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Research article

The effect of nitrogen-sulphur fertilizer with nitrification inhibitor on winter wheat (*Triticum aestivum* L.) nutrition

Jiří Antošovský, Petr Škarpa, Pavel Ryant *

Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Faculty of AgriScience, Mendel University in Brno, Zemědělská 1, 61300, Brno, Czech Republic

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A B S T R A C T

The high input of nitrogen is often required in today's agriculture, especially for the most cultivated crops largely involved in human and animal nutrition, such as winter wheat. Nitrogen is a mobile nutrient in the soil, and the high doses of N are often associated with possible losses through volatilization or leaching. One of the possible options to increase nitrogen use efficiency is the application of fertilizers with inhibitors. The main objective of the presented three-year experiment established under the field conditions at the two experimental sites was to examine the effect of nitrogen-sulphur fertilizer (ammonium nitrate sulphate) with the inhibitors of nitrification (IN) (dicyandiamide and 1,2,4 triazole). In addition to the nitrogen content in two forms, this fertilizer also contains sulphur, which can possibly enhance the utilization of nitrogen due to their well-known synergy. The treatments included in the experiment were: 1. Unfertilized, 2. N technology 3. N + S technology and 4. N + S + IN. The total dose of applied N for every fertilized treatment was 159 kg/ha. Treatments 2 and 3 were fertilized with three split doses of N, treatment 4 was fertilized only two times due to the addition of IN (a higher dose of fertilizer in the second application). The results obtained from the three-year experiment showed a significantly higher yield of grain (8.18 t/ha) after the fertilization with N + S + IN in comparison with N + S (7.67 t/ha) and N (7.61 t/ha), which proved the positive effect of IN on nitrogen use efficiency during the vegetation. The differences between qualitative parameters of wheat grain (hectolitre weight, protein and gluten content) were evaluated as statistically insignificant for each fertilized treatment. This similar result is likely due to the IN application, which provided a continuous nitrogen supply during vegetation comparable to the three split nitrogen applications. Thus, our results showed, that the addition of IN to the higher dose of fertilizer applied earlier in the vegetation can provide comparable results in terms of quality to the technologies based on three split fertilizations. The three-year experiment established at two experimental sites has proved, that the application of ammonium sulphate nitrate fertilizers with IN in a higher dose is a better option to the commonly used nitrogen technology, which was also supported by the economic evaluation and the highest net profit.

1. Introduction

The most dominant nutrient in crop production is nitrogen. The utilization of nitrogen fertilizers in agriculture represents more

* Corresponding author.
E-mail addresses: jiri.antosovsky@mendelu.cz (J. Antošovský), petr.skarpa@mendelu.cz (P. Škarpa), pavel.ryant@mendelu.cz (P. Ryant).

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than half of the overall fertilizer's consumption [1]. A large amount of nitrogen has been applied to agricultural ecosystems to meet the growing global demand for food [2]. Several studies [3,4] have described the positive effect of N fertilization on the grain yield and its quality. However, nitrogen is characterized by possible significant loss (up to 70 %) due to the high mobility in the environment [5]. The average nitrogen use efficiency (NUE) ranges between 32 and 53 % in common field conditions [6,7]. The NUE can be possibly increased up to 50–70 % [8] if the N is supplied to the plants at the time of their greatest need. The percentage of N loss could be affected by the term of application, method of fertilization and form of nitrogen, incorporation of fertilizers into the soil, and especially by the course of weather [9]. Low efficiency of N fertilizers not only causes economic losses and a decrease in soil fertility, but it is also environmentally unfriendly and represents a possible risk to human and animal health due to the leaching [10,11] of NO_3^-, volatilization of NH_3, and emission of other N-containing gases [12,13]. The main problems associated with low NUE have a significant impact on climate change and possible environmental toxicity [14,15], eutrophication and nitrate pollution, ozone and air quality degradation, and emissions of greenhouse gases [16]. Therefore, improving the nitrogen use efficiency from fertilization should be the focus for sustainability in agriculture.

It was estimated that global production will increase by 70–100 % to maintain a world population of 9 billion people [17]. This would require a heavy reliance on the synthesis of N fertilizers through the Haber-Bosch process [18] and doubling the nitrogen doses applied globally [19]. This approach could possibly lead to a very high [20] annual loss of nitrogen (6.15 x 10^7 t). On the contrary, there is social and legislative pressure to prevent the negative impacts on the environment. EU climate policy has set out a target for a 55 % reduction in greenhouse gases below 1990 levels by 2030, and to become climate neutral by 2050 (European Commission No. 82/2021). The reduction of ammonia losses is also mandated by the EU (European Commission No. 2284/2016). Unfortunately, the current emission of ammonia has increased by more than 50 % during the past 30 years with a continued trend of increased proportion originating from agriculture [21]. The nitrogen loss, combined with an increased economic incentive for farmers to optimize NUE associated with rising costs of synthetic fertilisers, has placed an emphasis and imperative to implement nutrient management strategies that reduce reactive N loss [22].

One of the commonly used methods to improve NUE is the split application of nitrogen. Thus, nitrogen is applied in smaller doses several times during the growing season. One of the more sophisticated possibilities to improve NUE is represented by the application of mineral fertilizers, which can control the release of nutrients according to the crop growth and development, or soil-weather conditions. These fertilizers can be characterized as slow-release fertilizers (SRF) with part of the nutrient in slowly soluble form, controlled-release fertilizers (CRF) coated with organic or inorganic substances or fertilizers with inhibitors. These effective and environmentally friendly fertilizers contain inhibitors that temporarily restrict N transformations in the soil (urea hydrolysis or nitrification inhibition). Most nitrification inhibitors (INs) affect the ammonia mono-oxygenase enzyme, which is responsible for the oxidation of ammonium into nitrite in the first step of nitrification. In other words, the INs are depressing the activity of *Nitrosomonas* bacteria, which also provides more time for the plant's uptake or for the microbial immobilization of NH_4^+. Therefore, the IN is responsible for the conservation of immobile NH_4^+ form in the soil for a longer period (4−10 weeks), which reduces the amount of very mobile and potentially leeched NO_3^- [23,24] or denitrified gaseous emissions to the atmosphere [25]. One of the most applied nitrogen fertilizers in the EU is calcium ammonium nitrate, which is currently the subject of interest in terms of fertilizer coating [26] and even combination with new IN. Other commonly used nitrogen fertilizers include ammonium sulphate (21 % $N–NH_4^+$; 23 % of water-soluble S) and ammonium sulphate nitrate (18.5 % $N–NH_4^+$; 7.5 % $N–NO_3$; 13 % of water-soluble S). Both fertilizers include sulphur, and both have commercially available alternatives with nitrification inhibitors. The advantage of ammonium sulphate nitrate is the content of nitrogen in both forms (ammonia and nitrate) and the less negative impact on soil pH.

The efficiency of nitrogen fertilization can be possibly enhanced by the addition of sulphur, as these nutrients applied in mineral fertilizers have been proven to have good synergy [27–30] and their co-application may increase the yield [31,32]. The deficiency of sulphur in recent years was described by many authors [33–38]. The content of S in the soil decreases mainly due to the reduction of air pollution, intensification of agriculture mostly via low or S-free mineral fertilizers (Urea, CAN), low utilization of organic fertilizers and leaching of sulphates. Sulphur fertilization is essential mostly for the oil or legumes crops, however, the addition of S can be also beneficial for cereals [39], even though their S requirement is relatively low [40]. The need to produce 1 t of winter grain and the respective amount of straw is estimated at about 4 kg of S. Sulphur is essential for the synthesis of cysteine, methionine, and some other sulphur amino acids and vitamins [41,42]. The vegetative growth of plants, nitrate reduction [43] and incorporation of nitrogen into the plant proteins are also supported by the optimal content of sulphur. The deficiency of sulphur in plants during the vegetation can also result in the lower qualitative parameters of harvested products [44,45]. In agriculture, sulphur is mainly applied in the form of mineral fertilizers. Besides these fertilisers, some wastes [46,47] from industrial production can be used as a source of S. Such reutilization represents a potential return of sulphur back into the nutrient cycle in the agroecosystem and decreases the consumption of mineral fertilizers.

The aim of this study was to verify the effect of winter wheat fertilization by solely nitrogen technology and nitrogen technology enhanced with IN. The fertilizer with IN used in the experiment also contained sulphur (ammonium sulphate nitrate), therefore, a similar treatment without the IN was also evaluated in the experiment. The first hypothesis was, that co-application of nitrogen and sulphur is going to have a positive effect on the winter wheat yield in comparison with fertilization based solely on nitrogen. However, the main hypothesis was, that the addition of IN is going to enhance the positive effect of this nitrogen-sulphur treatment due to the more gradual release of nitrogen. Three-year experiment (2016–2018) was established in the field conditions at the two experimental sites (Žabčice, Vatín) to examine these hypotheses.

2. Material and methods

2.1. Experimental sites and climate-soil conditions

The three-year experiment was established first in the autumn of 2015. The winter wheat, variety Bohemia (Oseva Bzenec, a.s.; maintainer: Selgen a.s.), was sown as a tested crop in every experimental year. The experiment was established simultaneously at two field experimental sites belonging to Mendel University in Brno. The first experimental area is located in Žabčice (49.0229836 N, 16.6175028E), the altitude is 180 m above sea level, and it is characterized predominantly by a warm and dry climate with an average annual temperature of about 10 °C. The possible limiting factor for crop production is the relative lack of precipitations (or rather its uneven distribution during the year) together with frequent drying winds. According to the long-term normal, Žabčice is characteristic with a low precipitation of about 490 mm per year. The second experimental area 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 and more precipitations in comparison with Žabčice. The average monthly temperatures and precipitation sums during the experimental years for both experimental sites are shown in Fig. 1 (Žabčice 1a-d; Vatín 1e-h), together with the long-term normal of 1960–1990.

Due to the permanent influence of groundwater, the soil type of experimental area Žabčice is the Gleyic Fluvisols with clay loam

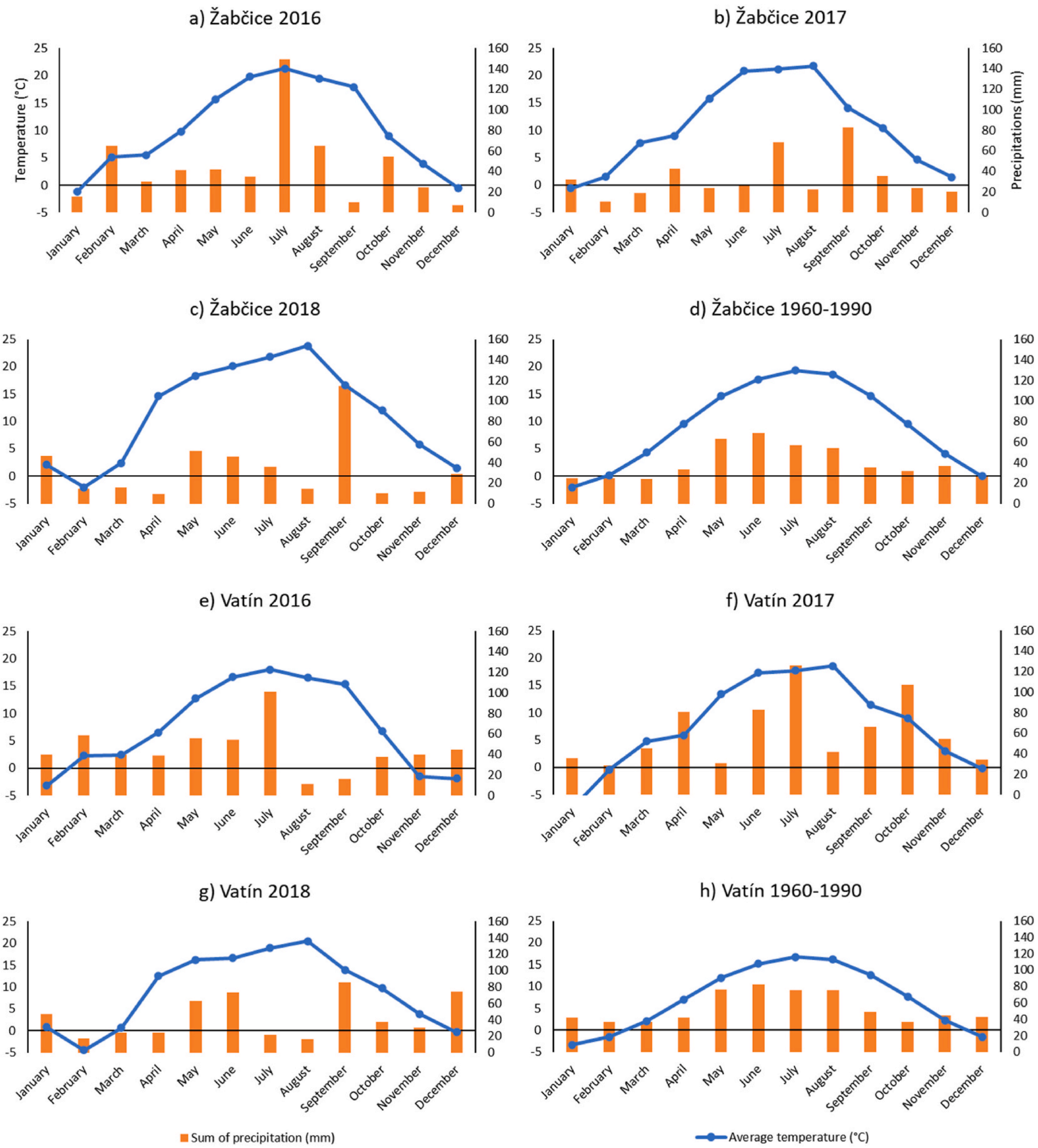

Fig. 1. Characteristics of average monthly temperatures and precipitation for locality Žabčice in 2016 (a), 2017 (b) and 2018 (c) and locality Vatín in 2016 (e), 2017 (f) and 2018 (g), with their comparison to the long-term normal (1960–1990) for Žabčice (d) and Vatín (h).

structure. The soil type at the locality Vatín is Cambisols, mostly with sandy soil structure. The average content of nutrients in the soil before sowing and exchangeable soil acidity was determined each year by the certified methodology [48]. Table 1 describes the basic agrochemical characteristics (average from 20016-2018) of both experimental sites. The content of nutrients in Vatín soils is mostly lower compared to Žabčice; the soil reaction at this locality is acidic.

2.2. Methodology of the experiment and field treatments

The Randomized Complete Block Design was used during the experiment. The examined treatments were unfertilized control**,** N technology, N + S technology and N + S + IN technology. The commonly used N technology was based on three split applications of nitrogen during the main stages [49] of winter wheat vegetation (tillering, stem elongation and beginning of heading). The nitrogen was applied in commonly used fertilizers calcium ammonium nitrate (CAN; 13.5 % $N–NH_4^+$ and 13.5 % $N–NO_3$; applied in tillering and stem elongation) and urea ammonium nitrate (UAN; 19.5 % $N–NH_2$, 9.75 % $N–NH_4^+$ and 9.75 % $N–NO_3$; applied at the beginning of heading). The N + S technology was also based on three split applications of nitrogen, one of them enhanced with sulphur. The nitrogen was applied in CAN (tillering), ammonium sulphate nitrate (ASN; 18.5 % $N–NH_4^+$; 7.5 % $N–NO_3$; 13 % of water-soluble S; applied in stem elongation) and UAN (heading). The technology with the N + S + IN was based only on two split applications of nitrogen during the vegetation (tillering and stem elongation). Fertilizer CAN was used for the first fertilization (tillering); for the second fertilization (stem elongation), ASN fertilizer enriched with a mixture of nitrification inhibitors dicyandiamide DCD (0.36–0.54 % w/w) and 1,2,4 triazole – TZ (0.032–0.048 % w/w) in 10:1 ratio was used. The total dose of nitrogen (159 kg/ha) was identical for every fertilized treatment (Table 2). Every treatment was established in four repetitions. The size of each experimental plot for the fertilization was 16.8 m^2 (11 rows with a length of 14 m and inter-row spacing of 0.12 m). All fertilizers were manually applied (broadcasted) to each field plot separately.

The winter wheat (variety Bohemia) was cultivated similarly every year. The pre-sowing preparation of soil (turn of July/August) after the same fore-crop harvest (winter wheat) was carried out using cultivators (plates or coulters as required) and harrows. The small plot sowing machine was used for wheat sowing (turn of September and October; sowing rate of 340 grain/m^2). The first application of nitrogen was performed at the end of February or at the beginning of March, depending on the course of weather and the crop vegetation. The second fertilization was performed at the turn of March and April, depending on the BBCH stage of winter wheat. The third application of nitrogen in common technologies (N or N + S) was performed at the turn of April and May. The winter wheat was treated with approved fungicides and insecticides as needed during the whole experiment. Upon the wheat fully ripening (at the start of July), plants were harvested from an area of 10 m^2 within each plot.

2.3. Plant and soil analysis

The aboveground biomass (AGB) of winter wheat was collected by cutting off of 16 plants above the soil surface before the beginning of heading (BBCH 49) from two repetitions of every examined treatment (8 plants from each repetition) every year as an evaluation of the different nitrogen fertilization performed in stem elongation (CAN vs ASN vs ASN + IN). The AGB of winter wheat was oven-dried at 60 °C for the first 2 h. The temperature was then reduced to 45 °C, where the samples were kept for 72 h. The dry weight of AGB was determined using a laboratory-scale PCB Kern (KERN & Sohn GmbH, Balingen, Germany) and expressed as the dry matter (DM) of 1 plant. Then, the dried AGB was crushed and homogenized by the laboratory grinder Grindomix GM200 (Retsch GmbH, Haan, Germany). The nitrogen content in AGB of winter wheat was determined by the Kjeldahl method (Kjeltec 2300 device, Foss Analytical, Hillerød, Denmark).

The chlorophyll content in winter wheat was measured using a Yara N-tester chlorophyll meter (Yara International ASA, Oslo,

Table 1
The characteristic of soil at both experimental sites.

Locality/Analysis	Žabčice	Vatín
$N–NH_4$	1.9	1.4
$N–NO_3$	6.2	5.3
N_{min}	8.2	6.7
P (mg/kg)	122.6	78.0
K (mg/kg)	256.3	241.0
Ca (mg/kg)	3679.3	2539.3
Mg (mg/kg)	377.8	114.3
S (mg/kg)	13.3	7.2
pH ($CaCl_2$)	6.8	5.4
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
C_{ox} (%)	1.3	1.8

Table 2
The experimental treatments.

Treatment	Tillering (BBCH 22–25)		Stem elongation start (BBCH 30–32)			Heading (BBCH 49–51)	
	N (kg/ha)	Fertilizer	N (kg/ha)	S (kg/ha)	Fertilizer	N (kg/ha)	Fertilizer
Unfertilized	–	–	–	–	–	–	–
N technology	54	CAN	65	–	CAN	40	UAN
N + S technology	54	CAN	65	32.5	ASN	40	UAN
N + S + IN technology	54	CAN	105	52.5	ASN + IN	–	–

BBCH - Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie, CAN - calcium ammonium nitrate, UAN - urea ammonium nitrate, ASN - ammonium sulphate nitrate, IN - inhibitors of nitrification.

Norway) at the middle of the heading (BBCH 55). The chlorophyll content was expressed as the "N-tester value." Measurements were performed at a wavelength range of 650–940 nm from 30 random plants (measurements) in every treatment's repetition [50].

The samples for determination of mineral nitrogen (NO_3^-, NH_4^+) and sulphur content in the soil after the winter wheat harvest were collected using a hand soil probe from the soil profile 0–30 cm from two repetitions of every examined treatment. The determination of mineral nitrogen (N_{min}) was performed according to the certified methodology [48], which described that nitrate and ammonium nitrogen were extracted from the soils with a solution of neutral salt (1 % of K_2SO_4). The NH_4^+ determination was carried out spectrophotometrically (λ660 nm) using the Unicam 8625 UV/VIS (Pye Unicam Ltd., Cambridge, UK). The NO_3^- contents were determined by ISE (Ion Selective Electrode). Sulphur in Mehlich 3 soil extract was determined using ICP-OES Spectro Arcos SOP 21A (Spectro GmbH., Kleve, Germany) [48].

2.4. *Yield and grain quality measurements*

The weight of grain (kg) from the harvested area (the harvest area of each plot was 10 m^2) was determined using the digital scale Kern ECE 20K–2N (KERN and Sohn GmbH, Balingen, Germany). The wheat grain moisture content was measured (portable moisture meter Wile 78 Crusher, Farmcomp Oy, Finland), and the final wheat grain yield was standardized at a 12.0 % moisture content and expressed as tons per hectare (t/ha). The agronomic nitrogen efficiency [51,52] (ANE) was expressed for the fertilized treatments as the increase in grain yield (kg) per unit of nitrogen applied (kg) according to equation (1):

$$\text{ANE (kg of grain)} = \frac{\text{yield of treatment fertilized (t)} - \text{yield of treatment unfertilized (t)}}{\text{nitrogen dose applied by fertilizers (kg)}} \times 1000 \tag{1}$$

The test weight scale Wile 241 (Farmcomp OY, Tuusula, Finland) was used to determine the hectolitre weight. The protein content in the grain was determined by the Kjeltec 2300 device (Foss, Hillerød, Denmark) followed by the multiplication by a 6.25 coefficient (the Kjeldahl method). The gluten content was estimated by the NIR (Near Infrared Spectroscopy) method on the Inframatic 9500 NIR grain analyzer (Perten Instruments, Hägersten, Sweden). The principle of the NIR method is the transmittance or reflectance measurement of radiation within the wavelength range of 800–2500 nm (12.500–4000 cm^{-1}), which is related to the different chemical groups contained in the sample [53,54].

2.5. *Economic analysis*

Economic analysis following a partial budget [55] was performed for compared fertilization technologies. By its nature, this procedure considers only major differences between technologies (fertilization) and does not take all costs and benefits into account. Therefore, only the cost of applied fertilizers, the number of their applications and the price of winter wheat grain were considered. The cost of 1 tonne of used fertilizers was: CAN – 333 €; UAN – 358 €; ASN – 370 €; ASN + IN – 412 €. The price of fertilizers for compared technologies has been recalculated according to the corresponding applied doses (Table 2). The cost of one application (fertilization in a selected BBCH stage) was 12 €/ha. The price of winter wheat was 209 €/t. The prices were actual for the end of the year of 2023. However, the prices of input (fertilizers) and outputs (harvested commodities) usually fluctuate, making it difficult for economic analysis. Therefore, additional sensitivity analysis [56] 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) an increase cost in N fertilizers by 10 % but fixed wheat grain price, (2) an increase in wheat grain price by 10 % with fixed N fertilizers cost, and (3) an increase in N fertilizers cost by 10 % and a decrease in wheat grain price by 10 % (the worst-case scenario from farmers' perspective). The average yield of winter wheat from both experimental sites was used for the economic evaluation.

2.6. *Statistical analysis*

The collected data (N-tester value, grain yield and qualitative parameters) were evaluated by the Statistica Software 14 CZ by multifactorial analysis of variance (ANOVA with factors: experimental year, locality, treatment) and subsequently by the Fisher's 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 error (SE). The contribution of monitored factors (experimental year, treatments, and locality) was expressed for the winter wheat yield using the partial eta squared according to equation (2) and it is described In Supplementary Data [57]:

$$\text{ETA squared} = \frac{\text{SSeffect}}{\text{SStotal}} \times 100 \tag{2}$$

SSeffect - The sum of squares of an effect for one variable; SStotal - The total sum of squares in the ANOVA model.

3. Results

3.1. Plant and soil analysis

The average weight of aboveground biomass, the average content of nitrogen in the plant tissue collected before heading and N-tester values are described in Table 3. The statistical difference was observed only in N-tester values (unfertilized control compared to fertilized treatments). The highest N-tester value (723) was measured after the N + S treatment, which correlates with the highest content of N in plant tissues (3.15 %). The highest DM of AGB (3.84 g) and N uptake by plants (112.90 mg) were observed after the treatment N + S + IN. The average site-specific results of plant analysis are available in Supplementary Data (Tables S1 and S2).

The content of residual mineral nitrogen (N_{min}) in the soil was evaluated after the harvest of winter wheat (Table 4). Although the content of mineral nitrogen in the soil after the harvest was not statistically significant, it is evident from these results that the N + S + IN treatment has provided the highest amount of slowly mobile NH_4^+ form of nitrogen in comparison with other treatments. In comparison, the treatment based only on N application without S and without IN has provided the highest proportion of very mobile nitrate. The statistical difference was observed in the content of S, as it mainly correlates with the dose of S applied in the fertilizers. The average site-specific results of soil analysis are available in Supplementary Data (Tables S3 and S4).

3.2. The grain yield of winter wheat, agronomic nitrogen efficiency and economic evaluation

The average yields of winter wheat grain and their statistical significance obtained during the three-year field experiment established at two different sites are described in Fig. 2. The highest yield of grain was observed after the N + S + IN treatment (8.18 t/ha). Technology without IN resulted in grain yield of 7.67 t/ha (by 0.45 t/ha lower), fertilization strictly by nitrogen resulted in a grain yield of 7.61 t/ha (by 0.51 t/ha lower). The unfertilized control treatment has provided the lowest yield (6.43 t/ha). The separate site-specific average yields are presented in Supplementary Data (Figs. S1 and S2) available online. The average agronomic nitrogen efficiency expressed as ANE is described in Table 5. These results are in correlation with grain yield (although not statistically significant), as the highest ANE was determined after the N + S + IN treatment (11.05 kg). The ANE determined for the treatment N + S without IN (7.79 kg) was lower by 2.26 kg compared to the N + S + IN. The lowest ANE was determined by solely N fertilization (7.46 kg). The average site-specific ANE is described in Supplementary Data (Tables S5 and S6). The contribution of monitored factors to yield variation of winter wheat over the three-year field experiment at two experimental sites were as follows: experimental year 42 % ($p < 0.001$), experimental treatment 18 % ($p < 0.001$) and locality 7 % ($p < 0.001$) (Table S7).

Table 6 describes the results of the economic evaluation of winter wheat production according to the examined technologies of fertilization under different price scenarios, considering the variable price of fertilizers and harvested product. In every scenario, the technology with IN resulted in higher total revenues despite the increase in fertilizers cost (due to the higher price of fertilizer with IN) in comparison with the N technology. Therefore, the technology with IN resulted in higher net profit in every examined scenario (from 65 to 97 €/ha). The technology with N + S without the addition of IN was profitable only in one scenario.

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

3.3. The qualitative parameters of winter wheat grain

The results of the examined qualitative parameters of winter wheat grain are given in Figs. 3–5. The fertilization did not have a significant effect on the hectolitre weight of wheat grain (Fig. 3), as every treatment provided similar values (75.7–76.1 kg/hl). The

Table 3
Plant analysis at the beginning of heading stage of winter wheat and their statistical significance (Žabčice and Vatín, 2016–2018).

Treatment	DM of AGB (g/plant)	N content (%)	N uptake by plant (mg/plant)	N-tester value
Unfertilized	3.36[a]	2.68[a]	88.45[a]	597[a]
N technology	3.66[a]	2.89[a]	105.74[a]	708[b]
N + S technology	3.65[a]	3.15[a]	106.02[a]	731[b]
N + S + IN technology	3.84[a]	2.90[a]	112.90[a]	723[b]

The values are expressed as the arithmetic mean. The mean marked by different letters indicates significant differences $p < 0.05$ (Fisher's LSD test).

Table 4
Soil analysis after the harvest and their statistical significance (Žabčice and Vatín, 2016–2018).

Treatment	N–NH_4 (mg/kg)	N–NO_3 (mg/kg)	N_{min} (mg/kg)	S (mg/kg)
Unfertilized	3.3[a]	8.8[a]	12.1[a]	11.7[ab]
N technology	3.6[a]	17.3[a]	20.9[a]	10.6[a]
N + S technology	3.6[a]	9.2[a]	12.8[a]	14.7[ab]
N + S + IN technology	9.9[a]	12.3[a]	22.2[a]	18.0[b]

The values are expressed as the arithmetic mean. The mean marked by different letters indicates significant differences $p < 0.05$ (Fisher's LSD test).

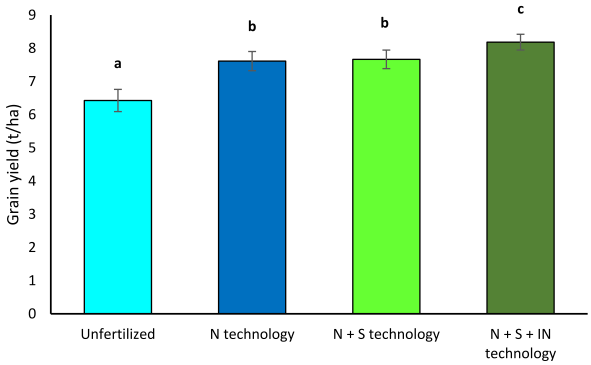

Fig. 2. The effect of examined treatments on the grain yield (t/ha) from Žabčice and Vatín (2016–2018). The same letters in the columns describe no statistically significant differences between treatments (LSD test; $p < 0.05$). Standard error (SE) is expressed by error bars.

Table 5
The agronomic N efficiency and their statistical significance (Žabčice and Vatín, 2016–2018).

Treatment	Average yield (t/ha)	Average yield difference (t/ha)[a]	Nitrogen dose (kg/ha)	ANE (kg)
N technology	7.61[a]	1.19[a]	159	7.46[a]
N + S technology	7.67[a]	1.24[a]		7.79[a]
N + S + IN technology	8.12[b]	1.76[a]		11.05[a]

[a] Average yield difference between fertilized treatments and unfertilized control; The values are expressed as the arithmetic mean. The mean marked by different letters indicates significant differences $p < 0.05$ (Fisher's LSD test). ANE – agronomic nitrogen efficiency.

statistical differences were observed between unfertilized and fertilized treatments in the average content of protein (Fig. 4a) in wheat grain protein production (Fig. 4b). Although the protein content was evenly balanced (12.7–13.1 %) between fertilized treatments, the production of protein expressed from average grain yield and average protein content was higher after the treatment with N + S + IN (1.05 t/ha) in comparison with N + S (1.01 t/ha) or N technology (0.98 t/ha), although not statistically. The statistical difference between gluten contents (Fig. 5) was again observed only between fertilized treatments and unfertilized control. Fertilized treatments were characteristic with similar content of gluten in grain (28.7–30 %). The separate site-specific average results of examined qualitative parameters are presented in Supplementary Data (Figs. S3–S10). The contribution of monitored factors to qualitative parameters of winter wheat over the three-year field experiment at two experimental sites are described in Tables S8–S11.

4. Discussion

4.1. Plant and soil analysis

The results of plant analyses and their statistical significance are described in Table 3. It is evident from these results that treatment without fertilization resulted in the lowest DM weight of AGB, which was lower by 10.8 % in comparison with commonly used N technology based on three split nitrogen applications, although the difference was not significant. The content of nitrogen in unfertilized plants was also the lowest. On the contrary, the average content of nitrogen in the plants fertilized with N and S was the highest, which supports the positive influence of S on N uptake and assimilation [58], even if the difference was not significant. However, it did not translate to the average DM weight of AGB, as it was identical to the treatment fertilized solely by nitrogen. The increase in DM weight by 5.2 % was observed after the treatment with N + S + IN compared to the N technology. The average content of N in leaves was admittedly lower (although not statistically) in comparison with N + S technology, probably as a result of higher production of AGB. The calculated nitrogen uptake by plant was almost similar between N and N + S technology, a non-significant increase was observed after the N + S + IN treatment, which may support the idea of higher utilization of inhibited nitrogen by plants, probably due to the lower risk of loss. The measurement of chlorophyl by Yara N-tester expressed as N-tester values was performed in the middle of

Table 6
Economic evaluation of average wheat yield according to the examined technologies (Žabčice and Vatín, 2016–2018).

Scenario	Treatment	Fertilizers cost			Increase in fertilizer cost * (€/ha)	Sales profit (wheat grain)			Revenue increase (€/ha)	Net profit (€/ha)
		Fertilizer price (€/ha)	Application cost (€/ha)	Total costs (€/ha)		Average yield (t/ha)	Purchase price (1 t of grain)	Total revenue (€/ha)		
2023	**N technology**	184	36	220	-	7.61	209	1593	–	–
	N + S technology	197	36	233	**13**	7.67	209	1605	**12**	**−1**
	N + S + IN technology	233	24	257	**37**	8.18	209	1712	**119**	**82**
1	**N technology**	202	36	238	-	7.61	209	1593	–	
	N + S technology	217	36	253	**15**	7.67	209	1605	**12**	**−3**
	N + S + IN technology	256	24	280	**42**	8.18	209	1712	**119**	**77**
2	**N technology**	184	36	220	-	7.61	230	1752	–	–
	N + S technology	197	36	233	**13**	7.67	230	1766	**14**	**1**
	N + S + IN technology	233	24	257	**37**	8.18	230	1883	**131**	**94**
3	**N technology**	202	36	238	-	7.61	188	1433	–	–
	N + S technology	217	36	253	**15**	7.67	188	1445	**11**	**−4**
	N + S + IN technology	256	24	280	**42**	8.18	188	1541	**107**	**65**

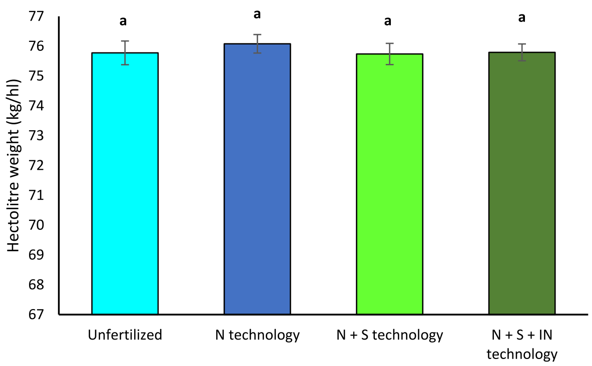

Fig. 3. The effect of examined treatments on hectolitre weight of grain (kg/hl) from Žabčice and Vatín (2016–2018). The same letters in the columns describe no statistically significant differences between treatments (LSD test; $p < 0.05$). Standard error (SE) is expressed by error bars.

the heading phase of winter wheat (Table 3). The results show that only the unfertilized treatment provided a significantly lower value of the N-tester compared to other treatments, which logically correlates with the lowest production of AGB and nitrogen content in leaves because of no nitrogen fertilization. The differences between fertilized treatments were not significant, as the N-tester values are primarily dependent on the fertilization and optimal supply of nitrogen. However, the highest N-tester value was recorded after the treatment with N + S without the IN, which logically suggests a higher available content or faster availability of applied nitrogen compared to the treatment enhanced with IN. On the contrary, the treatment N + S + IN resulted in lower (although not statistically) values of N-tester, probably because of nitrogen inhibition by IN, which is confirmed by the content of nitrogen in the soil after the harvest in Table 4.

The results of soil analyses and their statistical significance are described in Table 4. It is necessary to point out that the transformation of nitrogen and the effect of inhibitors are strongly influenced by environmental factors such as temperature, precipitation, or soil moisture [59–61]. The technology based on three split applications of nitrogen resulted in 20.9 mg/kg of N_{min} determined in the soil. A large part of this content consisted of a very mobile nitrate form of nitrogen with a high possibility of loss through leaching. On the contrary, a comparable amount of N_{min} was determined after the treatment with N + S + IN (22.2 mg/kg), however, the proportion of ammonia form of nitrogen in the soil was almost three times higher in comparison with the mentioned nitrogen technology. This is a result of nitrification inhibitors [62], which slow down the transformation process of ammonia to nitrate in soils. The ammonia form of nitrogen in the soil is more stable and can be fixed by soil particles and soil microbes, which presents a more optimal utilization of this

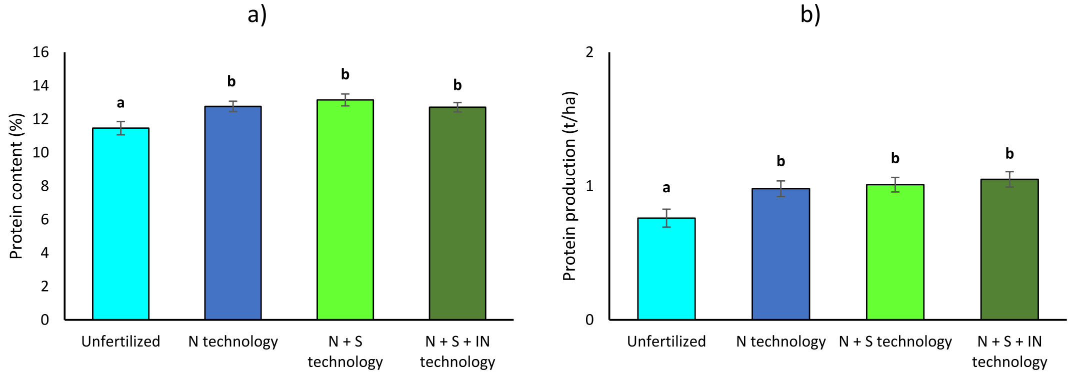

Fig. 4. The effect of examined treatments on the protein content (%) (a) and protein production (t/ha) (b) from Žabčice and Vatín (2016–2018). The same letters in the columns describe no statistically significant differences between treatments (LSD test; $p < 0.05$). Standard error (SE) is expressed by error bars.

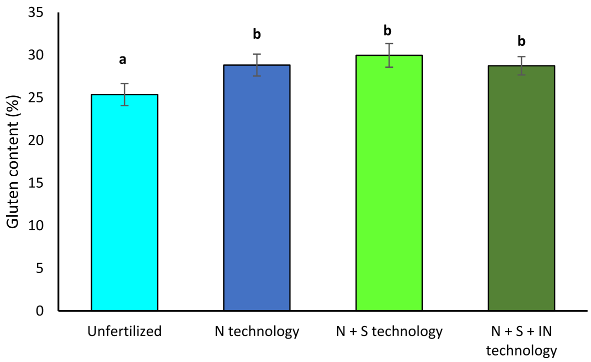

Fig. 5. The effect of examined treatments on the gluten content (%) from Žabčice and Vatín (2016–2018). The same letters in the columns describe no statistically significant differences between treatments (LSD test; $p < 0.05$). Standard error (SE) is expressed by error bars.

mineral nitrogen for the next crop (intercrop) or for the possible wheat straw mineralization, if it is left out on the field and incorporated to the soil. According to some authors [63,64], the IN can delay the nitrification of ammonium by more than 42 days. Interestingly, the content of N_{min} after the treatment with N + S technology was comparable to the unfertilized treatment, therefore lower than N and N + S + IN technology. This could be possibly explained by the well-known synergy between nitrogen and sulphur. Another possible explanation could be the higher proportion of ammonia nitrogen presented in ASN fertilizer in comparison with the common N technology (CAN). It has been suggested that nitrification may be inhibited by ammonium sulphate itself [65,66]. The suitable amount of sulphur is a good prerequisite for optimal nitrogen assimilation. This theory is supported by the plant analysis during the vegetation, as the highest N content, N uptake and N-tester values were observed in plants after both treatments with sulphur. The dose of sulphur applied in the N + S + IN technology was higher compared to the mentioned N + S technology, the higher content of mineral nitrogen in the soil determined after harvest on this treatment is therefore primarily a result of IN. The content of sulphur in the soil after the harvest is in correlation with application doses of S, as the unfertilized and N treatments were not fertilized with sulphur, and the content of S is, therefore, lower after these treatments. The content of S in the soil determined after the technology with N + S + IN was 1.2x higher in the comparison with N + S, while the applied dose of S was similarly higher by 1.6x.

4.2. The grain yield of winter wheat, agronomic nitrogen efficiency and economic evaluation

The average grain yield of winter wheat and their statistical significance are described in Fig. 2. It is evident, that nitrogen fertilization is essential for crop yield, as the unfertilized treatment resulted in statistically the lowest yield compared to the classic nitrogen technology by 15.6 % (1.18 t/ha). The commonly used technology based on three split applications of solely mineral nitrogen resulted in the average yield of 7.61 t/ha. The enhancement of this technology with sulphur applied at the stem elongation increased the grain yield of winter wheat by 0.7 % (7.67 t/ha). The effect of sulphur applied with nitrogen during the vegetation was therefore insignificant. This correlates with other published results [67–71], even if the increasing yield after the N + S application is often discussed. The non-significant result of co-application of N and S can be possibly explained by the relatively low S requirement of wheat and its late application in the BBCH 30–32. The recommendation for sulphur fertilization is usually before crop sowing, at the start of the vegetation, or in split doses during the whole vegetation [72–77]. Some authors have also reported that sulphur fertilization is only beneficial to a certain limit [39,78,79]. Several authors [80,81] have also found out an overall low sulphur use efficiency (SUE) not exceeding 10 %, although the world SUE for cereals was estimated up to 18 % [79]. 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 [82,83]. This idea can be supported by the low contents of sulphur (Table 4) estimated in the soil after the harvest in our experiment [84]. Another possible explanation of the insignificant effect of S fertilization was described by Dhillon et al. [85], 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. [86]. The S application at a higher dose has also decreased the crop yield in some cases [87]. The statistically highest yield of grain (8.18 t/ha) in our experiment was observed after the fertilization with N + S + IN treatment. The addition of IN resulted in an increase in grain yield by 7.5 % in comparison with common N technology and by 6.8 % compared to the N + S without the inhibitor. This represents an increase of 0.57 t/ha, respectively 0.51 t/ha, and it can be explained mostly by a more gradual release of nitrogen due to the IN. Thus, the gradual nitrification of ammonium nitrogen probably released nitrate nitrogen with respect to the yield-building needs of wheat. Another benefit of this treatment with IN is the reduction of N loss (Table 4) and the possibility of reducing the number of applications (Table 2). It was possible to combine the two-split application (at stem elongation and heading) of nitrogen in the commonly used technology to just one fertilization with a higher dose, which may represent a more economical (time saved, fuel and application cost saved) and ecological (soil compaction, nitrogen loss, legislative, higher NUE) option [88]. As it is evident from the results, such a combination resulted in a higher yield of winter wheat grain, the highest weight of AGB and the highest residual N after the harvest. The positive effect of IN application on the winter wheat yield is also described by several authors [89–93]. These increases in grain yield after IN application highlight the fact that application of the IN, which increases the proportion of mineral N in the NH_4^+ form, provided an equal opportunity to take up applied N as NH_4^+ form. NH_4^+ may be incorporated into organic compounds and finally into plant protein at less energy cost compared to the nitrate form of nitrogen, suggesting that the plant may be left with extra energy to allocate to growth and crop yields [94,95]. On the contrary, several studies [96–98] have also reported that the IN did not statistically influence the grain yield, although a trend in increased yields was observed. Similar results are described also by Wang et al. [99], as they refer to the good potential of inhibitors to increase the crop yield while reducing the emission of N_2O. It is necessary to mention, that the dose of applied sulphur at this treatment was higher in comparison with N + S technology treatment thanks to the single application of ASN. However, it is safe to assume, that the mentioned positive effects of N + S + IN technology was primarily caused by the IN, as the same treatment without the IN (although with a lower dose of S) had no statistical effect on the grain yield.

Another reason for this assumption is the agronomic nitrogen efficiency expressed as ANE (Table 5), which represents the increase in grain yield (in kg) caused by 1 kg N applied in fertilizers. It is evident from the results that the application of fertilizer with IN resulted in the highest utilization of nitrogen by wheat compared to the same total dose of nitrogen applied in classic nitrogen technology with three split nitrogen fertilization. A similar finding is also presented by Dawar et al. [91], where the utilization of IN in combination with Urea also increased the ANE, or by Shalmani et al. [89], 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 each treatment. For the N technology, the CAN fertilizer (balanced ammonium and nitrate content) was used for the first and second fertilization. The technology with IN was based on the fertilizer ASN, where a major part of nitrogen is presented in ammonium form. The IN are responsible for the slower nitrogen transformation from ammonia to nitrate [23–25]. Therefore, the plants fertilized with ASN, especially enhanced with nitrification inhibitors, probably had lower energy requirements for the assimilation of nitrogen into plants' protein. The NUE determined for the treatment N + S was comparable with N technology, which again signalizes the insignificant effect of sulphur fertilization in ASN in comparison with the enhancement of the same fertilizer by IN.

The positive effect of N + S + IN technology on the agronomic nitrogen efficiency and, therefore, grain yield, is also supported by economic evaluation following a partial budget method. It is evident from the presented results (Table 6) that the technology with the inhibitor addition resulted in the highest profit in every examined scenario when compared to the N technology and even N + S technology. The technology with N + S + IN is, however, characterized by the highest purchase price of fertilizers (addition of IN to the ASN), but the possibility of only two applications and especially the positive effect of IN on the grain resulted in the highest total revenues, therefore profit. The N + S technology (without IN) is characterized also by a higher cost of fertilizers (ASN compared to CAN), however, the effect of S addition to the grain was insignificant compared to the N technology, therefore, this technology was mostly unprofitable.

4.3. The qualitative parameters of winter wheat grain

The effects of the examined treatments on the qualitative parameters of winter wheat grain were tested every experimental year after the harvest, as they are important for the monetization of grain and for subsequent use. Besides fertilization, the most important factors with an effect on the qualitative parameters are crop variety and environmental conditions during the vegetation [100]. The hectolitre weight (HW), protein and gluten content were evaluated. The optimal requirement of HW of winter wheat grain used in food processing is according to the standard of the Commission Regulation (EC) No. 824/2000 76 kg/hl. It is evident from our result (**Fig. 3**), that only the treatment with solely nitrogen fertilization met this requirement (76.1 kg/hl). There are two possible explanations. Firstly, this treatment has provided the lowest grain yield in comparison with other fertilized treatments. The younger the plants are, the higher the nutrient concentrations of nutrients are. The more the plants grow and produce biomass, the lower the concentration of nutrients. Therefore, the lower values, although statistically insignificant, of HW determined after the fertilization with N + S (75.7 kg/hl) and N + S + IN (75.8 kg/hl) could be simply linked with the dilution effect [101]. For example [102], have also reported an increase in grain yield followed by a decrease in HW of grain after the wheat fertilization with sulphur. On the contrary [103], observed an increase in the hectolitre weight of wheat grain after the inhibitor application compared to the common nitrogen fertilizer. Even if the value of HW after the addition of IN was in our work relatively lower compared to the commonly used technology with three split applications of nitrogen, it is necessary to point out that the difference was insignificant, and it was a result of only two applications thanks to the inhibitor of nitrification. This is a welcome result, as it is evident from three-year averages, that the application of a higher dose of N and omitting one application is viable due to the gradual transformation and release of nitrogen. The second reason for the threshold values of HW around 76 kg/ha is the drought period in the critical time of vegetation in the first experimental year.

The minimal content of protein is 11.5 % (Commission Regulation EC No. 824/2000), although for higher-quality products or higher monetization of wheat grain, a demand for 12.5 % is the most usual in common praxis. The content of protein (%) determined in winter wheat grain is described in Fig. 4a. The unfertilized treatment provided the lowest, statistically significant content of protein 11.5 %, thus it would meet the criteria set by that standard. The differences between fertilized treatments were insignificant, although the treatment with nitrogen technology enhanced with sulphur (13.1 %) provided an increase of 3 % (relative %) in comparison with nitrogen fertilization without S (12.8 %). The positive effect of sulphur fertilization on the protein content was reported by Shahsavani and Gholami [104] or further by Klikocka et al. [105], as they also in addition to increased protein content observed a higher content of cysteine and methionine. On the contrary, the insignificant effect of S fertilization on the protein content was described by several authors [106–109]. The effect of N + S + IN (12.7 %) was comparable to the N treatment (12.8 %). This can be possibly explained by the missing nitrogen fertilization in the heading stage of winter wheat. The main objective of this relatively late fertilization is usually the enhancement of qualitative parameters [110,111]. Foliar application of liquid nitrogen fertilizer (for example UAN) is effective and has the benefit of increasing protein content in grain. On the contrary, the application of these fertilizers may present a possible risk due to the leaf burning and possibly yield reduction [112]. Due to the IN, the N fertilizer was applied earlier at the stem elongation in the higher dose. It is possible, that even if the nitrogen was supplied to the plants more gradually, which positively influenced the grain yield (**Fig. 2**), the amount of nitrogen was not sufficient to enhance the qualitative parameters even more positively. Very similar trends in qualitative parameters values of winter wheat grain after the fertilization with ASN or ASN with IN are described by Školníková et al. [113]. Several studies [63,93,98,114] have also described, that the IN application had no statistical effect on the quality of grain. During the four-year experiment, Huérfano et al. [115]) observed the effect of IN on wheat grain quality. It is evident from their results, that the IN did not influence the examined parameters, even if the number of fertilizations was the same between IN and IN-free treatments. Therefore, as mentioned by Thapa and Chatterjee [116], the important effect of inhibitors is their ability to reduce all possible loss of nitrogen, while simultaneously maintaining crop yield and quality. It was also stated by De Santis et al. [117], that the utilization of slow-release fertilizers can be recommended for biscuit making (better qualitative requirements) without compromising the grain yield. The production of protein (t/ha) was subsequently expressed from the grain yield of winter wheat (t/ha) and the content of protein (%) obtained from every repetition through experimental years. It is evident from Fig. 4b, that the treatment with N + S + IN has provided the highest production of protein (1.05 t/ha). It was an increase of 7.1 % in comparison with N technology and of 4 % compared to the N + S technology without IN. Although the differences were again statistically insignificant, the technology with IN appears to be most suitable in terms of production, especially due to the statistically highest yield, which in conclusion makes up for the relatively lower content of protein.

The gluten content (Fig. 5) is an important indicator of baking quality, as it directly influences the properties of dough and the quality of bakery products. Even so, it is not standardly used in the EU as a technological criterion for the winter wheat in food production. The minimal requirement is 23–24 % [118], for better monetization of wheat even higher (26–28 %). The content of gluten is directly influenced by nitrogen fertilization [119], similar to the content of protein, however, there is no correlation between the content and quality of gluten. The quality of gluten, especially the visco-elastic properties, can be for example positively influenced by sulphur fertilization [107,120,121]. In conclusion, sometimes the lower content of gluten may exhibit a higher quality. Both fertilized treatments without the IN have provided relatively higher content (28.8 % and 30.0 %) of gluten in comparison with the N + S + IN treatment (28.7 %), which is consistent with the studies [122,123] describing the positive effect of split application of nitrogen on the gluten content. The differences were again statistically insignificant. It can be concluded that the fertilization with sulphur in ASN fertilizer later in the vegetation did not overall result in increased yield or qualitative parameter in comparison with technology based only on N fertilizers. Although the same treatment with ASN enhanced with IN did also not significantly increase the parameters of quality, its result is comparable even with one less fertilization performed. In addition, the IN factor significantly increased the grain yield (Fig. 2).

5. Conclusion

The fertilizer with inhibitors provides a modern alternative to supply the nitrogen to the plants more gradually during the vegetation. The three-year experiment established at two experimental sites has proved that the application of ammonium sulphate nitrate fertilizers with IN in higher doses is a viable option, as it significantly increased the grain yield of winter wheat by 7.5 % in comparison with the nitrogen treatment without inhibitor applied three times during the vegetation. This increase also resulted in higher net profit, despite the higher cost of fertilizer in this technology. The benefit of IN application is also in the more gradual release of nitrogen to the soil environment and fertilization by higher doses of nitrogen combined in single or two applications. This presents an opportunity to decrease the amount of field crossing, which can positively influence the soil compaction while reducing the cost of fuel and fertilization. The qualitative parameters after the treatment with IN were comparable to the treatments without IN. This result can be considered positively if we take the omitted qualitative fertilization by nitrogen due to the IN fertilizer applied earlier in the vegetation in a higher dose into consideration. The treatment based on the split application of nitrogen with ammonium sulphate nitrate without the IN included in the experiment as a verification of sulphur factor did not have any significant effect on the yield nor the quality of winter wheat, possibly due to the late application of sulphur during the vegetation.

The possible limitations of the presented study can be found in the soil conditions and course of the weather at experimental sites. Although the fertilizer with IN proved to be a suitable alternative to the conventional nitrogen fertilization, a possible limitation in conventional farming is more difficult marketing associated with the often-conservative approach to the agriculture.

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Data availability

Data available on request from the authors.

CRediT authorship contribution statement

Jiří Antošovský: Writing – review & editing, Writing – original draft, Visualization, Investigation, Conceptualization. **Petr Škarpa:** Writing – review & editing, Visualization, Investigation. **Pavel Ryant:** Writing – review & editing, Project administration, Methodology, Conceptualization.

Declaration of competing interest

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. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33035.

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