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The effect of nitrification inhibitor on the yield and quality of *Triticum aestivum* L. and *Brassica napus* L. – A long-term experiment

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ABSTRACT

Purpose: The nitrogen is a crucial element in crop production, which can be associated with the environmental loss and low agronomic nitrogen efficiency. The utilization of fertilizers with inhibitors represents an economical option by lowering the number of applications, lowering the dose of nitrogen and limiting the risk of environmental loss of N.

Methods: The long-term effect of nitrogen fertilizer with nitrification inhibitors (alternative technology) in comparison with conventional fertilizers (prevalent technology) on yield and quality of winter wheat and oilseed rape cultivated in field conditions at two experimental localities was evaluated.

Results: The long-term average yields of both crops were significantly higher after the alternative technology with the NI (+0.4 t/ha wheat grain, +0.3 t/ha rape seed) in comparison with prevalent technology. The effect of NI also resulted in significantly higher average protein content (13 %), protein production (0.98 t/ha) and gluten content (29.5 %) in wheat grain in comparison with prevalent technology without NI (12.8 %; 1.04 t/ha; 28.7 %). The oil content of oilseed rape did not differ significantly between the compared fertilizer technologies. The alternative technology with NI resulted in significantly highest production of oil (+0.1 t/ha) in comparison with prevalent technology. The economic evaluation of alternative technology with NI resulted in net profit in comparison with prevalent technology in every scenario.

Conclusions: These long-term results are proving, that addition of NI to the conventional fertilizer applied in higher dose and less applications is more suitable choice compared to the classic split nitrogen fertilization.

1. Introduction

The average efficiency of nitrogen fertilization ranges between 32 % and 53 % in common field conditions (Dawar et al., 2021; Zhang et al., 2015a). The percentage of N loss could be affected by the term and method of fertilization, form of nitrogen in applied fertilizers, soil cultivation and especially by the course of weather (Gupta et al., 2023; Santillano-Cázares et al., 2018). Low efficiency of N fertilizers is not only environmentally unsafe (Li and Chen, 2020; Umar et al., 2020) and represents a possible risk to the human and animal health due to the leaching of NO_3^- (He et al., 2018; Wu et al., 2017), volatilization of NH_3 (Harty et al., 2017), and emission of other N-containing gases (Recio et al., 2018; Wu et al., 2021), but also causes the economic loss for farmers, reduced potential yield of crops and decrease in soil fertility (Lawrencia et al., 2021; Subbarao et al., 2012). Due to the increased rate of mineral N fertilizer application to meet global nutritional needs, the agriculture is accountable for over 70 % of total N_2O emissions (Adu-Poku et al., 2022). Therefore, improving the nitrogen use efficiency from fertilization should be the focus for sustainability in agriculture (Galloway et al., 2008).

A globally used method to improve efficiency of nitrogen fertilization in crop production is the split application of N contained in conventional mineral fertilizers. Thus, nitrogen is usually applied in lower doses several times during the main stages of vegetation with the goal of optimizing yields and limiting the environmental loss of N. However, the total amount of split applications represents a disadvantage because of additional field crossing, more fuel consumption, time consumption, etc. (Anas et al., 2020). A more sophisticated method to improve nitrogen efficiency is the utilization of mineral fertilizers, which can adjust the release date, release period or release amount of contained nutrients according to the crop growth and development, or soil-weather conditions.

According to the principle of nutrient release, these fertilizers can be classified as (1) slow-release fertilizers (SRF) or (2) controlled-release

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fertilizers (CRF) (Rajan et al., 2021). Recent research is also focusing on the application of hydrogels, especially with natural and biodegradable polymers in combination with nutrients as a possible alternative (Kriška et al., 2023; Skarpa et al., 2023). Another group (3) are fertilizers with inhibitors of urease or nitrification (NIs) or both. These effective and environmentally friendly fertilizers contain inhibitors that temporary restrict N changes in the soil (urea hydrolysis or nitrification inhibition). The most of NIs affects the bacterial ammonia mono-oxygenase enzyme, which is responsible for the oxidation of ammonium into nitrite in the first step of nitrification (Byrne et al., 2020). Therefore, the NIs inhibits the activity of *Nitrosomonas* bacteria, which steadily increases the concentrations of N in the NH_4^+ form (Lin and Hernandez-Ramirez, 2020) and secures more time for the plants to uptake it or for the microbial immobilization of NH_4^+ (Gupta et al., 2023). Therefore, the NIs are responsible for the conservation of immobile NH_4^+ form in the soil for a longer period (4 −10 weeks), which reduces the content of very mobile and potentially leeched NO_3^- (Cui et al., 2021; Jiang et al., 2023) or denitrified gaseous emissions to the atmosphere (Fan et al., 2018). The effectivity of NIs in the soil is directly related to the course of nitrification (and activity of nitrification bacteria), so it is influenced by the same factors (temperature, pH, humidity, soil moisture or precipitation). In Central Europe, the commonly used fertilizers with higher ammonium nitrogen content are ammonium sulphate nitrate (18.5 % N-NH_4^+; 7.5 % N-NO_3; 13 % of water-soluble S) and ammonium sulphate (21 % N-NH_4^+; 23 % of water-soluble S). Both fertilizers are also available in a nitrification inhibitor alternative. The advantage of ammonium sulphate nitrate is the content of nitrogen in both forms (ammonium and nitrate) and the less negative impact on soil pH.

The aim of this study was to compare the long-term effect of winter wheat and oilseed rape fertilization by prevalent nitrogen technology or alternative technology enhanced with NI on the yield and grain and seed quality, as these long-term yield-related effects of NIs has not yet been published. The prevalent technology was based on three split fertilizations with conventional N-fertilizers, the alternative technology was focused on different approach (NI, two applications, NS fertilizer). The main hypothesis was, that the alternative technology is going to have a positive effect on the winter wheat and oilseed rape yield and quality in comparison with prevalent fertilization based on three nitrogen applications. A long-term, ten-year experiment (2013–2022) was established in the field conditions at the two different experimental localities (Žabčice, Vatín) to examine the suggested hypothesis.

2. Material and methods

2.1. Experimental localities and climate-soil conditions

The long-term experiment was established in the autumn of 2012. The winter wheat (*Triticum aestivum* L., variety Bohemia; Selgen, Prague, Czech Republic) and oilseed rape (*Brassica napus* L., variety DK Excellium; DeKalb Genetics Corporation, Illinois, USA) were chosen as a model crop and were sown repeatedly in every growing season. The experiment was established parallelly at two field experimental stations, which belong to the Mendel University in Brno, Czech Republic. According to the Köppen climate classification system, our country generally falls into the "Cfb" category. It is characterised by a mild climate with warm summers (average temperatures above 10 °C) and cold winters below 0 °C. However, it is essential to note that there can be variations in local climate within due to the diverse topography. The first experimental station is located in Žabčice (49.0229836 N, 16.6175028E), the altitude is 184 m above sea level, and it is characteristic mostly by warm and drought climate. According to the long-term normal, Žabčice is characteristic with a low annual precipitation sum of about 490 mm per year. The average monthly temperatures and precipitation sums during the experiment are shown in Table S1 (in Supplementary Data) together with the comparison to the long-term normal 1990–2020. The second experimental station is located near Vatín (49.5170872 N, 15.9725964E). The altitude is 560 m above sea level, and it is characteristic with lower average temperatures (annual mean temperature is 7 °C) and more precipitation (annual precipitation sum is 620 mm) in comparison with Žabčice (lower risk of drought periods). The average monthly temperatures and precipitation sums during the experiment for this locality are shown in Table S2 (in Supplementary Data) together with the long-term normal 1990–2020.

The basic soil characteristic of both experimental localities is presented in Table 1. The average contents of macronutrients and exchangeable soil acidity from every growing season are described in Table S3 (in Supplementary Data).

2.2. Methodology of the experiment and field treatments

The Randomized Complete Block Design was used during the experiment. This study aims to compare the prevalent technology of fertilization based on split fertilizations with conventional nitrogen fertilizers applied three times during the main stages of model crops with an alternative approach to the fertilization. This technology was based on split fertilizations with alternative nitrogen fertilizers applied only two times during the vegetation due to the NI addition. The unfertilized treatment was also included for relevant comparison. The examined treatments (Table 2) were identical for both model crops and both experimental localities. The nitrogen in prevalent technology was applied in commonly used fertilizers: calcium ammonium nitrate (CAN; 13.5 % N-NH_4^+ and 13.5 % N-NO_3^-) and urea ammonium nitrate (UAN; 15.0 % N-NH_2, 7.5 % N-NH_4^+ and 7.5 % N-NO_3^-). The alternative technology with the NI was fertilized with CAN and ammonium sulphate nitrate (ASN + NI; 18.5 % N-NH_4^+; 7.5 % N-NO_3^-; 13 % of water-soluble S; ENSIN®), which included the nitrification inhibitors dicyandiamide (0.36–0.54 % w/w) and 4 amino 1,2,4 triazole (0.032–0.048 % w/w). The total doses of nitrogen at every treatment were identical for both model crops (160 kg/ha winter wheat; 195 kg/ha oilseed rape). Every treatment was established in four repetitions. The size of each experimental plot of wheat for the fertilization was 16.8 m^2 (10 rows with a length of 14 m and inter-row spacing of 0.12 m). The size of the experimental plots of oilseed rape was 18 m^2 (6 rows with a length of 12 m and inter-row spacing of 0.25 m). All the fertilizers were manually (analogy of broadcasting by spreader) applied on the soil surface of each field plot separately during the main vegetation stages described in Table 2.

The winter wheat and oilseed rape were cultivated identically in term of soil cultivation during the whole experiment at both experimental stations. The fertilization in tillering/side shot formation

Table 1
The soil characteristic of both experimental localities.

Analysis / Locality	Žabčice	Vatín
P (mg/kg)	123 (high content)	82 (good content)
K (mg/kg)	246 (good content)	274 (high content)
Ca (mg/kg)	3824 (good content)	2094 (good content)
Mg (mg/kg)	405 (very high content)	136 (good content)
S (mg/kg)	16 (low content)	9 (very low content)
pH (CaCl2)	6.5 (neutral)	5.4 (acidic)
clay fraction (<2 μm) (%)	27.3	11.7
silt (50–2 μm) (%)	26.0	34.9
sand (2000–50 μm) (%)	11.5	53.4
clay (<0.01 mm) (%)	31.0	26.2
bulk density (kg/m)	1370.0	1197.0
total porosity (%)	48.0	54.4
maximum capillary water capacity (%)	47.7	39.9
minimal air capacity (%)	11.5	14.5
Cox (%)	1.3	1.8
clay fraction (<2 μm) (%)	27.3	11.7

Table 2
The examined treatments (Žabčice and Vatín; 2013–2022).

Treatment (Winter wheat)	Tillering N (kg/ha)	Fertilizer	Stem elongation start N (kg/ha)	Fertilizer	Heading N (kg/ha)	Fertilizer
Unfertilized	-	-	-	-	-	-
Prevalent technology	*55 (50:50:0)	CAN	65 (50:50:0)	CAN	40	UAN (25:25:50)
Alternative technology with NI	55 (50:50:0)	CAN	105	ASN + NI (66:33:0)	-	-
Treatment (Oilseed rape)	**Side shoot formation N (kg/ha)**	**Fertilizer**	**Stem elongation start N (kg/ha)**	**Fertilizer**	**Flower bud emergence N (kg/ha)**	**Fertilizer**
Unfertilized	-	-	-	-	-	-
Prevalent technology	105 (50:50:0)	CAN	45	UAN (25:25:50)	45	UAN (25:25:50)
Alternative technology with NI	65 (50:50:0)	CAN	130	ASN + NI (66:33:0)	-	-

* Percentage of applied forms of N ($N\text{-}NH_4^+$; $N\text{-}NO_3^-$; $N\text{-}NH_2$) in the total N dose.

(wheat/rape) with CAN was usually performed at the end of February or at the beginning of March, depending on the course of weather and the crop vegetation. The fertilization in stem elongation (both crops) with common nitrogen fertilizers (CAN or UAN) or with fertilizer enriched by NI was then performed at the turn of March and April, depending on the growth stages of winter wheat and oilseed rape. The application of UAN in prevalent technology was performed at the turn of April and May. The winter wheat and oilseed rape were treated with permitted fungicides and insecticides as needed during the whole experiment. Upon the full ripening, both crops were harvested from the harvested area of 10 m^2 within each fertilized plot. Unfortunately, the long-term experiment with oilseed rape was negatively influenced by the course of weather. In the 2019, the oilseed rape at experimental locality Vatín was decimated by the hailstorm just before the harvest. Even though the experiment was still harvested, the seed yields from that year could not be evaluated because of the large loss. The experiment with oilseed rape at the locality Žabčice had to be cancelled in the year 2022, because of the warm winter and early overpopulation of *Psylliodes*. The winter wheat was cultivated at both localities without any problems in every growing season.

2.3. Yield, grain and seed quality measurements

The weight of winter wheat grain and oilseed rape seed (kg) from the harvested area (10 m^2) was determined using the digital scale Kern ECE 20K-2N (KERN and Sohn GmbH, Balingen, Germany). The moisture content of harvested products was measured subsequently (portable moisture meter Wile 78 Crusher, Farmcomp Oy, Finland). The final wheat grain yield was standardized at a 12.0 % moisture content and expressed as tons per hectare (t/ha), the final yield of rape seed was standardized at 8 % moisture content and also expressed as t/ha. The agronomic nitrogen efficiency (ANE) (Moitzi et al., 2020; Tian et al., 2018) was expressed for the fertilized treatments as the increase in grain yield (kg) per unit of nitrogen applied (kg) according to the Eq. (1), where Y_{FERT} is fertilized treatment yield of grain/seed, $Y_{CONTROL}$ is unfertilized treatment yield of grain/seed and N_{DOSE} is nitrogen rate applied by mineral fertilizer:

$$\text{ANE}\ (\text{kg}/\text{kg}) = \frac{Y_{FERT}\ (t/ha)\ -\ Y_{CONTROL}\ (t/ha)}{N_{DOSE}\ (t/ha)} \quad (1)$$

Winter wheat grain quality: The test weight scale Wile 241 (Farmcomp OY, Tuusula, Finland) was used for the determination of the hectolitre weight of wheat grains. The nitrogen content in the grain was determined according to the Kjeldahl method (Kirk, 1950) using Kjeltec 2300 device (Foss, Hillerød, Denmark), protein content was calculated by multiplying the nitrogen content by 5.7 coefficient. The gluten content and the sedimentation index known as the Zeleny-test (ZT) were estimated by the NIR (Near Infrared Spectroscopy) method on the Inframatic 9500 NIR grain analyzer (Perten Instruments, Hägersten, Sweden). The protein production (t/ha) was expressed from average yield (t/ha) and average protein content (%).

Oilseed rape seed quality: The oil content was determined gravimetrically after the extraction of the samples with diethyl ether using the Soxhlet method based on the NMR extraction of rapeseeds in a continuous flow extractor Minispec mq series TD NMR (Bruker Corporation, Ettlinger, Germany). The oilseed production (t/ha) was expressed from average yield (t/ha) and average oil content (%).

2.4. Economic analysis

Economic analysis following a partial budget (CIMMYT Economics Program, 1988) was performed for compared nitrogen fertilization technologies. By its nature, this procedure considers only major differences between technologies (fertilization) and did not take all costs and benefits into account. Therefore, only the cost of N fertilizers, the number of their applications and price of winter wheat grain or seed of oilseed rape were considered. The cost of 1 tonne of used fertilizers were: CAN – 333 €; UAN – 358 €; ASN + NI – 412 €. The price of fertilizers for compared technologies have been recalculated according to the corresponding applied doses (Table 2). The cost of one application (fertilization in selected growth stage) was 12 €. The prices of harvested commodities were: winter wheat (for food industry according to the standard of the Commission Regulation (EC) No. 824/2000) – 209 €/t; oilseed rape – 430 €/t. The prices were actual for the end of the year of 2023. However, the prices of input (fertilizers) and outputs (wheat grain/seed of oilseed rape) usually fluctuates, making it difficult for economic analysis. Therefore, additional sensitivity analysis (Mohammed et al., 2022) was performed under three different scenarios to accommodate possible market dynamics and to see the effects of input and output price changes on the compared technologies of fertilization. These scenarios were: (1) increase cost of N fertilizers by 10 % but fixed commodity price, (2) increase commodity price by 10 % with fixed N fertilizers cost and (3) increase N fertilizers cost by 10 % and decrease commodity price by 10 % (worst case scenario from farmers' perspective). The average yield of winter wheat and oilseed rape from both experimental localities over the ten years of the experiment was used for the economic evaluation.

2.5. Statistical analysis

The collected data (grain / seed yield and quality) were evaluated by the Statistica Software 14 CZ by multifactorial analysis of variance (ANOVA with factors: growing season, locality, treatment) and subsequently by the Fisher's Least Significant Difference (LSD) test at the 5 % level ($p \leq 0.05$) of significance. Normality and homogeneity of variances were checked using the Shapiro-Wilk test and Levene's test. The results were expressed as the arithmetical mean ± standard deviation (SD). The results were also evaluated by the multifactorial analysis of variance (factor: growing season, treatment) for both experimental stations separately. Finally, the results presented in the Supplementary Data were evaluated by single factorial ANOVA (factor: treatment).

3. Results

3.1. Grain yield of winter wheat and agronomic efficiency

The average grain yield of winter wheat and average agronomic nitrogen efficiency (ANE) over the ten years of the experiment with the addition of nitrification inhibitors to the nitrogen fertilizer are described in Table 3. It is evident that the alternative technology with NI resulted in higher yield at both experimental localities compared to the prevalent technology. At experimental station Žabčice, the fertilization with nitrogen enhanced with NI resulted in average yield 8.7 t/ha, which represents an increase by 3.6 % in comparison with classic nitrogen fertilization without the inhibitors (8.4 t/ha). The statistically lowest yield was determined after the unfertilized treatment (7.1 t/ha). Similarly, the statistically lowest yield (5.2 t/ha) was determined after the same treatment at the experimental locality Vatín. The alternative technology with NI (7.4 t/ha) resulted in an increase in grain yield by 8.8 % in comparison with the prevalent technology (6.8 t/ha). On average from both localities and every growing season, the alternative technology with NI resulted in the statistically highest yield of wheat grain (8.0 t/ha). The yield determined after the prevalent technology without NI was statistically lower by 0.4 t/ha (5.3 %). The unfertilized treatment resulted in statistically lowest average yield 6.16 t/ha. The average yields from every growing season and their statistical differences are described in the Supplementary Data (Tables S4-S5).

ANE represents the increase in grain yield (in kg) caused by 1 kg of N in applied fertilizer. These results are in correlation with grain yield, as the ANE were statistically significant on average from both experimental localities. According to these results, fertilization with NI (11.6 kg) resulted in statistically higher (by 2.6 kg) utilization of applied nitrogen in comparison with prevalent technology (9.0 kg). The ANE determined separately at Žabčice and Vatín stations were also higher (by 1.7 and 3.4 kg), but not statistically.

Table 3
The average yield (t/ha) of winter wheat grain and agronomic N efficiency of winter wheat treatments and their statistical differences (Žabčice and Vatín; 2013–2022).

Locality / Treatment		Average yield – Žabčice (t/ha)	Yield difference (t/ha)	Nitrogen dose (kg/ha)	ANE (kg/kg)
Žabčice	**Unfertilized**	7.1 ± 2.3 a	-	-	-
	Prevalent technology	8.4 ± 1.8 b	1.3 ± 1.2 a	160	7.8 ± 1.2 a
	Alternative technology with NI	8.7 ± 1.7 b	1.5 ± 1.4 a		9.5 ± 1.4 a
Vatín	**Unfertilized**	5.2 ± 1.6 a	-	-	-
	Prevalent technology	6.8 ± 1.8 b	1.6 ± 1.9 a	160	10.3 ± 1.9 a
	Alternative technology with NI	7.4 ± 1.7 b	2.2 ± 1.7 a		13.7 ± 1.4 a
Average	**Unfertilized**	6.2 ± 2.2 a	-	-	-
	Prevalent technology	7.6 ± 2.0 b	1.5 ± 1.6 a	160	9.0 ± 1.1 a
	Alternative technology with NI	8.0 ± 1.8 c	1.9 ± 1.6 b		11.6 ± 1.1 b

The values are expressed as the arithmetic mean ± standard deviation (SD). The mean marked by different letters indicate significant differences $p < 0.05$ (Fisher's LSD test). ANE – agronomic nitrogen efficiency.

3.2. Winter wheat grain quality

The average values of winter wheat grain quality are described in Table 4. The fertilization of winter wheat with three separate doses of nitrogen applied in conventional fertilizers have on average provided statistically similar values of hectolitre weight (76.9 kg/hl) in comparison with two nitrogen applications with the NI (76.6 kg/hl). At Žabčice, both fertilized treatments resulted in statistically higher HW compared to the unfertilized treatment, the differences between HW values obtained from Vatín were insignificant. The statistically highest protein content was determined after the alternative technology with NI (13.0 %). This represents an increase in protein content by 1.9 % (in relative %) compared to the prevalent technology (12.8 %). The lowest values were determined after the unfertilized treatment (11.2 %). The protein content determined in wheat grain separately for Žabčice and Vatín was not statistically different between the fertilized treatments, although the values were relatively higher after the fertilization with NI (by 1.6 and 2.1 % in relative %) at both localities. The highest production of protein was determined after the treatment with NI, which was statistically higher by 6.1 % in comparison with prevalent technology without inhibitors. The use of NI represents an increase in protein production compared to prevalent technology by 5.3 % and 9.5 % in Žabčice and Vatín respectively. However, these differences were evaluated as statistically insignificant. The highest content of gluten was on average determined after the alternative technology with NI (29.5 %). The ZT was on average highest after the alternative technology with NI (39 ml), although the difference by 4.9 % between the prevalent technology was evaluated as not significant (38 ml). Significantly lowest mean values were found on the unfertilized treatment (24 ml). Similarly, to previous results, the alternative technology with NI resulted in higher values of ZT (by 2.5 and 5.8 %) in comparison with prevalent technology, however, the differences determined separately at Žabčice and Vatín were again not statistically significant between these fertilized treatments.

3.3. Economic evaluation of winter wheat production

The Table 5 is describing the results of economic evaluation of winter

Table 4
The winter wheat grain quality and their statistical differences (Žabčice and Vatín; 2013–2022).

Parameter	Localities/ Average	Treatment		
		Unfertilized	Prevalent technology	Alternative technology with NI
Hectolitre weight (kg/hl)	Žabčice	76.9 ± 2.2 a	78.1 ± 2.2 b	78.0 ± 2.2 b
	Vatín	75.7 ± 2.4 a	75.7 ± 2.7 a	75.2 ± 3.3 a
	Average	76.3 ± 2.4 a	76.9 ± 2.7 b	76.6 ± 3.1 ab
Protein content (%)	Žabčice	11.7 ± 2.2 a	13.5 ± 1.7 b	13.7 ± 1.4 b
	Vatín	10.7 ± 1.4 a	12.1 ± 1.7 b	12.3 ± 1.7 b
	Average	11.2 ± 1.9 a	12.8 ± 1.8 b	13.0 ± 1.7 c
Protein production (t/ha)	Žabčice	0.9 ± 0.3 a	1.1 ± 0.3 b	1.2 ± 0.3 b
	Vatín	0.6 ± 0.2 a	0.8 ± 0.2 b	0.9 ± 0.2 b
	Average	0.7 ± 0.3 a	* 1.0 ± 0.3 b	* *1.0 ± 0.3 c
Gluten content (%)	Žabčice	25.5 ± 6.0 a	30.4 ± 4.4 b	31.3 ± 4.1 b
	Vatín	23.2 ± 3.7 a	27.0 ± 4.6 b	27.7 ± 4.6 b
	Average	24.3 ± 5.1 a	28.7 ± 4.8 b	29.5 ± 4.7 c
Zeleny test (ml)	Žabčice	28 ± 18 a	43 ± 15 b	44 ± 13 b
	Vatín	20 ± 11 a	32 ± 14 b	34 ± 15 b
	Average	24 ± 15 a	38 ± 15 b	39 ± 15 b

The values are expressed as the arithmetic mean ± standard deviation (SD). The mean marked by different letters indicate significant differences $p < 0.05$ (Fisher's LSD test). For all monitored parameters, differences between localities and their average were statistically evaluated separately. * 0.98 (t/ha), * * 1.04 (t/ha).

Table 5
Economic evaluation of average wheat yield according to the examined technologies (Žabčice and Vatín; 2013–2022).

Scenario	Treatment	Fertilizers cost			Increase in fertilizer cost (€/ha)*	Sales profit (wheat grain)			Revenue increase (€/ha)* *	Net profit (€/ha) * **
		Price of fertilizers (€/ha)	Application cost (€/ha)	Total costs (€/ha)		Average yield (t/ha)	Purchase price (1 t of grain)	Total revenue (€/ha)		
2023	**Prevalent technology**	184	36	**220**	-	7.61	209	1 593	**-**	**-**
	Alternative technology with NI	233	24	**257**	**37**	8.02	209	1 679	**86**	**49**
1	**Prevalent technology**	202	36	**238**	-	7.61	209	1 593	**-**	**-**
	Alternative technology with NI	256	24	**280**	**42**	8.02	209	1 679	**86**	**44**
2	**Prevalent technology**	184	36	**220**	-	7.61	230	1 750	**-**	**-**
	Alternative technology with NI	233	24	**257**	**37**	8.02	230	1 845	**95**	**58**
3	**Prevalent technology**	202	36	**238**	-	7.61	188	1 431	**-**	**-**
	Alternative technology with NI	256	24	**280**	**42**	8.02	188	1 508	**77**	**35**

Scenario: 2023 – actual prices at the end of the year 2023; 1 - increase cost of N fertilizers by 10 % but fixed commodity price; 2 - increase commodity price by 10 % with fixed N fertilizers cost; 3 - increase N fertilizers cost by 10 % and decrease commodity price by 10 %. * Increase in fertiliser costs (€/ha) = Total fertilizer cost of Alternative technology with NI (€/ha) - Total fertilizer cost of Prevalent technology (€/ha). * * Revenue increase (€/ha) = Total revenue of Alternative technology with NI (€/ha) – Total revenue of Prevalent technology (€/ha). * ** Net profit (€/ha) = Revenue increase (€/ha) - Increase in fertiliser costs (€/ha).

wheat. In every scenario, the alternative technology with NI resulted in higher total revenues despite the increase in fertilizers cost. Therefore, the alternative technology with NI resulted in higher net profit in every examined scenario (from 35 to 58 €/ha).

3.4. Seed yield of oilseed rape and agronomic efficiency

The average seed yield of oilseed rape and average agronomic nitrogen efficiency over the whole experiment with the NI utilization in nitrogen fertilization are described in Table 6. The result obtained from Žabčice and Vatín separately are similar to winter wheat, therefore relatively higher yield (by 5.8 % at Žabčice and by 12.5 % at Vatín) after the alternative technology with NI (3.6 t/ha Žabčice, 3.6 t/ha Vatín) in comparison with prevalent technology (3.4 t/ha Žabčice, 3.2 t/ha Vatín), although these differences between fertilized treatments were evaluated as statistically insignificant. The unfertilized treatment provided the lowest yield of seed at both localities (2.8 t/ha Žabčice, 2.6 t/ha Vatín).

Table 6
The average yield (t/ha) of oilseed rape seed and agronomic N efficiency of oilseed rape treatments and their statistical differences (Žabčice and Vatín; 2013–2022).

Locality / Treatment		Average yield – Žabčice (t/ha)	Yield difference (t/ha)	Nitrogen dose (kg/ha)	ANE (kg/kg)
Žabčice	**Unfertilized**	2.8 ± 1.0 a	-	-	-
	Prevalent technology	3.4 ± 1.0 b	0.6 ± 0.6 a	195	3.1 ± 0.5 a
	Alternative technology with NI	3.6 ± 1.1 b	0.9 ± 0.5 b		4.5 ± 0.4 b
Vatín	**Unfertilized**	2.6 ± 1.0 a	-	-	-
	Prevalent technology	3.2 ± 1.6 ab	0.6 ± 1.1 a	195	2.9 ± 1.0 a
	Alternative technology with NI	3.6 ± 1.6 b	1.0 ± 1.1 a		4.9 ± 0.9 a
Average	**Unfertilized**	2.7 ± 1.0 a	-	-	-
	Prevalent technology	3.3 ± 1.4 b	0.6 ± 0.9 a	195	3.0 ± 0.5 a
	Alternative technology with NI	3.6 ± 1.4 c	0.9 ± 0.8 b		4.7 ± 0.5 b

The values are expressed as the arithmetic mean ± standard deviation (SD). The mean marked by different letters indicate significant differences $p < 0.05$ (Fisher's LSD test). ANE – agronomic nitrogen efficiency.

On average from both localities and every growing season, the alternative technology with NI resulted in the statistically highest yield of oilseed rape (3.6 t/ha). The seed yield determined after the prevalent technology without NI was statistically lower by 0.3 t/ha (9.1 %). The unfertilized treatment resulted in statistically lowest average yield 2.7 t/ha. The average yields of oilseed rape from every growing season and their statistical differences are described in the Supplementary Data (Tables S6-S7).

The ANE were statistically significant at Žabčice station, where the alternative technology with NI resulted in statistically higher utilization of applied nitrogen (4.5 kg) in comparison to the prevalent technology (3.1 kg). Same results were determined on average from both experimental stations, the alternative technology with NI resulted in higher ANE by 1.7 kg compared to the prevalent technology. The differences at experimental station Vatín were insignificant, however, relatively higher ANE was observed after the treatment with NI.

3.5. Oilseed rape seed quality

The average oil content (%) of oilseed rape and oil production (t/ha) expressed from average seed yield (t/ha) and average oil content are described in Table 7. It is evident from these results, that the oil content determined separately from each experimental stations was highest after the unfertilized treatment (41.8 % Žabčice, 45.4 % Vatín), the alternative technology with NI resulted in statistically lowest oil content (40.6 % Žabčice, 42.1 % Vatín). On average, the statistical differences were determined in order: unfertilized > prevalent technology

Table 7
The oilseed rape seed quality and their statistical differences (Žabčice and Vatín; 2013–2022).

Parameter	Localities/ Average	Treatment		
		Unfertilized	Prevalent technology	Alternative technology with NI
Oil content (%)	Žabčice	41.8 ± 1.8 b	41.1 ± 1.6 ab	40.6 ± 1.3 a
	Vatín	45.4 ± 1.3 b	44.7 ± 2.5 b	43.5 ± 1.9 a
	Average	43.6 ± 2.4 c	42.9 ± 2.7 b	42.1 ± 2.2 a
Oil production (t/ha)	Žabčice	1.2 ± 0.4 a	1.4 ± 0.4 b	1.5 ± 0.5 b
	Vatín	1.2 ± 0.5 a	1.4 ± 0.7 ab	1.6 ± 0.7 b
	Average	1.2 ± 0.4 a	1.4 ± 0.6 b	1.5 ± 0.6 c

The values are expressed as the arithmetic mean ± standard deviation (SD). The mean marked by different letters indicate significant differences $p < 0.05$ (Fisher's LSD test). For all monitored parameters, differences between localities and their average were statistically evaluated separately.

> alternative technology with NI. On the contrary, the average oil production in tonnes per hectare were determined in the opposite order with the statistical differences due to the higher yield of fertilized treatments: unfertilized < prevalent technology < alternative technology with NI.

3.6. Economic evaluation of oilseed rape production

The Table 8 is describing the results of economic evaluation of oilseed rape production under examined technologies of fertilization under different price scenarios, considering the variable price of fertilizers and harvested products. In every scenario, the alternative technology with NI resulted in higher total revenues in comparison with prevalent technology despite the increase in fertilizers cost (due to the higher price of fertilizer with NI). Therefore, the alternative technology with NI resulted in higher net profit in every examined scenario (from 79 to 96 €/ha).

4. Discussion

From our results presented in Tables 3 and 6 is evident, that the alternative technology of nitrogen fertilization enhanced with NI resulted in overall highest yields of wheat grain and rape seed observed during the ten years of the experiment. Due to the addition of nitrification inhibitors, the alternative technology with NI resulted in significantly higher grain yield of winter wheat (8.2 t/ha) and seed yield of oilseed rape (3.61 t/ha) in comparison with the prevalent technology (7.61 t/ha; 3.27 t/ha). The main goal of presented long-term study was to evaluate the effect of crop fertilization enhanced with NI on yield and quality, e.g. especially from the practical point of view, as the effect of inhibitors to the nitrogen transformation in the environment is already well documented (Byrne et al., 2020; Gupta et al., 2023; Woodward et al., 2021). The obtained results are suggesting that alternative technology of fertilization with NI represents an opportunity to optimise food production.

The purpose of the incorporation of NI to the commonly available nitrogen fertilizers is its more gradual release, therefore the possibility of simplifying the strategies of fertilization (de Souza et al., 2023; Torabian et al., 2023) like reducing the number of applications or possibly reducing the overall dose nitrogen (lowering the environmental loss). Thus, the gradual nitrification of ammonium nitrogen releases the nitrate nitrogen with the respect to the yield-building needs of crops while limiting the risk of leaching, which resulted in overall higher yield of grain yield by 5.4 % and by 10.2 % of seed yield in comparison with prevalent technology, where the nitrogen dose had to be split into more applications. The positive effect of nitrogen fertilization enhanced with NI on the grain yield of winter wheat is also described by several authors (Dawar et al., 2022; Shalmani et al., 2022; Tao et al., 2021; Zheng et al., 2021). Similar results are described also by Wang et al. (2021), as they refer to good potential of inhibitors to increase the crop yield while reducing the emission of N_2O. The positive effect of inhibitors to the crop yield was also described by several authors for maize (Borzouei et al., 2021; Dawar et al., 2021; Steusloff et al., 2019; Yang et al., 2016), rice (Linquist et al., 2013), oilseed rape (Li et al., 2008), vegetables (Fan et al., 2018; Zhang et al., 2015b) or flax (Kakabouki et al., 2020). A

Table 8
Economic evaluation of average oilseed rape yield according to the examined technologies (Žabčice and Vatín; 2013–2022).

Scenario	Treatment	Fertilizers cost			Increase in fertilizer cost (€/ha)*	Sales profit (oilseed rape)			Revenue increase (€/ha)* *	Net profit (€/ha) * **
		Price of fertilizers (€/ha)	Application cost (€/ha)	Total costs (€/ha)		Average yield (t/ha)	Purchase price (1 t of seed)	Total revenue (€/ha)		
2023	**Prevalent technology**	211	36	247	-	3.27	430	1 408	-	-
	Alternative technology with NI	285	24	309	**62**	3.61	430	1 552	**144**	**82**
1	**Prevalent technology**	252	36	288	-	3.27	430	1 408	-	-
	Alternative technology with NI	313	24	337	**49**	3.61	430	1 552	**144**	**95**
2	**Prevalent technology**	211	36	247	-	3.27	473	1 549	-	-
	Alternative technology with NI	285	24	309	**62**	3.61	473	1 707	**158**	**96**
3	**Prevalent technology**	252	36	288	-	3.27	387	1 268	-	-
	Alternative technology with NI	313	24	337	**49**	3.61	387	1 396	**128**	**79**

Scenario: 2023 – actual prices at the end of the year 2023; 1 - increase cost of N fertilizers by 10 % but fixed commodity price; 2 - increase commodity price by 10 % with fixed N fertilizers cost; 3 - increase N fertilizers cost by 10 % and decrease commodity price by 10 %. * Increase in fertiliser costs (€/ha) = Total fertilizer cost of Alternative technology with NI (€/ha) - Total fertilizer cost of Prevalent technology (€/ha). * * Revenue increase (€/ha) = Total revenue of Alternative technology with NI (€/ha) – Total revenue of Prevalent technology (€/ha). * ** Net profit (€/ha) = Revenue increase (€/ha) - Increase in fertiliser costs (€/ha).

meta-analysis by Abalos et al. (2014) indicated that, on average, the use of inhibitors led to a 7.5 % increase in yield, which correlates with our results. These increases in crop yield are caused by the increased proportion of mineral nitrogen in ammonia form (due to the NIs effect), which provided a more opportunity for plants to take up applied N as NH_4^+ form. NH_4^+ may be incorporated into amino acids and finally into plant protein at less energy cost compared to nitrate form of N, suggesting that the plants may be left with extra energy to allocate to the growth and crop yields (Aulakh et al., 2001; Dawar et al., 2011). Several studies (Arregui and Quemada, 2008; Cardenas et al., 2019; De Antoni Migliorati et al., 2014; Nauer et al., 2018; Polychronaki et al., 2012) have also reported, that the NI did not statistically influence the crop yield, although the trend in relatively increased yields was usually observed.

Another possible approach to evaluate the effectiveness of the examined technologies is ANE (Tables 3 and 6). The agronomic nitrogen efficiency represents an increase in crop yield (in kg) caused by 1 kg of nitrogen in applied fertilizers. Overall, the alternative technology with NI (two applications) resulted in higher utilization of nitrogen by cultivated crops (11.6 kg winter wheat; 4.7 kg oilseed rape) compared to the same total dose of nitrogen applied in prevalent nitrogen technology with three split nitrogen fertilization (9.0 kg winter wheat; 3.0 kg oilseed rape). Similar finding is also described by (Dawar et al., 2022), where the utilization of NI in combination with Urea also increased the ANE, or by Shalmani et al. (2022), where the application of IN with ammonium sulphate increased the ANE even in the drought stress conditions. A possible explanation can be also in the forms of nitrogen applied in both technologies. For the prevalent N technology, the CAN fertilizer (balanced ammonium and nitrate content) or UAN (three form of N with majority in NH_2) were used for the first and second fertilization of model crops. The alternative technology with NI was based on the fertilizer ASN, where a major part of nitrogen is presented in ammonium form. The NI are responsible for the slower nitrogen transformation from ammonia to the nitrate (Cui et al., 2021; Galloway et al., 2008; Ruser and Schulz, 2015). Therefore, the plants fertilized with ASN, especially enhanced with nitrification inhibitors, probably had lower energy requirement for the assimilation of nitrogen into plants protein.

For farmers, higher yields simply represent a possible higher profit, therefore a possible innovation in growing technologies or less dependency on subsidies. However, the prices of input (fertilizers, seed, fuel, pesticides, …) and outputs (harvested commodities) usually fluctuates, making it very difficult for economic analysis, However, we have tried to at least partially evaluate the economic aspects of both technologies (Tables 5 and 8) in several scenarios. It is evident from these calculations that the alternative technology with NI resulted in optimal profit every scenario for both model crops. Therefore, although the total cost of fertilizers used in alternative technology with NI is higher (in every scenario) due to the addition of NI (more expensive fertilizer) even with the reduced amount of applications (two-times), the higher yields achieved by this fertilization technology resulted in higher total net profit in every scenario compared to the prevalent technology based on conventional fertilizers (lower price) applied three times during the vegetation. Same results with the identical fertilizer (ASN with NI) are also presented by Slamka et al. (2016). The alternative technology with NI therefore represents a more economical (time saved, fuel and application cost saved) and ecological (reduction of soil compaction, reduction of nitrogen loss, legislative, higher NUE) option (Chien et al., 2009). In addition, the utilization of INs (or inhibitors generally) is in correlation with the legislative changes. For presented partial economical evaluation, the price of harvested products in food quality were considered. In real conditions, the quality of harvested products can heavily influence the final price of the product, especially for winter wheat. The result obtained from our ten-year experiment proved, that the addition of NI to conventional nitrogen fertilizer have positive effect on production of protein and oil, gluten content and even index of sedimentation (Tables 4 and 7). The minimal required content of protein is 11.5 % (Commission Regulation EC No. 824/2000) was met by both technologies, the fertilization with NI (13.0 %) resulted in statistically higher values by 1.9 % (rel. %) compared to the prevalent technology (12.8 %). Identically, the content of gluten was higher by 2.7 % (rel. %) (29.5 % alternative technology with NI; 28.7 % prevalent technology). Very similar trend in grain quality of winter wheat grain after the fertilization with ASN or ASN with NI are described by Školníková et al. (2022) or several other studies (Arregui and Quemada, 2008; Duncan et al., 2017; Liu et al., 2013; Villar and Guillaumes, 2010). The hectolitre weight was only wheat grain parameter, which was lower (statistically not significantly) after the alternative technology with NI compared to the prevalent fertilization. This could be simply explained by dilution effect (Jarrell and Beverly, 1981). For example, Hoel (2011) have also reported an increase in grain yield followed by the decrease in HW of grain after the wheat fertilization, Poblaciones et al. (2014) suggested an inversely related grain quality to higher yield. Even if the value of HW after the addition of NI was in our work relatively lower compared to the prevalent technology with three split applications of nitrogen, it is necessary to point out, that the difference was insignificant. This is a welcome result, as it is evident from ten-year averages, that the application of higher dose of N with NI and omitting one application is viable due to the gradually transformation and release of nitrogen. Similar situation can be observed in oil content detected in oilseed rape.

It is necessary to point out, that there is a 13 % of sulphur in ammonium sulphate nitrate used in the alternative technology with IN (ASN + IN). It is well known fact, that the sulphur and nitrogen have a good synergy and that the addition of sulphur may have a positive effect on the nitrogen fertilization (Dubousset et al., 2010; Klikocka et al., 2017; Tabak et al., 2020). Therefore, the sulphur applied in alternative technology with NI to the winter wheat and oilseed rape in addition to identical dose of nitrogen compared to the prevalent technology may be another reason for increased yield and ANE in our experiment. However, to filter the effect of NI and S addition, we have established an identical three-year experiment (2016–2018) at the same experimental localities, where the effect of additional treatment with ASN without NI was evaluated (Antošovský et al., 2024). It is evident from this manuscript, that the same treatment with ASN without NI did not result in significantly higher yield in comparison with prevalent N technology (without S and NI). The significantly higher yields were identically determined only after the alternative technology with NI. The ANE observed between technology fertilized only by nitrogen and technology based on ASN without IN were also almost identical, the ANE provided by alternative technology with NI was considerably higher. Therefore, it is safe to assume the positive effects of alternative technology with NI (ASN + NI) was primary caused by the addition of inhibitors, not the additional content of sulphur. There are several authors (Abit et al., 2017; Ercoli et al., 2011; Kulhánek et al., 2014; Ryant and Skládanka, 2009), whose also described the insignificant result of S fertilization. The non-significant effect of fertilization with N and S can be possibly explained by its late application in the stem elongation. The most optimal period for sulphur fertilization is according to the literature usually before crop sowing or very early, at the start of the vegetation, or in split doses during the whole vegetation (Ahmad et al., 2005; Eriksen and Mortensen, 2002; Grant et al., 2003; Habtegebrial and Singh, 2006; Khan et al., 2019). Several authors (Gupta and Jain, 2008; Haque et al., 2015) have also found out an overall low sulphur use efficiency (SUE) not exceeding 10 %. The low SUE is usually attributed to the leaching of sulphate anion from the soil profile, adsorption to clay hydrous oxides and anion exchange sites, or sulphur retention in crop residues (Singh Shivay et al., 2014; Singh et al., 2014). Another possible explanation of insignificant effect of S fertilization was described by Dhillon et al. (2019), as they are referring to the adequate supply of S from the mineralization of soil organic matter, which is also mentioned by Mahal et al. (2022).

5. Conclusion

From the presented and discussed results obtained from ten growing seasons with winter wheat and nine growing seasons with oilseed rape grown at two different localities, it is evident that the application of nitrogen fertilizer with NI in higher dose is a very suitable alternative to the conventional fertilization practice of several split nitrogen fertilization. The use of fertilizers with NI represents a possible reduction of field crossing (higher dose, less applications), thus saving costs and reducing the burden on the environment while maintaining comparable yield and quality of harvested products. The long-term average yields of winter wheat grain and seed of oilseed rape determined from two different localities were significantly higher after the fertilization with the NI in comparison with prevalent technology. The effect of inhibitor addition also resulted in significantly higher average protein content, protein production and gluten content in wheat grain in comparison with prevalent technology without NI. The hectolitre weight and index of sedimentation were similar for both fertilized treatments. The oil content of oilseed rape was, on the contrary, significantly lower on both fertilized treatments compared to unfertilized control. However, the alternative technology with NI resulted in significantly highest production of oil due to the highest seed yield. These long-term results are proving, that addition to NI to the conventional fertilizer applied in higher dose and less applications is optimal choice compared to the classic split nitrogen fertilization due the higher yields of model crops and similar quality (despite the missing nitrogen application). The novelty of this work is the continuous yield and quality related results obtained from ten growing seasons at two different localities with two different crops, as such results was not yet published. Second benefit is the examined fertilizer (ammonium sulphate nitrate) with NI, as most of the published articles are focused on urea.

The possible limitations of the presented study can be found in the soil conditions and course of the weather at the experimental localities, although the experiment was conducted for ten years at two different localities. The alternative technology with NI appeared to be a suitable fertilization strategy in comparison with the prevalent nitrogen fertilization, a possible limitation of this study might be in different fertilizers used.

CRediT authorship contribution statement

Antošovský Jiří: Writing – original draft, Visualization, Investigation, Data curation. **Škarpa Petr:** Writing – review & editing, Validation, Supervision, Investigation. **Ryant Pavel:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors declare that they have not used any AI-supportive technologies during the writing process.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fcr.2025.109906.

Data availability

Data will be made available on request.

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