adoption of technologies such as controlled-release fertilizers and urease and nitrification inhibitors (NIs) can mitigate NO_3^- loss from the soil–plant system. Research suggests that the use of NIs is a promising approach to the reduction of nitrate leaching [13,14]. NIs diminish the transformation of NH_4^+ to NO_3^- in soil by reducing the activity of nitrifying microorganisms, with the benefit of decreasing NO_3^- leaching potential [15].

Common fertilizers usually contain N in one or more of the following forms: NO_3^-, NH_3, NH_4^+, or $CO(NH_2)_2$. Each form has specific properties determining its suitability for use. Most N fertilizers are used in the form of NH_4^+ or $CO(NH_2)_2$, which are easily converted to NO_3^- in the nitrification process. Previous studies have shown that adding NIs to NH_4^+ or NH_4-containing fertilizers decreases NO_3^--N formation, N leaching, and denitrification process, thus retaining N at the root zone, which is where the crops need it [16,17]. Lin and Hernandez-Ramirez [18] reported that NIs steadily increase concentrations of N in the NH_4^+ form. The stabilization of NH_4^+ by NIs allows for simplified fertilization strategies with reduced fertilizer applications [19,20]. This stabilization may increase crop yield while reducing negative environmental impacts. Bhatia et al. [21] showed that the application of urea with NIs *[S-benzylisothiouronium butanoate* (SBT-*butanoate) and S-benzylisothiouronium furoate* (SBT-*furoate)]* improves wheat yield. Liu et al. [22] revealed that NIs (*dicyandiamide* and DMPP) increase grain yield and NUE in a wheat–maize cropping system. Ma et al. [23] stated that the application of *dicyandiamide* and *chlorinated pyridine* as NIs increased wheat yield in conventional and no-till practices. In a recent study, Dawar et al. [24] showed that NIs preserve N in the rhizosphere and improve NUE in wheat. During the wet season in Montana, the application of Agrotain® Ultra (urease inhibitor, Koch Agronomic Services) with urea increased winter wheat yield, although no noticeable increase was found during the dry season [25].

There are some inconsistent reports about the efficiency of NIs. Dawar et al. [26] observed that urease inhibitor N-(n-butyl) *thiophosphoric triamide* (NBPT) reduced NH_4^+ concentrations for the first 5 days following fertilizer application. However, afterward, NH_4^+ concentration did not differ from the urea control. Chen et al. [27] observed that under moist (60% water-filled pore space) and mild conditions (15 °C), the effect of NI declined substantially after 14 days. Zaman and Blennerhassett [28] reported that spring applications of NIs did not significantly reduce NO_3^- leaching in a pasture system. They attributed this lack of response to a nine-month delay between the NIs application and the first leaching event. During this period, NIs may have been rendered ineffective by soil microorganisms. Following the application, bacteria gradually decompose NIs and they can be leached down the soil profile. NIs effectiveness is, therefore, governed by factors such as temperature, rainfall/drainage levels, soil organic matter, and pH [29–32]. It has been reported that the half-life of *dicyandiamide* (DCD) was 111–116 days at a soil temperature of 8 °C, while it became 18–25 days at a soil temperature of 20 °C [33]. The low effectiveness of DCD was attributed to late-season drainage occurring when soil DCD concentrations were likely low [34]. Suter et al. [35] reported that neither DMPP- nor NBPT-coated urea increased pasture yields. Cookson and Cornforth [36] did not find any increase in pasture dry matter from DCD use. Because Instinct® II (Cortiva Agriscience) did not induce positive effects on corn growth or yield, Sassman et al. [37] concluded that Instinct® II use with UAN solution at spring pre-plant would not be effective in enhancing fertilizer N availability to the crop, nor to increase corn production. Owing to the inconsistent efficiency of NIs under different climate conditions, further investigations are needed to optimize NUE from NI use.

Oregon's Columbia Basin is a main crop production region in the U.S. Soils are generally coarse-textured with low soil organic matter content and low water holding capacity. Therefore, there is a great potential for nitrate leaching, particularly in irrigated systems. Deteriorating groundwater quality has increased regulatory pressure to reduce nitrate leaching. Identification of optimal fertilization strategies could sustain or improve crop production while minimizing environmental hazards. However, little information is available on how nitrogen fertilizer sources, rates, and application methods impact crops and soils in the region. NIs might be effective tools to overcome nitrate-leaching issues in the Columbia Basin. Agrotain® Ultra urease inhibitor is marketed as an effective product for reducing N losses and improving crop NUE. Instinct® II is a nitrogen stabilizer containing nitrapyrin that delays the nitrification of ammoniacal and urea N fertilizers in soils by controlling the nitrification process. Thus, it can sustain or increase crop yield while reducing environmental issues. Both products are available in the region, but the information on their effectiveness is very limited. Therefore, comprehensive studies to evaluate the efficacy of these products in managing N could be of great importance in this region. For this purpose, we carried out two field trials with spring wheat and winter wheat from 2014 to 2016, using two kinds of NIs (Instinct® II and Agrotain® Ultra) on two N fertilizer sources, i.e., urea [$CO(NH_2)_2$] and urea ammonium nitrate UAN (liquid form; UAN32) with two N rates (85% vs. 100%) and two application methods (single application vs. split-application). We measured soil and plant parameters, including mineral soil N content (NO_3^--N, NH_4^+-N and total mineral nitrogen-TMN), grain yield (GY), SPAD (leaf greenness), and grain protein (GP). These findings will provide growers with insights about N management strategies, improve NUE, and reduce environmental contamination.

2. Materials and Methods

2.1. The Experiments and Growing Conditions

Two field trials were conducted at the Oregon State University-Hermiston Agricultural Research and Extension Center, Hermiston, OR (Latitude: 45°50′43.9548″ N, Longitude: 119°17′33.5076″ W, elevation 140 m above sea level). The trial with spring wheat (*Triticum aestivum* L.) was conducted in 2014; the trial with winter wheat was conducted during the 2015−2016 growing seasons. The climate in the region is classified as *Csa* (= temperate, dry, and hot summer) by the Köppen-Geiger system [38]. In the 2014 growing season (March to July), cumulative precipitation was 60.0 mm, and the mean air temperature was 16.4 °C. March, with a mean temperature of 8.1 °C, was the coldest month, while July, with a mean temperature of 25.3 °C, was the warmest month (Figure 1). In the 2015–2016 growing season (October 2015 to July 2016), precipitation was 175.4 mm, and the mean air temperature was 11.2 °C. January was the coldest month, with a mean temperature of 2.0 °C, and July was the warmest month, with a mean temperature of 22.5 °C (Figure 1). Both trials were conducted on an Adkins fine sandy loam (Adkins coarse-loamy, mixed, superactive, *mesic Xeric Haplocalcid*). The basic soil properties for a soil depth of 0–30 cm were pH of 6.3, soil organic matter of 0.8%, soil available P of 39 mg kg^{-1}, soil available K of 330 mg kg^{-1}, and soil S of 49 mg kg^{-1}. Soil nitrogen (N) of 0–60 cm before the trials are compiled in Table 1.

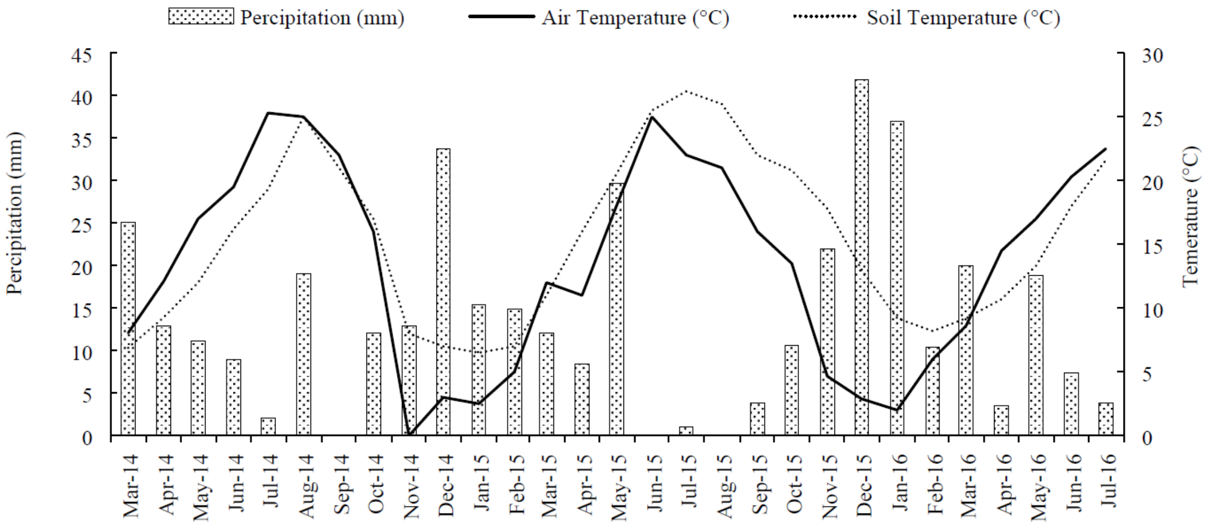


Figure 1. Average monthly air and soil temperatures (20 cm depth) and amount of precipitation during the two growing seasons (2014 for spring wheat and 2015−2016 for winter wheat).

Table 1. Soil nitrogen (mg kg^{-1} soil) before field trial establishment at Hermiston, OR, 2014–2016.

Growth Season	$NH4^+$-N	NO_3^--N	$NH4^+$-N	NO_3^--N
	(0–30 cm)		(30–60 cm)	
2014 (spring wheat)	10.3	34.0	10.8	16.0
2015–2016 (winter wheat)	7.1	17.1	4.1	9.2

In the spring wheat trial, the variety 'Westbred 528′ was sown on March 10, 2014, at 135 kg ha^{-1} and harvested on July 23, 2014. In the winter wheat trial, the variety 'LCS Jet' was sown on 29 October 2015 at 135 kg ha^{-1} and harvested on 15 July 2016. Growers' standard pest and weed controls were applied throughout the growing season.

In both trials, each experimental plot was 9×9 m^2 in size containing 35 rows. The row-to-row distance was 0.26 m. The experiments were laid out as a randomized complete block design (RCBD), having eleven treatments with five replications in 2014 and four treatments with four replications in the 2015−2016 growing season.

The treatments in the 2014 trial included two sources of N, i.e., urea and UAN32, with two application rates (100% and 85%) in combination with two NIs as follows: (1) No-fertilizer Control (CK), (2) 85% Urea (85U), (3) 85% Urea + Instinct® II (85U + I), (4) 85% Urea + Agrotain® Ultra (85U + A), (5) 100% Urea (100U), (6) 100% Urea + Instinct® II (100U + I), (7) 85% UAN (85UAN), (8) 85% UAN + Instinct® II (85UAN + I), (9) 85% UAN + Agrotain® Ultra (85UAN + A), (10) 100% UAN (100UAN), and (11) 100% UAN + Instinct® II (100UAN + I). The treatments were applied three days after sowing. The N application rate of 100% U or 100% UAN was equivalent to 225 kg ha^{-1}. Both NIs were mixed with fertilizers before application. The mixing rates of Agrotain® Ultra to urea and UAN were 3.1 and 1.55 L ton^{-1}, respectively. The mixing rate of Instinct® II was according to its label rate of 1.1 kg ha^{-1}.

In the 2015–2016 trial, four treatments consisted of the following fertilizer-NIs combinations: (1) Single application of UAN + Instinct® II (100UAN + I), (2) Single application of UAN (100UAN), (3) Split application of UAN + Instinct® II (60% UAN in fall + Instinct® II and 40% UAN in spring; 60/40UAN + I), and (4) Split application of UAN (60% UAN in fall and 40% UAN in spring; 60/40UAN). For the treatments of 100 UAN + I and 100 UAN, the fertilizer was applied on October 27, while the Instinct® II was applied on October 28. For the treatments of 60/40UAN + I or 60/40UAN, 60% of UAN was applied on October 27, and the Instinct® II was applied on October 28, 2015, while 40% of UAN was applied on April 6 at the stem elongation stage. The N rate for the 100 UAN was 280 kg ha^{-1}. Fertilizers were applied by hand uniformly on the ground. The Instinct® II was only applied in fall with a rate of 1.1 kg ha^{-1} with the purpose of reducing nitrate loss through the wet winter.

2.2. Sampling and Measurements

2.2.1. Wheat Plant Parameters

The extended BBCH scale [39] was used to describe the phenological development of 50% of the plants in each treatment. During 2014, wheat parameters such as plant height (PH), leaf greenness (SPAD values), and total N content of flag leaf were measured at the flag leaf stage (BBCH stage 39). PH was recorded from 10 randomly selected plants from the inner plant rows in each plot. The SPAD meter readings were used as an indicator of leaf chlorophyll content per unit leaf area [40,41] and were determined on the blades, midway between the leaf edge and midrib [42] of fully expanded flag leaves using a SPAD-502 m (Minolta, Plainfield, IL, USA). Measurements were taken early in the morning and recorded as the mean of 10 randomly selected fully expanded leaves per plot. Total N content of the flag leaves was determined by the Kjeldhal method [43]. Moreover, at physiological maturity (BBCH stage 91 and 92), grain yield (GY) was assessed on a per plot basis and converted to tons per hectare (t ha^{-1}) after adjusting to 13% moisture content. Grain moisture (%) (GM) and grain protein content (%) (GP) were also measured. In the 2015–2016 trial, data collection was generally similar to the 2014 trial, with exceptions for PH and total N content of flag leaves.

2.2.2. Soil Nitrogen Content

In both trials, representative soil samples were collected to assess the contents of NH_4^+-N, NO_3^--N, and total mineral N (TMN) from 0–30 cm and 30–60 cm soil depths. Five well-distributed locations per plot were selected for soil sampling. The soils from the same depth were mixed uniformly into a composite sample and submitted for analysis. Soil sampling was conducted between irrigation events. The contents of NH_4^+-N and NO_3^--N were determined by potassium chloride extraction combined with cadmium reduction [44,45]. The TMN was calculated as the sum of the NH_4^+-N and NO_3^--N.

For the 2014 trial, soil N was measured at the 2nd, 4th, 6th, and 8th weeks after plant emergence (WAE). For the 2015–2016 trial, soil N was measured 3 weeks before the second split application and at the 4th and 8th week after the second split application.

2.3. Statistical Analyses

Data were subjected to analysis of variance (ANOVA) using the PROC GLM procedure of SAS (SAS version 9.4) for a randomized complete block design after checking for the normalcy of the variables with the Kolmogorov–Smirnov test. Means were compared using Fisher's least significant difference test (LSD) at $p \leq 0.05$. Data on NH_4^+-N, NO_3^--N, and TMN contents were analyzed with a split plot in time arrangement based on randomized complete block design because there were multiple measurements on the same experimental unit. Treatment and sampling were considered as main plot and subplot, respectively. It is noted that the presented table is a slice of the complete analysis. Figures were prepared in Excel version 2016 64-Bit Edition.

3. Results

3.1. First Experiment: Spring Wheat

Treatments significantly affected PH, leaf greenness (SPAD), and GY of spring wheat, and no effects were found for total leaf N content, GM, and GP (Table 2). Compared to control (no-fertilizer), PH, SPAD, and GY were 29.0%, 20.8%, and 31.0% higher when fertilizer/NIs combinations were applied. However, among all fertilization treatments, there was no significant difference in terms of GY. The tallest plants were recorded at 100U, followed by 85U + A and 85U + I, while the shortest plants were recorded at the treatments that included either 85U and 100U + I or any other UAN combination with NIs. The highest values of SPAD meter readings were recorded at 100UAN (Table 2).

Table 2. Analysis of variance (*p* values) of N fertilizer-nitrification inhibitor combinations on spring wheat plant height (PH), leaf greenness (SPAD), total N content of flag leaves measured at flag leaf stage, and grain yield (GY), grain moisture (GM) and protein content (GP) at physiological maturity in 2014.

Source of Variation	df	PH (cm)	SPAD	Total N of Flag Leaf mg kg^{-1}	GY (t ha^{-1})	GM (%)	GP (%)
Rep	4	0.61	0.28	0.01	0.08	0.25	0.78
Treatment	10	<0.01	<0.01	0.50	0.05	0.36	0.16
Control		47 ± 1.1^{d}	37 ± 1.3^{d}	3.5 ± 0.4	4.06 ± 0.45^{b}	5.5 ± 0.2	13.0 ± 0.6
85U		60 ± 0.4^{bc}	44 ± 0.4^{ab}	3.5 ± 0.2	5.30 ± 0.45^{a}	5.1 ± 0.1	14.2 ± 0.7
85U + I		62 ± 0.9^{ab}	46 ± 0.9^{ab}	4.1 ± 0.3	5.55 ± 0.41^{a}	5.3 ± 0.1	13.6 ± 0.3
85U + A		63 ± 1.2^{ab}	45 ± 1.0^{ab}	4.0 ± 0.2	4.91 ± 0.55^{a}	5.0 ± 0.1	14.5 ± 0.7
100U		64 ± 0.7^{a}	46 ± 0.7^{ab}	3.8 ± 0.1	5.45 ± 0.25^{a}	5.5 ± 0.1	14.8 ± 0.4
100U + I		60 ± 1.4^{bc}	43 ± 1.3^{bc}	3.9 ± 0.2	5.32 ± 0.30^{a}	5.3 ± 0.1	14.2 ± 0.5
85UAN		59 ± 0.8^{c}	41 ± 2.3^{c}	4.2 ± 0.4	5.45 ± 0.35^{a}	5.3 ± 0.2	13.0 ± 0.5
85UAN + I		59 ± 1.0^{c}	45 ± 0.6^{ab}	3.7 ± 0.2	5.48 ± 0.22^{a}	5.2 ± 0.1	14.5 ± 0.5
85UAN + A		60 ± 0.9^{bc}	45 ± 0.7^{ab}	3.9 ± 0.2	5.63 ± 0.23^{a}	5.2 ± 0.1	14.1 ± 0.5
100UAN		61 ± 0.5^{bc}	47 ± 1.1^{a}	3.6 ± 0.1	5.15 ± 0.25^{a}	5.1 ± 0.1	15.2 ± 0.5
100UAN + I		60 ± 1.2^{bc}	45 ± 0.2^{ab}	3.8 ± 0.3	4.95 ± 0.34^{a}	5.5 ± 0.1	14.5 ± 0.2

Means are averages of five replicates ±SE (standard error). Different letters within columns indicate means with significant differences according to least significant difference (LSD) at $p < 0.05$. No-fertilizer (Control), 85% Urea (85U), 85% Urea + Instinct® II (85U + I), 85% Urea + Agrotain® Ultra (85U + A), 100% Urea (100U), 100% Urea + Instinct® II (100U + I), 85% UAN (85UAN), 85% UAN + Instinct® II (85UAN + I), 85% UAN + Agrotain® Ultra (85UAN + A), 100% UAN (100UAN), 100% UAN + Instinct® II (100UAN + I).

The treatment effects, sampling time, and their interaction on NH_4^+-N, NO_3^--N, and TMN of soil are shown in Table 3. Compared to the control, all fertilizer treatments increased NH_4^+-N, NO_3^--N, and TMN contents in 0–30 cm significantly ($p < 0.01$). The use of UAN increased the average NH_4^+-N content of soil compared to urea. The highest soil NH_4^+-N content (14.6 mg N kg^{-1} soil) was found at 100UAN + I, followed by 100UAN, while the lowest one (9.7 mg N kg^{-1} soil) was observed in the treatment of 85U + I (Table 3).

The 100U treatment was associated with the highest soil NO_3^--N content, followed by 100U + I, 85U + A, and 85U, while the lowest NO_3^--N content (12.2 mg N kg^{-1} soil) was found in 85UAN + I, followed by 85UAN, 85U + I, and 85UAN + A. The 100U treatment was associated with the highest TMN at 0–30 cm. Except for the control (8.9 mg N kg^{-1} soil), the 85UAN + I treatment was associated with the lowest TMN (24.0 mg N kg^{-1} soil), followed by 85U + I, 85UAN, and 85UAN + A (Table 3). Urea application generally resulted in higher soil NO_3^--N and TMN than UAN, while the NH_4^+-N content was slightly higher following UAN applications. The effects of NIs on N forms found in soils were relatively limited when speciation was compared to the treatments without NIs. Between the two NIs, soil N contents tended to be higher with Agrotain® Ultra application.

Figure 2a–c show the NH_4^+-N, NO_3^--N, and TMN content in soil (0–30 cm depth). In all treatments, NH_4^+-N content was highest at 2 WAE; the highest value was shown in the treatment of 100UAN + I, while the lowest value was observed in the control, followed by 100U + I and 85U + I. In general, a sharp reduction was found from the 2nd WAE to the 4th WAE, and afterward, the reduction tended to be smoother (Figure 2a). At 8 WAE, no difference was found among the treatments.

Compared to control, the soil NO_3^--N content increased considerably by the second WAE. The NO_3^--N content increased continually up to the maximum at 4 WAE; the highest values were observed in the 85U and 85U + A treatments, while the lowest was observed in the 85UAN + I treatment. Afterward, NO_3^--N content decreased steadily. As a consequence, the lowest NO_3^--N content was obtained at 8 WAE (Figure 2b). On average, NO_3^--N content was less in treatments with UAN than with urea at all sampling events. The addition of Instinct® II tended to decrease NO_3^--N content. As expected, NO_3^--N content was lower in the treatments with lower N application rates. At the last sampling event (8 WAE), the highest and lowest NO_3^--N contents were found in the 100U and 85UAN + I, respectively.

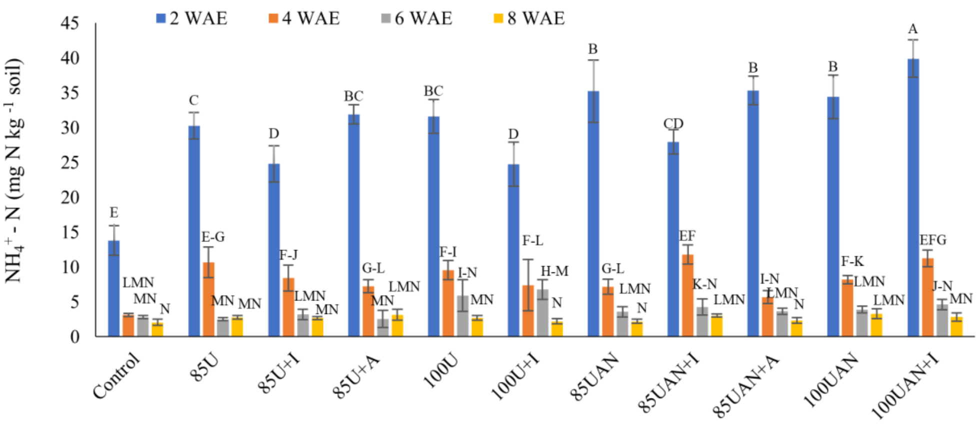
Table 3. Analysis of variance of N fertilizer-nitrification inhibitor combinations and sampling time on soil NO_3^--N, NH_4^+-N, and total mineral N (TMN) at 0–30 cm and 30–60 cm in spring wheat, 2014.

Source of Variation	df	NH_4^+-N	NO_3^--N	TMN	NH_4^+-N	NO_3^--N	TMN
			(mg kg^{-1} soil)			(mg kg^{-1} soil)	
			0–30 cm			30–60 cm	
Rep	4	0.50	0.02	0.03	0.02	0.03	0.09
Treatment	10	<0.01	<0.01	<0.01	0.07	<0.01	0.09
Sampling	3	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Sampling × Treatment	30	<0.01	<0.01	<0.01	0.25	0.29	0.19
Treatment							
Control		5.4 ± 1.2 ^{d}	3.4 ± 0.5 ^{d}	8.9 ± 1.5 ^{f}	5.3 ± 1.2	6.4 ± 0.8 ^{b}	11.7 ± 0.9
85U		12.1 ± 2.6 ^{b}	23 ± 3.8 ^{ab}	33.9 ± 4.4 ^{abc}	5.7 ± 1.4	8.5 ± 1.1 ^{ab}	14.3 ± 1.2
85U + I		9.7 ± 2.1 ^{c}	15.8 ± 2.3 ^{c}	24.9 ± 3.7 ^{de}	5.3 ± 1.4	9.3 ± 1.1 ^{a}	14.6 ± 1.3
85U + A		11.7 ± 2.7 ^{bc}	24.5 ± 3.4 ^{a}	36.4 ± 5 ^{ab}	5.3 ± 2.4	7.9 ± 1.3 ^{ab}	13.3 ± 3.3
100U		12.4 ± 2.7 ^{b}	26.7 ± 2.9 ^{a}	38.1 ± 4.4 ^{a}	5.1 ± 1.3	9.9 ± 0.9 ^{a}	15 ± 1.1
100U + I		10.6 ± 2.2 ^{bc}	26.1 ± 2.9 ^{a}	37.5 ± 4.2 ^{ab}	5.6 ± 1.3	8.2 ± 1 ^{ab}	14 ± 1.6
85UAN		10.9 ± 2.9 ^{bc}	14.1 ± 2.4 ^{c}	25.3 ± 4 ^{de}	5.7 ± 1.5	9.1 ± 0.8 ^{a}	14.8 ± 2.0
85UAN + I		11.7 ± 2.3 ^{bc}	12.2 ± 2 ^{c}	24.0 ± 3.6 ^{e}	6.8 ± 1.6	9.4 ± 0.8 ^{a}	16.3 ± 1.5
85UAN + A		11.7 ± 3.1 ^{bc}	16.4 ± 2.9 ^{c}	28.2 ± 4.2 ^{cde}	6.0 ± 1.4	8.3 ± 0.9 ^{ab}	14.3 ± 1.1
100UAN		12.4 ± 3 ^{ab}	17.7 ± 2.3 ^{bc}	30.1 ± 3.8 ^{bcd}	6.5 ± 1.8	10 ± 0.9 ^{a}	16.5 ± 1.7
100UAN + I		14.6 ± 3.4 ^{a}	16.8 ± 2.4 ^{c}	31.4 ± 5.2 $^{a\text{-}d}$	6.5 ± 1.7	8.8 ± 0.7 ^{a}	15.4 ± 1.8
Sampling							
2 WAE		29.9 ± 1.1 ^{a}	17.4 ± 1.2 ^{b}	46.8 ± 1.9 ^{a}	16.7 ± 0.4 ^{a}	4.7 ± 0.2 ^{d}	21.6 ± 0.7 ^{a}
4 WAE		8.3 ± 0.5 ^{b}	32.1 ± 1.8^{a}	40.3 ± 2.1 ^{b}	3.5 ± 0.3 ^{b}	13 ± 0.3 ^{a}	16.6 ± 0.5 ^{b}
6 WAE		4.1 ± 0.4 ^{c}	15.5 ± 1.3 ^{b}	19.8 ± 1.6 ^{c}	1.8 ± 0.8 ^{c}	10.4 ± 0.5 ^{b}	12.2 ± 1.2 ^{c}
8 WAE		2.6 ± 0.1 ^{d}	6.56 ± 0.8 ^{c}	9.2 ± 0.8 ^{d}	1.3 ± 0.1 ^{c}	6.6 ± 0.4 ^{c}	7.97 ± 1.2 ^{d}

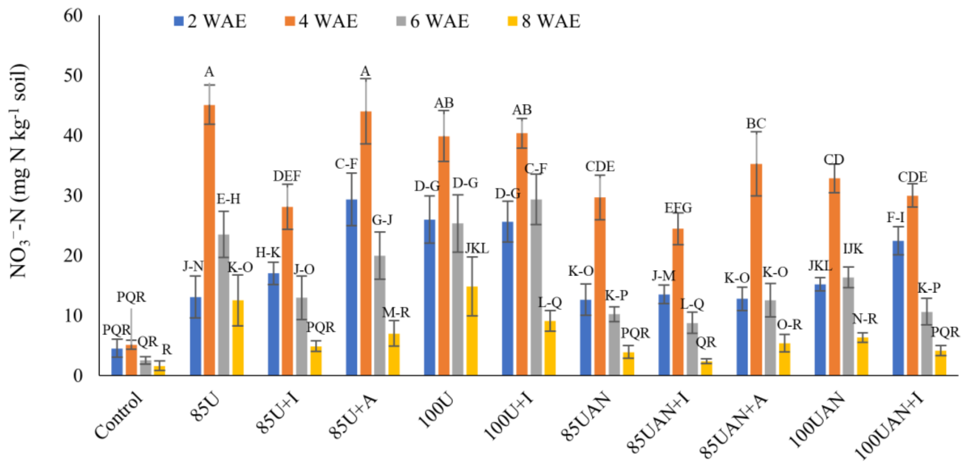
Means are averages of five replicates ± SE (standard error). Different letters within columns indicate means with significant differences according to least significant difference (LSD) at $p < 0.05$. No-fertilizer (Control), 85% Urea (85U), 85% Urea + Instinct® II (85U + I), 85% Urea + Agrotain® Ultra (85U + A), 100% Urea (100U), 100% Urea + Instinct® II (100U + I), 85% UAN (85UAN), 85% UAN + Instinct® II (85UAN + I), 85% UAN + Agrotain® Ultra (85UAN + A), 100% UAN (100UAN), 100% UAN + Instinct® II (100UAN + I). WAE (Weeks after Emergence).

The highest soil TMN content was found at 2 WAE, with the exception of 85U and 100U + I, which happened at 4 WAE (Figure 2c). Afterward, the TMN decreased gradually. At the final sampling event (8 WAE), the highest TMN content occurred in the 100U treatment, and the lowest were in the 85UAN and 85UAN + I treatments. A comparison of the two N sources indicated that TMN content was lower in the UAN treatments. The lower N rate naturally had the lower TMN content. The application of Instinct® II generally did not impact the TMN content or slightly reduced TMN content, while the application of Agrotain® Ultra tended to increase the TMN (Figure 2c).

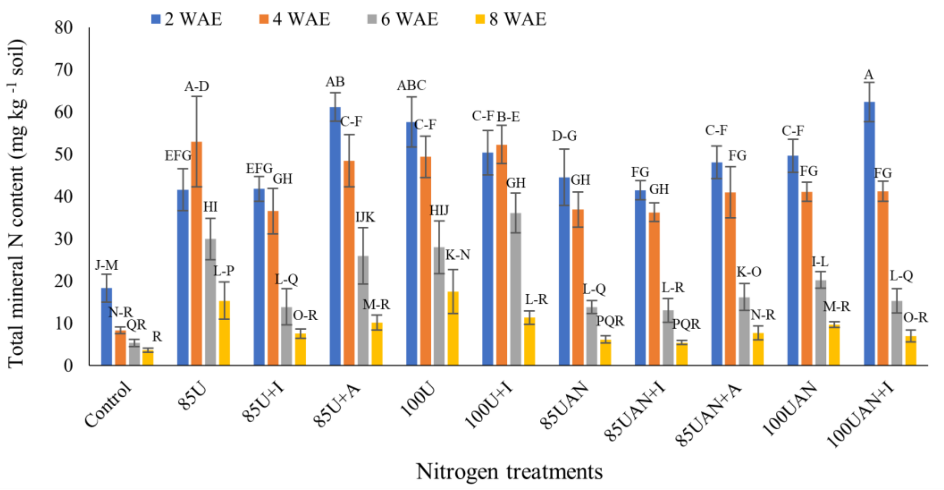
In the 30–60 cm soil profile, fertilization treatments had a significant effect only on NO_3^--N content. The lowest NO_3^--N content (6.4 mg N kg^{-1} soil) was found in the control. Among the fertilization treatments, 85U, 85U + A, 100U + I, 85UAN + A had slightly lower NO_3^--N content. As with 0–30 cm, sampling time significantly affected mineral soil N content at the 30–60 cm soil depth. NH_4^+-N and TMN content in 30–60 cm decreased with sampling time; the lowest NH_4^+-N and TMN contents were observed at 8 WAE. Consistent with the pattern in 0–30 cm, the NO_3^--N content increased 176% at 4 WAF compared to 2 WAE and then gradually decreased (Table 3).



(**a**)



(**b**)



(**c**)

Figure 2. Changes of (**a**) NH_4^+-N, (**b**) NO_3^--N, and (**c**) total mineral N content of soil during sampling times in spring wheat field at 0–30 cm. Data represent the averages of five replicates. Vertical bars indicate standard errors (±SE). Different letters indicate means with significant differences according to least significant difference (LSD) at $p < 0.05$. WAE (Weeks after Emergence). No-fertilizer (Control), 85% Urea (85U), 85% Urea + Instinct® II (85U + I), 85% Urea + Agrotain® Ultra (85U + A), 100% Urea (100U), 100% Urea + Instinct® II (100U + I), 85% UAN (85UAN), 85% UAN + Instinct® II (85UAN + I), 85% UAN + Agrotain® Ultra (85UAN + A), 100% UAN (100UAN), 100% UAN + Instinct® II (100UAN + I).

3.2. Second Experiment: Winter Wheat

Analysis of variance results showed that fertilization treatments, the combination of N fertilization and NIs, had no significant effects on flag leaf greenness (SPAD), GY, GM, and GP of winter wheat (Table 4). GY ranged from 8.51 t ha^{-1} (100UAN) to 9.01 t ha^{-1} (60/40UAN + I), GM ranged from 5.0% (100UAN +I) to 5.3% (100UAN), GP ranged from 9.4% (60/40UAN + I) to 10.0% [(100UAN + I) and (100UAN)], and leaf greenness ranged from 52 (100UAN + I) to 53 (Table 4).

Table 4. Analysis of variance of N fertilizer-nitrification inhibitor-application time combinations on leaf greenness (SPAD) at flag leaf stage and grain yield (GY), grain moisture (GM), and protein (GP) of winter wheat in 2015−2016.

Source of Variation	df	SPAD	GY (t ha^{-1})	GM (%)	GP (%)
Rep	3	0.35	0.015	0.78	0.19
Treatment	3	0.86	0.49	0.19	0.54
100UAN + I		52 ± 1.0	8.53 ± 0.51	5.0 ± 0.04	10.0 ± 0.2
100UAN		53 ± 0.9	8.51 ± 0.36	5.3 ± 0.09	10.0 ± 0.4
60/40UAN + I		53 ± 0.5	9.01 ± 0.22	5.1 ± 0.07	9.4 ± 0.2
60/40UAN		53 ± 0.8	8.77 ± 0.49	5.1 ± 0.10	9.8 ± 0.5

Means are averages of four replicates ± standard error (SE). Single application of UAN + Instinct® II (100UAN + I), single application of urea (100UAN), split application of UAN + Instinct® II (60% UAN in fall + Instinct® II and 40% UAN in spring; 60/40UAN + I), and split application of UAN (60% UAN in fall and 40% UAN in spring; 60/40UAN).

Fertilization treatments did not change NH_4^+-N, NO_3^--N, and TMN content in the 0–30 cm soil profile (Table 5). However, the soil N parameters differed significantly with sampling time ($p < 0.01$). Soil NH_4^+-N, NO_3^--N, and TMN contents decreased 17%, 69%, and 51%, respectively, at 4 weeks after the second split application (WAT), and 60%, 74%, and 69%, respectively, at 8 WAT compared to those found before the second split application (Table 5).

Table 5. Analysis of variance of treatment effects and sampling time on soil NO_3^--N, NH_4^+-N, and TMN at 0–30 cm and 30–60 cm in winter wheat in 2015−2016.

Source of Variation	df	NH_4^+-N	NO_3^--N	TMN	NH_4^+-N	NO_3^--N	TMN
		(mg kg^{-1} soil)			(mg kg^{-1} soil)		
		0–30 cm			30–60 cm		
Rep	3	0.92	0.60	0.51	0.81	0.16	0.17
Treatment	3	0.70	0.14	0.18	0.49	0.05	0.05
Sampling	2	<0.01	<0.01	<0.01	0.002	<0.01	<0.01
Sampling × Treatment	6	0.73	0.19	0.64	0.54	0.23	0.24
Treatment							
100UAN + I		3.6 ± 0.4	4.4 ± 1.1	8.1 ± 1.4	2 ± 0.3	6.8 ± 1.4 ab	8.9 ± 1.5 ab
100UAN		3.2 ± 0.3	5.2 ± 1.3	8.4 ± 1.5	1.7 ± 0.1	7.6 ± 2.5 a	10.1 ± 2.6 a
60/40UAN + I		3.2 ± 0.4	3.3 ± 0.5	6.5 ± 0.9	2 ± 0.2	4.3 ± 1 b	6.3 ± 1 b
60/40UAN		3.5 ± 0.4	4 ± 0.7	7.6 ± 1.1	1.8 ± 0.2	6.1 ± 1.6 ab	8 ± 1.7 ab
Sampling							
Before the second split application		4.6 ± 0.3 a	8.3 ± 0.9 a	13 ± 1 a	2.1 ± 0.2 a	12.9 ± 1.6 a	14.4 ± 1.6 a
4 WAT		3.8 ± 0.3 a	2.5 ± 0.1 b	6.3 ± 0.4 b	2.2 ± 0.2 a	3.8 ± 0.8 b	6.1 ± 0.9 b
8 WAT		1.8 ± 0.3 b	2.1 ± 0.1 b	4 ± 0.2 c	1.3 ± 0.1 b	2.8 ± 0.6 b	4.2 ± 0.6 b

Means are averages of four replicates ± SE (standard error). Different letters within columns indicate means with significant differences according to least significant difference (LSD) at $p < 0.05$. Single application of UAN + Instinct® II (100UAN + I), single application of urea (100UAN), split application of UAN + Instinct® II (60% UAN in fall + Instinct® II and 40% UAN in spring; 60/40UAN + I), and split application of UAN (60% UAN in fall and 40% UAN in spring; 60/40UAN). WAT (Weeks after the second split application).

Unlike 0–30 cm soil depth, the fertilization treatments in the 30–60 cm soil depth affected soil NO_3^--N and TMN content significantly. The highest levels of NO_3^--N and TMN were associated with 100UAN (7.6 mg kg^{-1} and 10.1 mg kg^{-1}, respectively) (Table 5). In contrast, the lowest NO_3^--N and TMN contents were associated with 60/40U + I (4.3 mg kg^{-1} and 6.3 mg kg^{-1}, respectively). On average, a split application of UAN reduced NO_3^--N and TMN contents by 27% and 24%, respectively, in comparison to a single UAN application.

Compared to treatments that did not include NIs, the addition of Instinct® II reduced NO_3^--N and TMN contents by 19% and 16%, respectively. Reduction trends were observed for NH_4^+-N, NO_3^--N, and TMN contents in the 30–60 cm soil profile. At 8 WAT, NH_4^+-N, NO_3^--N, and TMN levels were 38%, 78%, and 70% lower, respectively, than those before the second split application (Table 5).

4. Discussion

This study revealed that GY and GP of spring or winter wheat were not affected by the different N fertilizer treatments regardless of NIs application or N rates, although the PH and leaf greenness (SPAD) of spring wheat differed. Consistent with our results, some studies reported that the GY of barley [46], maize [46–48], and winter wheat [46,47] were not affected by NIs application. Other studies reported that NIs had a limited effect on biomass yield and crude protein of grass [14,49]. Several studies in pastures reported no significant effect of NIs on yields [50–53] nor in vegetable production [54,55]. However, a number of studies observed that the use of nitrification and urease inhibitors significantly increased wheat [24,56], maize [57–59], and vegetable yields [60]. A meta-analysis by Abalos et al. [61] indicated that, on average, the use of nitrification and urease inhibitors led to a 7.5% increase in yield, while effectiveness depended on environmental and management factors. Meng et al. [14] pointed out that yield improvement through NIs addition might be expected only when N is the limiting factor to plant growth. In our study, both the N application rates and the soil N data indicated sufficient N supply to the crops, and as a result, the response of N treatments on wheat yield and protein content was similar. Moreover, regardless of NIs use, treatments at a lower N rate did not affect the GY of spring wheat, which further confirmed that excessive N application occurred. Zhu [62] indicated that the N rate could be reduced by 7–24% without yield loss of rice or wheat.

Note that in the winter wheat trial, the numeric GY was higher, but the numeric GP was lower in the treatments with split-N fertilization, implying the advantages of split fertilization in improving NUE. Other studies showed that supplying a small portion of total N at planting coupled with multiple applications of the rest N according to crop N requirements can increase NUE and yield of rice, barley, wheat, potato, and maize [63–66].

In the trial with spring wheat, UAN treatments resulted in higher soil NH_4^+-N content and lower NO_3^--N content than urea treatments, which might be due to the fertilizer property, as UAN itself contain NH_4^+-N while urea may quickly convert to NO_3^--N. The higher NH_4^+-N contents in the soils indicate an increased risk of ammonia volatilization, another main pathway of N loss in agricultural systems [67,68]. Of the two N forms, NO_3^--N content was generally higher than NH_4^+-N, and TMN broadly followed the same pattern as NO_3^--N (Figure 2).

It was reported that NIs slow bacterial oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) in soils by depressing the activity of ammonia mono-oxygenase released by *Nitrosomas* bacteria [15], extending the retention of NH_4^+-N in soil [69]. In addition, less NO_3^--N is produced, and NO_3^--N leaching potential is reduced as well. In our trial, we did not observe a significant effect of NIs on soil N contents. This may be related to the environmental conditions, as our studies were conducted under irrigation; frequent irrigation might have an impact on the properties of the NIs. Moreover, although NIs repeatedly have been shown to reduce N_2O and NO emissions from agricultural soils, their mitigation effect varies greatly, and the mechanism is still not well explored [70,71].

Similar to the surface soil depth of 0–30 cm, the N content in the 30–60 cm was not affected by different N treatments, suggesting that the NIs application did not affect the nitrate leaching potential. Over the monitoring period, the increase in soil NO_3^--N content from 2 WAE to 4 WAE may be sourced from nitrification. Afterward, it naturally decreased with time due to the plant uptake.

In the winter wheat field trial, the split application of N (60% UAN in the fall and 40% UAN in the spring) generally reduced NO_3^--N and TMN contents in comparison to a single application (100% UAN in the fall), suggesting a lower NO_3^--N leaching potential. When all N was applied at sowing, intensive and heavy precipitation plus irrigation could lead to NO_3^--N being leached more deeply into the soil [63].

Throughout the soil profile to 60 cm depth, in the spring wheat trial, soil NH_4^+-N, NO_3^--N, and TMN contents at 0–30 cm soil depth were higher than those recorded at 30–60 cm soil depth. However, in the winter wheat trial, the NH_4^+-N content decreased but NO_3^--N content increased with the soil depth because the former is relatively immobile while the latter is highly mobile. Differences in N distribution between trials might be due to the longer growing season for winter wheat.

Apart from the spring wheat trial, the application of Instinct® II reduced NO_3^--N and TMN contents, compared to the no-application of NI in the winter wheat trial, suggesting that Instinct® II reduced nitrification. Studies reported that the performance of NIs is significantly affected by the timing of application (growth stage), type of application (single or split), and rate of application [72–75]. Moreover, the variability of weather conditions, especially soil temperature, affects the effectiveness of NIs [28]. Because NIs degradation and nitrification increase with the increasing soil temperatures, the efficiency of NIs in winter wheat field were more pronounced, perhaps due to the decreasing soil temperature. Thus, the greater effectiveness of NIs in winter wheat could have been a result of overall reduced nitrification activity. Nair et al. [76] reported that the efficiency of NIs may be affected by soil conditions (texture, temperature, pH, and organic matter), through the activity of nitrifiers and denitrifiers, and through N distribution. The effectiveness of nitrapyrin at decreasing nitrification in soils depends on a number of interacting factors besides soil temperature [77]. Raza et al. [78] showed that nitrification was significantly affected by soil temperature and moisture levels. Soil temperature controls the persistence and performance of DMPP as a NI [79]. However, gross nitrification rates were reduced in the presence of nitrapyrin at both 20 °C and 40 °C soil temperatures [80]. Other studies have found that nitrapyrin can decrease nitrification at temperatures from 25 °C to 35 °C [27].

In general, there is no unwavering confirmation regarding the behavior of NIs in soil. It remains unclear how long NIs remain effective and exactly what factors can affect their efficiency. Therefore, there is a need for more studies to elucidate the influence factors on NIs efficiency. Such information would assist growers in using NIs correctly.

5. General Remark

The present findings were obtained from field trials with spring and winter wheat and indicated that the crops received sufficient N. Thus, a reduced N rate (e.g., 15% reduction) could result in similar yields. Between the two N sources, urea and urea ammonium nitrate-UAN, we observed that the application of UAN could significantly reduce soil NO_3^--N content in the 0–30 cm soil depth and may provide environmental benefits by reducing nitrate leaching potential and denitrification risk. Hence, the environmental advantages of UAN as an N source outweigh urea. Furthermore, splitting N applications could reduce soil NO_3^--N content compared to a single application. Application of Instinct® II with lower-rate urea and with UAN during cool temperatures seems to be a suitable strategy to reduce NO_3^--N leaching potential, while Agrotain® Ultra did not show any considerable effect. Our results demonstrated that selecting effective NIs, suitable N sources, reducing N rate, and splitting N fertilizers during the growing season can be regarded as practical strategies to reduce NO_3^--N leaching while not compromising crop yield. Although the findings from this were based on two crops, it should be noted that the data were only from a single-season observation for either crop. Ideally, it will be necessary to carry out trials based on multiple years and locations to make a solution conclusion on the effects of NIs and N management on potential yield benefits and the N dynamics of soils. In such trials, at least some treatments supplying suboptimal N should be included, as NIs might show their potential to significantly increase crop yields. Moreover, frequent field measurements on N contents should be conducted before and after fertilization as the N transformation occurs very rapidly.

Author Contributions: All authors contributed substantially to the work reported in this paper. Data analyzing, S.T.; writing—original draft preparation, S.T. and S.F.-A.; review and editing, R.Q., C.N. and G.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Acknowledgments: We would like to express our appreciation for conducting field trials by Sandy DeBano, Dave Wooster, and Don Horneck from Oregon State University-Hermiston Agricultural Research and Extension Center, Hermiston, OR, 97838. Tim Weinke, students, and interns provided support in field measurements and data collection. Linda Brewer from Oregon State University provided thorough editorial comments for improving the written quality.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. von Wirén, N.; Gazzarrini, S.; Frommer, W.B. Regulation of mineral nitrogen uptake in plants. *Plant Soil* **1997**, *196*, 191–199. [CrossRef]
2. Fageria, N.K.; Baligar, V.C. Enhancing nitrogen use efficiency in crop plants. *Adv. Agron.* **2005**, *8*, 97–185.
3. Xu, A.; Li, L.; Xie, J.; Wang, X.; Coulter, J.A.; Liu, C.; Wang, L. Effect of long-term nitrogen addition on wheat yield, nitrogen use efficiency, and residual soil nitrate in a semiarid area of the loess plateau of China. *Sustainability* **2020**, *12*, 1735. [CrossRef]
4. Ahmed, M.; Rauf, M.; Mukhtar, Z.; Saeed, N.A. Excessive use of nitrogenous fertilizers: An unawareness causing serious threats to environment and human health. *Environ. Sci. Pollut. Res.* **2017**, *24*, 26983–26987. [CrossRef] [PubMed]
5. Omara, P.; Aula, L.; Oyebiyi, F.; Raun, W.R. World cereal nitrogen use efficiency trends: Review and current knowledge. *Agrosyst. Geosci. Environ.* **2019**, *2*, 1–8. [CrossRef]
6. Cameron, K.C.; Di, H.J.; Moir, J.L. Nitrogen losses from the soil/plant system: A review. *Ann. Appl. Biol.* **2013**, *162*, 145–173. [CrossRef]
7. Farzadfar, S.; Knight, J.D.; Congreves, K.A. Soil organic nitrogen: An overlooked but potentially significant contribution to crop nutrition. *Plant Soil* **2021**, *462*, 7–23. [CrossRef]
8. Gaudin, R.; Dupuy, J. Ammoniacal nutrition of transplanted rice fertilized with large urea granules. *Agron. J.* **1999**, *91*, 33–36. [CrossRef]
9. Salsac, L.; Chaillou, S.; Morot-Gaudry, J.F.; Lesaint, C.H.; Jolivet, E. Nitrate and ammonium nutrition in plants. *Plant Physiol. Biochem.* **1987**, *25*, 805–812.
10. Bloom, A.J.; Meyerhoff, P.A.; Taylor, A.R.; Rost, T.L. Root development and absorption of ammonium and nitrate from the rhizosphere. *J. Plant Growth Regul.* **2002**, *21*, 416–431. [CrossRef]
11. Fageria, N.K. *The Use of Nutrients in Crop Plants*; CRC Press: Boca Raton, FL, USA, 2016.
12. Kishchenko, O.; Stepanenko, A.; Straub, T.; Zhou, Y.; Neuhäuser, B.; Borisjuk, N. Ammonium Uptake, Mediated by Ammonium Transporters, Mitigates Manganese Toxicity in Duckweed, Spirodela polyrhiza. *Plants* **2023**, *12*, 208. [CrossRef]
13. Barth, G.; von Tucher, S.; Schmidhalter, U.; Otto, R.; Motavalli, P.; Ferraz-Almeida, R.; Meinl Schmiedt Sattolo, T.; Cantarella, H.; Vitti, G.C. Performance of nitrification inhibitors with different nitrogen fertilizers and soil textures. *J. Plant Nutr. Soil Sci.* **2019**, *182*, 694–700. [CrossRef]
14. Meng, Y.; Wang, J.J.; Wei, Z.; Dodla, S.K.; Fultz, L.M.; Gaston, L.A.; Xiao, R.; Park, J.H.; Scaglia, G. Nitrification inhibitors reduce nitrogen losses and improve soil health in a subtropical pastureland. *Geoderma* **2021**, *388*, 114947. [CrossRef]
15. McCarty, G.W. Modes of action of nitrification inhibitors. *Biol. Fertil. Soils* **1999**, *29*, 1–9. [CrossRef]
16. Jiang, R.; Yang, J.; Drury, C.F.; Grant, B.B.; Smith, W.N.; He, W.; Reynolds, D.W.; He, P. Modelling the impacts of inhibitors and fertilizer placement on maize yield and ammonia, nitrous oxide and nitrate leaching losses in southwestern Ontario, Canada. *J. Clean. Prod.* **2023**, *384*, 135511. [CrossRef]
17. Sanz-Cobena, A.; Sánchez-Martín, L.; García-Torres, L.; Vallejo, A. Gaseous emissions of N_2O and NO and NO_3- leaching from urea applied with urease and nitrification inhibitors to a maize (*Zea mays*) crop. *Agric. Ecosyst. Environ.* **2012**, *149*, 64–73. [CrossRef]

18. Lin, S.; Hernandez-Ramirez, G. Nitrous oxide emissions from manured soils as a function of various nitrification inhibitor rates and soil moisture contents. *Sci. Total Environ.* **2020**, *738*, 139669. [CrossRef] [PubMed]
19. de Souza, T.L.; de Oliveira, D.P.; Santos, C.F.; Reis, T.H.P.; Cabral, J.P.C.; da Silva Resende, É.R.; Fernandes, T.J.; de Souza, T.R.; Builes, V.R.; Guelfi, D. Nitrogen fertilizer technologies: Opportunities to improve nutrient use efficiency towards sustainable coffee production systems. *Agric Ecosyst. Environ.* **2023**, *345*, 108317. [CrossRef]
20. Fettweis, U.; Mittelstaedt, W.; Schimansky, C.; Führ, F. Lysimeter experiments on the translocation of the carbon-14-labelled nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) in a gleyic cambisol. *Biol. Fertil. Soils* **2001**, *34*, 126–130. [CrossRef]
21. Bhatia, A.; Sasmal, S.; Jain, N.; Pathak, H.; Kumar, R.; Singh, A. Mitigating nitrous oxide emission from soil under conventional and no-tillage in wheat using nitrification inhibitors. *Agric. Ecosyst. Environ.* **2010**, *136*, 247–253. [CrossRef]
22. Liu, C.; Wang, K.; Zheng, X. Effects of nitrification inhibitors (DCD and DMPP) on nitrous oxide emission, crop yield and nitrogen uptake in a wheat–maize cropping system. *Biogeosciences* **2013**, *10*, 2427–2437. [CrossRef]
23. Ma, Y.; Sun, L.; Zhang, X.; Yang, B.; Wang, J.; Yin, B.; Yan, X.; Xiong, Z. Mitigation of nitrous oxide emissions from paddy soil under conventional and no-till practices using nitrification inhibitors during the winter wheat-growing season. *Biol. Fertil. Soils* **2013**, *49*, 627–635. [CrossRef]
24. Dawar, K.; Rahman, U.; Alam, S.S.; Tariq, M.; Khan, A.; Fahad, S.; Datta, R.; Danish, S.; Saud, S.; Noor, M. Nitrification Inhibitor and Plant Growth Regulators Improve Wheat Yield and Nitrogen Use Efficiency. *J. Plant Growth Regul.* **2022**, *41*, 1–11. [CrossRef]
25. Mohammed, Y.A.; Chen, C.; Jensen, T. Urease and nitrification inhibitors impact on winter wheat fertilizer timing, yield, and protein content. *Agron. J.* **2016**, *108*, 905–912. [CrossRef]
26. Dawar, K.; Zaman, M.; Rowarth, J.S.; Blennerhassett, J.; Turnbull, M.H. The impact of urease inhibitor on the bioavailability of nitrogen in urea and in comparison, with other nitrogen sources in ryegrass (*Lolium perenne* L.). *Crop Pasture Sci.* **2010**, *61*, 214–221. [CrossRef]
27. Chen, D.; Suter, H.C.; Islam, A.; Edis, R. Influence of nitrification inhibitors on nitrification and nitrous oxide (N_2O) emission from a clay loam soil fertilized with urea. *Soil Biol. Biochem.* **2010**, *42*, 660–664. [CrossRef]
28. Zaman, M.; Nguyen, M.L.; Šimek, M.; Nawaz, S.; Khan, M.J.; Babar, M.N.; Zaman, S. Emissions of Nitrous Oxide (N_2O) and Di-nitrogen (N_2) from the Agricultural Landscapes, Sources, Sinks, and Factors Affecting N_2O and N_2 Ratios. In *Greenhouse Gases—Emission, Measurement and Management*; Liu, G.X., Ed.; InTech.: London, UK, 2012; pp. 1–32.
29. Amberger, A. Research on dicyandiamide as a nitrification inhibitor and future outlook. *Commun. Soil Sci. Plant Anal.* **1989**, *20*, 1933–1955. [CrossRef]
30. Kelliher, F.M.; Clough, T.J.; Clark, H.; Rys, G.; Sedcole, J.R. The temperature dependence of dicyandiamide (DCD) degradation in soils: A data synthesis. *Soil Biol. Biochem.* **2008**, *40*, 1878–1882. [CrossRef]
31. Peixoto, L.; Petersen, S.O. Efficacy of three nitrification inhibitors to reduce nitrous oxide emissions from pig slurry and mineral fertilizers applied to spring barley and winter wheat in Denmark. *Geoderma Reg.* **2023**, *32*, e00597. [CrossRef]
32. Kim, D.G.; Palmada, T.; Berben, P.; Giltrap, D.; Saggar, S. Seasonal variations in the degradation of a nitrification inhibitor, dicyandiamide (DCD), in a Manawatu grazed pasture soil. In *Advanced Nutrient Management: Gains from the Past—Goals for the Future*; Currie, L.D., Christensen, C.L., Eds.; Massey University: Auckland, New Zealand, 2012; 7p.
33. Di, H.J.; Cameron, K.C. Treating grazed pasture soil with a nitrification inhibitor, eco-n™, to decrease nitrate leaching in a deep sandy soil under spray irrigation—A lysimeter study. *N. Z. J. Agric. Res.* **2004**, *47*, 351–361. [CrossRef]
34. Smith, L.C.; Orchiston, T.; Monaghan, R.M. The effectiveness of the nitrification inhibitor dicyandiamide (DCD) for mitigating nitrogen leaching losses from a winter grazed forage crop on a free draining soil in Northern Southland. *Proc. N. Z. Grassl. Assoc.* **2012**, *74*, 39–44.
35. Suter, H.; Lam, S.K.; Walker, C.; Chen, D. Nitrogen use efficiency for pasture production–impact of enhanced efficiency fertilisers and N rate. In Proceedings of the 17th Australian Society of Agronomy Conference, Hobart, Australia, 20–24 September 2015; pp. 20–24.
36. Cookson, W.R.; Cornforth, I.S. Dicyandiamide slows nitrification in dairy cattle urine patches: Effects on soil solution composition, soil pH and pasture yield. *Soil Biol. Biochem.* **2002**, *34*, 1461–1465. [CrossRef]
37. Sassman, A.M.; Barker, D.W.; Sawyer, J.E. Corn Response to Urea–Ammonium Nitrate Solution Treated with Encapsulated Nitrapyrin. *Agron. J.* **2018**, *110*, 1058–1067. [CrossRef]
38. Peel, M.C.; Finlayson, B.L.; McMahon, T.A. Updated world map of the Köppen-Geiger climate classification. *Hydrol. Earth Syst. Sci.* **2007**, *11*, 1633–1644. [CrossRef]
39. Meier, U. *Growth Stages of Mono- and Dicotyledonous Plants. BBCH Monograph*, 2nd ed.; Blackwell Science: Berlin, Germany, 2001; p. 158.
40. Chapman, S.C.; Barreto, H.J. Using a chlorophyll meter to estimate specific leaf nitrogen of tropical maize during vegetative growth. *Agron. J.* **1997**, *89*, 557–562. [CrossRef]
41. Sadras, V.O.; Echarte, L.; Andrade, F.H. Profiles of leaf senescence during reproductive growth of sunflower and maize. *Ann Bot.* **2000**, *85*, 187–195. [CrossRef]
42. Peterson, T.A.; Blackmer, T.M.; Francis, D.D.; Schepers, J.S. *G93-1171 Using a Chlorophyll Meter to Improve N Management*; University of Nebraska: Lincoln, NE, USA, 1993.

43. Bremner, J.M.; Mulvaney, C.S. Nitrogen-total. In *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*; Page, A.L., Miller, R.H., Keeney, D.R., Eds.; American Society of Agronomy, Soil Science Society of America: Madison, WI, USA, 1982; pp. 595–624.
44. Miller, R.O.; Gavlak, R.; Horneck, D. Soil Nitrate N, NO_3-N Cadmium Reduction S-3.10. In *Soil, Plant, and Water Reference Methods for the Western Region*, 4th ed.; Western Rural Development Center: UT, USA, 2013; p. 39.
45. Miller, R.O.; Gavlak, R.; Horneck, D. Soil Ammonium Nitrogen S-3.50. In *Soil, Plant, and Water Reference Methods for the Western Region*, 4th ed.; Western Rural Development Center: UT, USA, 2013; p. 43.
46. Weiske, A.; Benckiser, G.; Herbert, T.; Ottow, J.C.G. Influence of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) in comparison to dicyandiamide (DCD) on nitrous oxide emissions, carbon dioxide fluxes and methane oxidation during 3 years of repeated application in field experiments. *Biol. Fertil. Soils* **2001**, *34*, 109–117.
47. De Antoni Migliorati, M.; Scheer, C.; Grace, P.R.; Rowlings, D.W.; Bell, M.; McGree, J. Influence of different nitrogen rates and DMPP nitrification inhibitor on annual N2O emissions from a subtropical wheat-maize cropping system. *Agric. Ecosyst. Environ.* **2014**, *186*, 33–43. [CrossRef]
48. Yuan, L.; Chen, X.; Jia, J.; Chen, H.; Shi, Y.; Ma, J.; Liang, C.; Liu, Y.; Xie, H.; He, H.; et al. Stover mulching and inhibitor application maintain crop yield and decrease fertilizer N input and losses in no-till cropping systems in Northeast China. *Agric. Ecosyst. Environ.* **2021**, *312*, 107360. [CrossRef]
49. Perez-Castillo, A.G.; Chinchilla-Soto, C.; Elizondo-Salazar, J.A.; Barboza, R.; Dong-Gill, K.I.M.; Muller, C.; Alberto, S.C.; Borzouei, A.; Dawar, K.; Zaman, M. Nitrification inhibitor nitrapyrin does not affect yield-scaled nitrous oxide emissions in a tropical grassland. *Pedosphere* **2021**, *31*, 265–278. [CrossRef]
50. Merino, P.; Menéndez, S.; Pinto, M.; González-Murua, C.; Estavillo, J.M. 3,4-Dimethylpyrazole phosphate reduces nitrous oxide emissions from grassland after slurry application. *Soil Use Manage* **2005**, *21*, 53–57. [CrossRef]
51. Dougherty, W.J.; Collins, D.; Van Zwieten, L.; Rowlings, D.W. Nitrification (DMPP) and urease (NBPT) inhibitors had no effect on pasture yield, nitrous oxide emissions, or nitrate leaching under irrigation in a hot-dry climate. *Soil Res.* **2016**, *54*, 675. [CrossRef]
52. Cardenas, L.M.; Bhogal, A.; Chadwick, D.R.; McGeough, K.; Misselbrook, T.; Rees, R.M.; Thorman, R.E.; Watson, C.J.; Williams, J.R.; Smith, K.A.; et al. Nitrogen use efficiency and nitrous oxide emissions from five UK fertilised grasslands. *Sci. Total Environ.* **2019**, *661*, 696–710. [CrossRef] [PubMed]
53. Nauer, P.A.; Fest, B.J.; Visser, L.; Arndt, S.K. On-farm trial on the effectiveness of the nitrification inhibitor DMPP indicates no benefits under commercial Australian farming practices. *Agric. Ecosyst. Environ.* **2018**, *253*, 82–89. [CrossRef]
54. Asing, J.; Saggar, S.; Singh, J.; Bolan, N.S. Assessment of nitrogen losses from urea and organic manure with and without nitrification inhibitor, dicyandiamide, applied to lettuce under glasshouse conditions. *Aust. J. Soil Res.* **2008**, *46*, 535–541. [CrossRef]
55. Pfab, H.; Palmer, I.; Buegger, F.; Fiedler, S.; Müller, T.; Ruser, R. Influence of a nitrification inhibitor and of placed N-fertilization on N2O fluxes from a vegetable cropped loamy soil. *Agric. Ecosyst. Environ.* **2012**, *150*, 91–101. [CrossRef]
56. Tao, R.; Li, J.; Hu, B.; Shah, J.A.; Chu, G. A 2-year study of the impact of reduced nitrogen application combined with double inhibitors on soil nitrogen transformation and wheat productivity under drip irrigation. *J. Sci. Food Agric.* **2021**, *101*, 1772–1781. [CrossRef]
57. Zheng, J.; Wang, H.; Fan, J.; Zhang, F.; Guo, J.; Liao, Z.; Zhuang, Q. Wheat straw mulching with nitrification inhibitor application improves grain yield and economic benefit while mitigating gaseous emissions from a dryland maize field in northwest China. *Field Crops Res.* **2021**, *265*, 108125. [CrossRef]
58. Borzouei, A.; Mander, U.; Teemusk, A.; Alberto, S.C.; Zaman, M.; Dong-Gill, K.I.M.; Muller, C.; Kelestanie, A.A.; Amin, P.S.; Moghiseh, E.; et al. Effects of the nitrification inhibitor nitrapyrin and tillage practices on yield-scaled nitrous oxide emission from a maize field in Iran. *Pedosphere* **2021**, *31*, 314–322. [CrossRef]
59. Dawar, K.; Sardar, K.; Zaman, M.; Mueller, C.; Alberto, S.C.; Aamir, K.; Borzouei, A.; Perez-Castillo, A.G. Effects of the nitrification inhibitor nitrapyrin and the plant growth regulator gibberellic acid on yield-scale nitrous oxide emission in maize fields under hot climatic conditions. *Pedosphere* **2021**, *31*, 323–331. [CrossRef]
60. Zhang, M.; Fan, C.H.; Li, Q.L.; Li, B.; Zhu, Y.Y.; Xiong, Z.Q. A 2-yr field assessment of the effects of chemical and biological nitrification inhibitors on nitrous oxide emissions and nitrogen use efficiency in an intensively managed vegetable cropping system. *Agric. Ecosyst. Environ.* **2015**, *201*, 43–50. [CrossRef]
61. Abalos, D.; Jeffery, S.; Sanz-Cobena, A.; Guardia, G.; Vallejo, A. Meta-analysis of the effect of urease and nitrification inhibitors on crop productivity and nitrogen use efficiency. *Agric. Ecosyst. Environ.* **2014**, *189*, 136–144. [CrossRef]
62. Zhu, Z.L. Fate and management of fertilizer nitrogen in agro-ecosystems. In *Nitrogen in Soils of China*; Springer: Dordrecht, Germany, 1997; pp. 239–279.
63. Cai, J.; Jiang, D.; Liu, F.; Dai, T.; Cao, W. Effects of split nitrogen fertilization on post-anthesis photoassimilates, nitrogen use efficiency and grain yield in malting barley. *Acta Agric. Scand B Soil Plant Sci.* **2011**, *61*, 410–420. [CrossRef]
64. Rahman, M.A.; Sarker, M.A.Z.; Amin, M.F.; Jahan, A.H.S.; Akhter, M.M. Yield response and nitrogen use efficiency of wheat under different doses and split application of nitrogen fertilizer. *Bangladesh J. Agric. Res.* **2011**, *36*, 231–240. [CrossRef]
65. Chen, Y.; Peng, J.; Wang, J.; Fu, P.; Hou, Y.; Zhang, C.; Fahad, S.; Peng, S.; Cui, K.; Nie, L.; et al. Crop management based on multi-split topdressing enhances grain yield and nitrogen use efficiency in irrigated rice in China. *Field Crops Res.* **2015**, *184*, 50–57. [CrossRef]

66. Zhou, Z.; Plauborg, F.; Liu, F.; Kristensen, K.; Andersen, M.N. Yield and crop growth of table potato affected by different split-N fertigation regimes in sandy soil. *Eur. J. Agron.* **2018**, *92*, 41–50. [CrossRef]
67. Li, H.; Chen, Y.X.; Liang, X.Q.; Lian, Y.F.; Li, W.H. Mineral-nitrogen leaching and ammonia volatilization from a rice–rapeseed system as affected by 3,4-dimethylpyrazole phosphate. *J. Environ. Qual.* **2009**, *38*, 2131–2137. [CrossRef]
68. Coskun, D.; Britto, D.T.; Shi, W.M.; Kronzucker, H.J. Nitrogen transformations in modern agriculture and the role of biological nitrification inhibition. *Nat. Plants* **2017**, *3*, 17074. [CrossRef]
69. Qiao, C.; Liu, L.; Hu, S.; Compton, J.E.; Greaver, T.L.; Li, Q. How inhibiting nitrification affects nitrogen cycle and reduces environmental impacts of anthropogenic nitrogen input. *Glob. Chang Biol.* **2015**, *21*, 1249–1257. [CrossRef]
70. Pereira, J.; Fangueiro, D.; Chadwick, D.R.; Misselbrook, T.H.; Coutinho, J.; Trindade, H. Effect of cattle slurry pre-treatment by separation and addition of nitrification inhibitors on gaseous emissions and N dynamics: A laboratory study. *Chemosphere* **2010**, *79*, 620–627. [CrossRef]
71. Ruser, R.; Schulz, R. The effect of nitrification inhibitors on the nitrous oxide (N2O) release from agricultural soils-a review. *J. Plant. Nutr. Soil Sci.* **2015**, *178*, 171–188. [CrossRef]
72. Singh, A.; Kumar, A.; Jaswal, A.; Singh, M.; Gaikwad, D. Nutrient use efficiency concept and interventions for improving nitrogen use efficiency. *Plant Arch.* **2018**, *18*, 1015–1023.
73. Li, T.; Zhang, X.; Gao, H.; Li, B.; Wang, H.; Yan, Q.; Ollenburger, M.; Zhang, W. Exploring optimal nitrogen management practices within site-specific ecological and socioeconomic conditions. *J. Clean Prod.* **2019**, *241*, 118295. [CrossRef]
74. Janke, C.K.; Moody, P.; Bell, M.J. Three-dimensional dynamics of nitrogen from banded enhanced efficiency fertilizers. *Nutr. Cycl. Agroecosyst.* **2020**, *118*, 227–247. [CrossRef]
75. Souza, E.F.; Soratto, R.P.; Sandaña, P.; Venterea, R.T.; Rosen, C.J. Split application of stabilized ammonium nitrate improved potato yield and nitrogen-use efficiency with reduced application rate in tropical sandy soils. *Field Crop. Res.* **2020**, *254*, 107847. [CrossRef]
76. Nair, D.; Abalos, D.; Philippot, L.; Bru, D.; Mateo-Marín, N.; Petersen, S.O. Soil and temperature effects on nitrification and denitrification modified N_2O mitigation by 3, 4-dimethylpyrazole phosphate. *Soil Biol. Biochem.* **2021**, *157*, 108224. [CrossRef]
77. Slangen, J.H.G.; Kerkhoff, P. Nitrification inhibitors in agriculture and horticulture: A literature review. *Fertil. Res.* **1984**, *5*, 1–76. [CrossRef]
78. Raza, S.; Jiang, Y.; Elrys, A.S.; Tao, J.; Liu, Z.; Li, Z.; Chen, Z.; Zhou, J. Dicyandiamide efficacy of inhibiting nitrification and carbon dioxide emission from calcareous soil depends on temperature and moisture contents. *Arch. Agron. Soil Sci.* **2021**, *68*, 1–17. [CrossRef]
79. Pasda, G.; Hähndel, R.; Zerulla, W. Effect of fertilizers with the new nitrification inhibitor DMPP (3, 4-dimethylpyrazole phosphate) on yield and quality of agricultural and horticultural crops. *Biol. Fertil. Soils.* **2001**, *34*, 85–97. [CrossRef]
80. Fisk, L.M.; Maccarone, L.D.; Barton, L.; Murphy, D.V. Nitrapyrin decreased nitrification of nitrogen released from soil organic matter but not amoA gene abundance at high soil temperature. *Soil Biol. Biochem.* **2015**, *88*, 214–223. [CrossRef]