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Partial Factor Productivity as a Tool to Enhance Wheat Seed Quality and Yield Through Nitrogen Fertilization Management

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Abstract

Even though wheat's response to nitrogen (N) is well studied, practical optimization remains challenging because yield and seed quality often react inconsistently across seasons. For winter wheat, the simultaneous quantification of efficiency indicators that capture N losses and diminishing returns is important. This study evaluated nitrogen (N) fertilization in two growing seasons. This study aimed to adjust N fertilization strategy through different combinations of granular N timing and foliar applications to improve winter wheat yield and technological seed quality while maintaining high fertilization efficiency. Field experiments were conducted over two growing seasons (2021/2022 and 2022/2023) using seven fertilization treatments (Control, TSE_1, TSE_2, TSEH_1, TSEH_2, TSEH_3, and TSH, which correspond to growth stage T—tillering stage; SE—stem elongation phase; H—heading stage) in the range of 140.5 to 194.5 kg ha^{-1} N. Seed yield and seed quality traits (moisture, hectoliter weight, starch, protein, gluten, and sedimentation value) were measured, and treatment effects were evaluated with ANOVA, correlation, and regression analyses. In 2021/2022, no significant treatment effects were detected for yield or seed quality parameters, indicating that environmental variability dominated crop response. In contrast, in 2022/2023, seed yield, hectoliter weight, gluten content, and starch yield showed significant treatment effects ($p \leq 0.05$–0.01), with fertilized variants generally outperforming the Control. Across both seasons, seed quality traits displayed strong internal structure: protein, gluten, and sedimentation were strongly positively correlated, while starch was strongly negatively correlated with these traits and the yield correlated positively with hectoliter weight but negatively with protein and gluten, highlighting a yield–quality trade-off. Importantly, partial factor productivity (*PFP*) and nitrogen use efficiency (*NUE*) showed the strongest treatment sensitivity, demonstrating their value for identifying efficient N strategies even when yield and quality responses were season-dependent. Regression analyses confirmed that seasonal conditions modulated nitrogen responsiveness, with *NUE* and starch yield showing much stronger relationships with nitrogen input in 2021/2022 and 2022/2023, respectively.

Keywords: calcium ammonium nitrate; urea; growth phase; foliar application; protein; gluten; nitrogen use efficiency; environment protein; starch

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1. Introduction

Among many cultivated plant species, winter wheat has irreplaceable importance and role as a food, feed, and raw material. In comparison with all worldwide cereal

harvested area in the last five years, wheat occupies 1/3 or 218,007,683 ha [1]. Due to its wide distribution area and pronounced polymorphism, it is cultivated on all continents and in different agro-ecological conditions, but the common feature of cultivation is mineral fertilization. Considering that it has an extremely important influence on yield and quality, producers around the world pay special attention to it. Although wheat needs many macro- and microelements [2–5] in practice, only three basic elements are added, which are nitrogen (N), phosphorus (P), and potassium (K), while the others are added in case of extremely unfavorable soil fertility. N has the strongest effect on seed yield and quality. In the context of seed development, N management influences the allocation of this crucial nutrient to storage compounds within the seed, which, in turn, significantly affects the seed's functional properties, like viability, vigor, and storability. According to Cummane et al. [6], nitrogen availability impacts the synthesis of key molecules, particularly storage proteins and other nitrogenous compounds essential for successful germination and stand establishment. The other two elements are more important in the contexts of physiology, morphology and genotype [7–10].

Based on agronomic recommendations for Croatia, the typical total nitrogen rate for winter wheat is 140–200 kg N/ha, usually applied in 2–4 split applications (basal + one, two, or three top-dressings). In practice, Croatian growers commonly use one main (basal) application and many supplementary N applications, giving a total in the upper recommended range [11]. However, due to the drastic price increase, especially for nitrogen fertilizers, producers are combining different quantities and compositions in order to maintain high productivity and reduce investment costs [12,13]. Furthermore, since N is an unstable element and has negative pressure on the air, soil, and groundwater, the correct application in the right amounts and at the right time is extremely important [14,15].

In Croatia, the most common nitrogen fertilizers used are urea and calcium ammonium nitrate (CAN) [16,17]. Urea is a highly concentrated fertilizer with 46% nitrogen in the amide form, which must be transformed into a form accessible to wheat. Also, its advantage is prolonged action, and its disadvantage is a high degree of acidity. CAN contains nitrogen (27%) in two forms, ammonium and nitrate in equal quantities, and is immediately available to the wheat. It contains small amounts of CaO and MgO; it is suitable for use on acidic soil and in colder climates but does not have a prolonged effect.

However, in addition to all characteristics, the application and effect of nitrogen fertilizers are closely related to weather conditions (e.g., amount of precipitation), soil properties, and the phenological phase of the plant and its needs at a certain stage [18–21]. In winter wheat growth, the exact timing of stem tillering depends on the region, soil temperature, weather conditions, and cultivar, but the typical phenological pattern for winter wheat in Croatia is well known. The tillering mostly occurs from late winter to early spring, typically in late February to March. At this time, daytime temperatures rise above ~5–7 °C, which allows the plant to resume active growth after winter dormancy. This timing corresponds to the period when Croatian growers normally apply the first nitrogen top-dressing. Stem elongation usually begins in early to mid-April, depending on weather conditions in a given year. This matches the period when the second nitrogen application is typically made (if used) and when temperatures commonly rise to around 10 °C, enabling rapid vegetative growth and internode extension.

The wheat has two basic roles. Its seed represents an important source of food and/or raw material for processing and an irreplaceable seed material. In both cases, the structure and chemical composition of the seed/seed must be at a high level. The main indicators of wheat seed quality are quantity reserves that support germination and early seedling growth [22,23]. First, seed protein status is commonly associated with seedling vigor [24] and parental nitrogen supply and can influence both total seed protein and subsequent

seedling establishment; however, changes in gluten protein composition (e.g., gliadin and glutenin balance) should be viewed as an indirect indicator of nitrogen partitioning and storage-protein allocation rather than a direct predictor of viability, unless germination and vigor tests are performed [25]. Second, starch represents the major energy reserve in wheat seeds and is actively mobilized during germination, and therefore, differences in starch quality can be linked to germination energy through the efficiency of starch degradation and carbohydrate supply to the embryo. Hu et al. [26] reported that the starch structure subsequently influences the processing property of wheat. According to Faměra et al. [27], higher starch contents in wheat were observed in crops cultivated in cooler, higher-altitude regions. According to Wen et al. [28], an optimal or elevated nitrogen fertilization level affects seed protein and starch content and high vigor seed production due to increased regulation of pathways included in nutrient mobilization (starch, sugar, nitrogen and linoleic acid metabolism) and the antioxidant system (tricin synthase and glutathione metabolism).

The seed quality parameter hectoliter weight (test weight) reflects kernel density and physical soundness and is widely used as a practical indicator of physical seed quality [29]. As a seed quality proxy, it is relevant for storability and handling because denser, well-filled kernels are generally less prone to mechanical damage and perform better during processing and storage, although storage outcomes remain strongly dependent on seed moisture and storage conditions.

Drought and heat during the late reproductive (post-anthesis) period are increasingly common in winter wheat and can severely depress photosynthesis, seed filling, and overall productivity, yet the extent to which nitrogen management can buffer these stresses is still not fully resolved [30–34]. In a two-season field experiment, Ru et al. [35] reported that drought–heat stress strongly reduced gas exchange, seed-filling rate, and nitrogen accumulation, but moderate or reduced N supply mitigated these penalties, with medium N giving the highest net photosynthesis and instantaneous water use efficiency (WUE). Notably, under combined stress, lower N increased yield, WUE-biomass, and biomass compared with high N, and multi-criteria evaluation ranked reduced N as the best overall strategy for maintaining performance under post-anthesis stress.

Nutrient use efficiency (*NUE*) is a critical indicator in crop production, particularly for wheat, as it reflects how effectively plants utilize applied nutrients to produce yield [36]. Nitrogen use efficiency in wheat is influenced by complex genetic and environmental interactions, as nitrogen is taken up and redistributed throughout the plant's entire life cycle [37]. To improve *NUE* in future cultivars, breeders must evaluate diverse germplasm under both high- and low-nitrogen conditions, as seed yield alone does not reliably indicate true *NUE* potential. High *NUE* not only enhances seed yield and quality but also reduces the need for excessive fertilizer application and lowers production costs. Moreover, efficient nutrient use mitigates environmental impacts, such as nutrient leaching, water pollution, and greenhouse gas emissions. Therefore, improving *NUE* is essential for sustainable and economically viable wheat production, and it provides a practical framework for optimizing fertilization strategies, including the evaluation of partial factor productivity (*PFP*). The *PFP* is an indicator used to assess the efficiency of nutrient use in crop production. It is calculated as the ratio of crop yield (e.g., seed yield) to the amount of applied fertilizer (or a specific nutrient), reflecting how effectively the applied input contributes to production. Higher *PFP* values indicate more efficient use of fertilizers, helping optimize both yield and input costs. *PFP* and *NUE* are not traditional yield or seed quality traits for assessing seed quality; rather, they provide complementary and often more decision-relevant information because they quantify how efficiently nitrogen inputs are converted into output. Unlike traditional yield parameters, which represent an aggregated outcome influenced by multiple environmental

and management factors, *PFP* and *NUE* are more sensitive to nitrogen availability and uptake dynamics.

This sensitivity is particularly relevant for seed quality research, as nitrogen use efficiency directly affects the accumulation and partitioning of storage proteins and carbohydrates that determine seed physiological performance. This manuscript has been revised to explicitly clarify that *PFP* and *NUE* provide a more mechanistic link between nitrogen management and seed quality outcomes. Nitrogen fertilization management (form and split timing during reproductive development) alters wheat seed biochemical composition—specifically the protein–starch balance and gluten-related traits—and these compositional shifts predict seed performance, such that seeds with higher absolute protein reserves show improved vigor and storability without compromising viability. Thus, this study aimed to examine the common and modified practice of nitrogen application in granulated and foliar application for winter wheat and its influence on the seed yield and quality during two vegetation seasons.

2. Materials and Methods

2.1. Field Trial

The field experiments were conducted at the following geographic coordinates: 45.536000 N, 18.672479 E for the 2021–2022 season and 45.529455 N, 18.676740 E for the 2022–2023 season. These coordinates correspond to a site in eastern Croatia, within the Slavonian region. Precrop in both seasons was soybean.

Wheat variety Kraljica (originating from the Agricultural Institute Osijek, Osijek, Croatia) was sown at the seeding rate of 700 seeds per m^2. Sowing was done on 21 October 2021 and 14 October 2022 with a sowing machine, Wintersteiger Tool Carrier 2700 (Ried im Innkreis, Austria). The experiment was set up as a randomized block design, and the basic plot was 10 m^2.

Soil sampling was carried out on a homogeneous field area, with 10 subsamples collected per hectare. Subsamples were taken using a non-systematic sampling approach following a Z-shaped pattern, which is commonly considered a simple and reliable method for agricultural fields of this size. Individual subsamples were evenly distributed along the imaginary Z transect while avoiding field edges, roads, and drainage canals.

Sampling was performed under optimal soil conditions after crop harvest and before fertilizer application for the subsequent production cycle. Subsamples were collected from the 0–30 cm soil layer and, after thorough mixing, combined into one composite sample (1.5 kg) representing the average chemical and physical properties of the soil across the entire area.

The composite sample was properly prepared, labeled, and delivered to an accredited laboratory for analysis. The analytical results served as the basis for fertilization recommendations and further planning of agricultural production.

Based on the soil analysis, the characteristics in both years were quite similar. In the 2021/2022 growing season, the soil was slightly acidic, and in the 2022/2023 growing season, pH was near neutral (Table 1). Further, both soils had a low content of humus, were poorly supplied with organic matter, and were well supplied with phosphorus and potassium.

Table 1. Soil analysis during two tested years.

Parameter	2021/2022	2022/2023	Unit/Note
Humus	1.65	1.80	%
pH (H_2O)	6.27	6.70	
pH (KCl)	5.2	5.7	%
Phosphorus (P_2O_5)	28.7	29.6	mg/100 g
Potassium (K_2O)	38.4	33.6	mg/100 g

2.2. Fertilization Treatment

In the 2021/2022 season, basal fertilization was carried out by incorporating 500 kg ha^{-1} of NPK 7:20:30 and 100 kg ha^{-1} of urea into the soil prior to sowing. Additionally, a pre-sowing application of 70 kg ha^{-1} of CAN (calcium ammonium nitrate) was applied. In 2022/2023, basal fertilization consisted of incorporating 500 kg ha^{-1} of NPK 7:20:30 together with 100 kg ha^{-1} of urea prior to sowing. In addition, a pre-sowing application of 71 kg ha^{-1} of CAN was applied. For top-dressing, fertilizer (urea or CAN) was applied at a rate of 100 kg ha^{-1}, dissolved in 1000 L of water per hectare, corresponding to an application volume of 1 L of solution per 10 m^2. Thus, in each season, 100 kg/ha N was added as basal fertilization.

During the wheat vegetation season, different treatments were applied as top-dressing according to wheat developmental stages, with applications at tillering, stem elongation, and heading (Table 2).

Table 2. Fertilizer application timing in relation to wheat growth stages and BBCH.

Developmental Stage	BBCH Stage	Application Date 2022	Application Date 2023
Tillering	BBCH 21–25	3 February 2022	9 February 2023
Stem elongation	BBCH 30–32	12 Aprile 2022	11 Aprile 2023
Heading	BBCH 51–59	20 May 2022	22 May 2023

BBCH—Federal Biological Institute, Federal Plant Variety Office, and Chemical Industry (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie).

The experiment was conducted to evaluate the effects of different nitrogen fertilization strategies on wheat. The following fertilization top-dressing treatments were applied as it is described in Table 3. All treatments were arranged in a randomized complete block design with four replicates per treatment. Treatment codes were assigned according to the timing of fertilizer application and the type of fertilizer used: Control, TSE_1, TSE_2, TSEH_1, TSEH_2, TSEH_3, and TSH. Here, T denotes the tillering stage, SE denotes the stem elongation phase, and H denotes the heading stage. Fertilization was applied at three growth stages of wheat because, in winter wheat production, in the tillering stage, it is important to add fertilizer to stimulate the development of tillers, increasing the potential number of spikes. Another important stage for winter wheat top-dressing is the stem elongation phase to support stem and spike growth, ensuring proper formation of seeds per spike. The last top-dressing in the heading stage is often not performed in practice, but it is important to enhance seed filling and final yield by providing sufficient nutrients during the critical reproductive phase. Fertilization at the heading stage (H) is generally not performed in practice. The detailed amounts of fertilizers applied in each treatment are provided in Tables 3 and 4.

Table 3. Winter wheat top-dressing fertilization treatments in 2021–2022 and 2022–2023 vegetation.

Treatment No.	Top-Dressing Tillering Stage	Stem Elongation	Heading
Control	150 kg/ha granulated CAN	–	–
TSE_1		150 kg/ha granulated CAN	–
TSE_2		100 kg/ha foliar UREA	–
TSEH_1		100 kg/ha granulated CAN +	100 kg/ha granulated CAN
TSEH_2		100 kg/ha granulated CAN	100 kg/ha foliar CAN
TSEH_3		100 kg/ha foliar CAN	100 kg/ha foliar CAN
TSH		–	100 kg/ha foliar UREA

T—tillering stage; SE—stem elongation phase; H—heading stage; CAN—calcium ammonium nitrate.

Table 4. Total amount of N applied.

Treatment	N from CAN (kg N/ha)	N from Urea (kg N/ha)	Total Top-Dressing N (kg N/ha)	Total N Basal (100) + Top-Dressing (kg N/ha)
Control	40.5	–	40.5	140.5
TSE_1	81.0	–	81.0	181.0
TSE_2	40.5	46.0	86.5	186.5
TSEH_1	94.5	–	94.5	194.5
TSEH_2	94.5	–	94.5	194.5
TSEH_3	94.5	–	94.5	194.5
TSH	40.5	46.0	86.5	186.5

T—tillering stage; SE—stem elongation phase; H—heading stage.

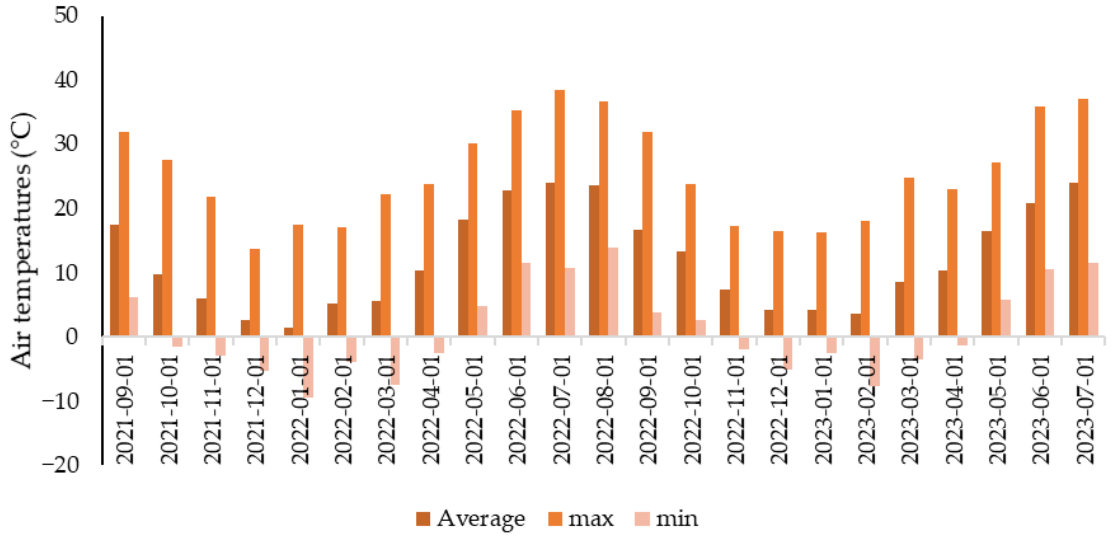
Nitrogen was applied in different growth stages depending on the treatment, as shown in Table 4. All treatments received 100 kg N ha^{-1} as basal fertilization, incorporated into the soil before sowing, to ensure early growth and establishment. Additional nitrogen was supplied through top-dressings applied during specific phenological stages, tillering, stem elongation, and heading, using either calcium ammonium nitrate (CAN, 27% N), urea (46% N) or their combination.

Applications were carried out in the morning (between 8:00 and 11:00 a.m.) to minimize nitrogen losses and maximize uptake, particularly for foliar fertilizers. This design allowed us to evaluate the effectiveness of commonly used fertilizers and formulations as well as potential differences between granular and foliar applications and the timing of the nitrogen supply. The fertilizers were applied according to standard agricultural practice, in which the solid fertilizer was completely dissolved in water prior to application to ensure uniform distribution and effective foliar uptake by plants.

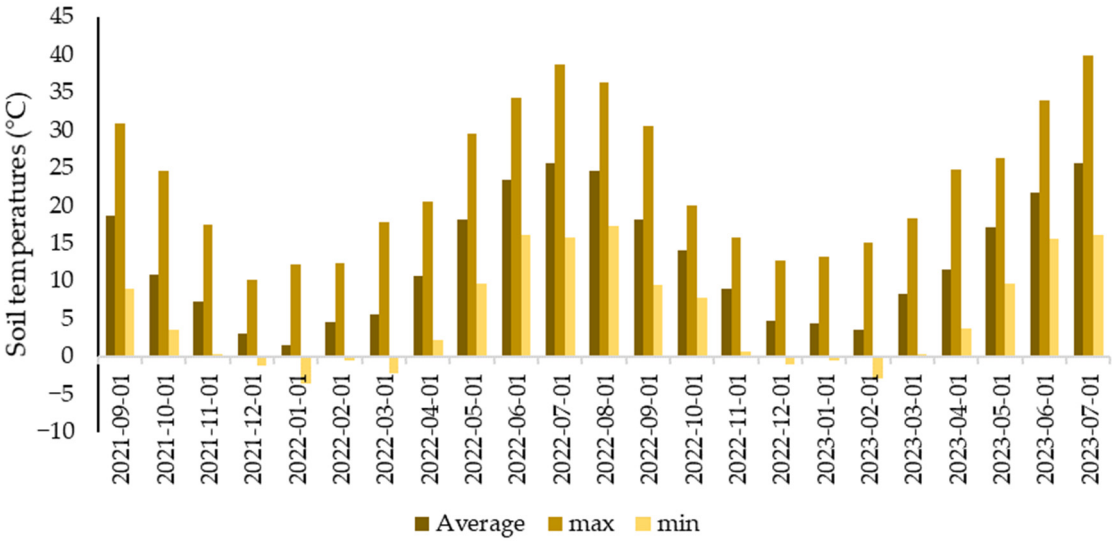
2.3. Weather Characteristic

Climatic data for the experimental period were obtained from the FieldClimate platform [38] by Pessl Instruments, based on measurements recorded by the local agro-meteorological station. Meteorological data were obtained from an automatic weather station located at the Centre for Seed and Seedling Production (Osijek, Croatia). During the 2021/2022 growing season, the weather station was 950 m from the experimental site, whereas during the 2022/2023 season, the distance was 450 m.

Figure 1a illustrates the monthly average, maximum, and minimum air temperatures recorded from September 2021 to July 2023. The temperature patterns follow the expected seasonal dynamics for a temperate continental climate. Minimum temperatures frequently dropped below 0 °C during the winter months, particularly from December to February, while maximum temperatures peaked above 35 °C during the summer months of 2022 and 2023.



(**a**)



(**b**)

Figure 1. Temperatures in the winter wheat vegetation period. (**a**) Minimum, maximum, and average monthly air temperature (°C) from September 2021 to July 2023; (**b**) minimum, maximum, and average monthly soil temperature (°C) from September 2021 to July 2023 (axis format year—month—hour).

Spring periods (March–May) show a consistent rise in average temperatures, marking the beginning of the main vegetative growth phase for winter wheat, whereas autumn temperatures (September–November) were moderate and suitable for crop establishment. The warmest months were July 2022 and July 2023, both reaching maximum temperatures above 36 °C. Overall, the dataset shows substantial seasonal variability, with clear climatic differentiation between the two growing seasons, which likely influenced crop development, nitrogen uptake, and final yield performance.

In the context of precipitation, the first tested year was characterized by a significantly lower amount (total of 408.4 mm from September 2021 to July 2022 and total of 697.6 mm from September 2022 to July 2023) in comparison with the second year. During the sowing to four-leaf stage period of 2021, an adequate amount of precipitation fell, while the January–March period of 2022 had the lowest values (38.0 mm in total). Precipitation in September 2022 was very high (130.4 mm) (Figure 2a), which was favorable for seed germination and

early root establishment. October and November 2022 showed moderate rainfall (12.4 and 67.0 mm, respectively), sufficient to support vegetative growth, though October's lower value (~10 mm) could cause temporary moisture stress if soil water retention is low. The winter period (December 2022–February 2023) showed higher precipitation values than wheat needs because it is in a dormant and slow growth phase. March showed a mix of low and moderate precipitation (~30–50 mm), which may slow early spring growth. In April and May, there were larger amounts of rainfall (76.2, and 99.0 mm, respectively), providing good conditions for tillering and stem elongation, supporting yield potential.

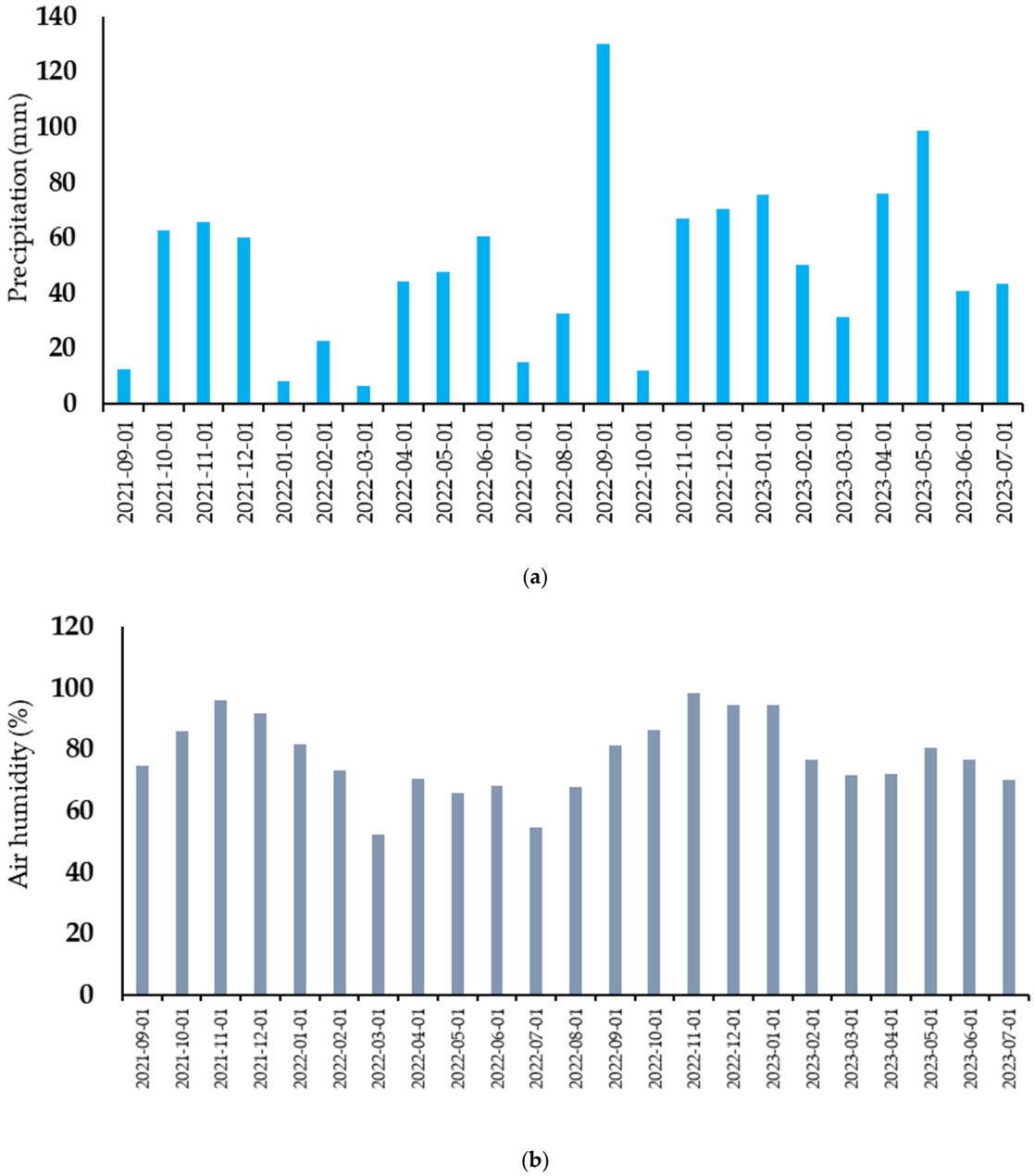


Figure 2. Precipitation and air moisture in the winter wheat vegetation period. (**a**) Monthly precipitation (mm) from September 2021 to July 2023; (**b**) monthly humidity (%) from September 2021 to July 2023 (axis format year–month–hour).

Relative air humidity showed clear seasonal fluctuations across all three years. In 2021, humidity values were generally high, ranging from approximately 75% to 95%, with peak values recorded in late autumn and early winter (Figure 2b). During 2022, humidity

displayed a more variable pattern, with noticeably lower values during spring and early summer (around 60–70%) before increasing again in autumn, reaching nearly 100% in November. In 2023, the pattern resembled that of 2022, with moderate humidity in winter, a slight decrease during early spring (around 70%), and values stabilizing between 70% and 80%.

2.4. Harvest and Quality Parameter Determination

Harvesting was carried out on 5 July 2022 and 12 July 2023 with a Wintersteiger Classic Plus plot combine (Ried im Innkreis, Austria), equipped with a Harvest Master H2 (W1) seed measurement system (Ried im Innkreis, Austria). The Wintersteiger plot combine harvests and threshes small field plots, after which the HarvestMaster W1 system automatically measures seed weight, moisture, and test weight. The W1 unit uses load cells (for mass) and moisture sensors to provide real-time, high-accuracy measurements during harvest.

Seed quality parameters were determined using a Foss Infratec 1241 NIR (Near-Infrared Reflectance) instrument (Hillerød, Denmark). The Infratec 1241 determines seed quality (moisture, protein, starch, gluten, sediment value) using near-infrared reflectance spectroscopy. Infratec 1241 is a near infrared transmission (NIT) monochromator instrument scanning the 570–1100 nm range (optical bandwidth 7 nm, 265 data points per scan). Predictions are obtained by applying embedded application models (AMs), which may be based on PLS or ANN calibration techniques. Due to unverified calibration for this specific dataset, NIR-derived values represent relative differences between treatments under identical measurement conditions rather than absolute biochemical values.

Approximately 0.5 kg of seed each treatment was used for quality parameter determination in four replications. The manufacturer indicates that, for whole seed measurements, no sample preparation is necessary because the instrument uses transmission absorption. The wheat seed was handled as whole-kernel material (no milling), visible impurities were removed, and samples were equilibrated to laboratory conditions before analysis.

2.5. Partial Factor Productivity and Nitrogen Use Efficiency

Partial factor productivity (*PFP*) is expressed as kg seed/kg N, calculated as a ratio of seed yield (kg/ha) and added N (N kg/ha) as follows:

$$PFP = \frac{Seed\ yield\ (\mathrm{kg/ha})}{Applied\ N\ (\mathrm{kg/ha})}$$

Moreover, nitrogen use efficiency (*NUE*) was calculated based on seed N removal on the seed protein content. This approach allows the estimation of the amount of nitrogen exported from the field in the harvested seed. Firstly, seed N removed was estimated using the seed protein content, using a correction factor of 5.7 according to the following equation:

$$Seed\ N\ removed\ (\mathrm{kg/ha}) = Seed\ yield\ (\mathrm{kg/ha}) \times \frac{protein\ (\%)}{5.7}$$

The *NUE* was then calculated as the ratio of seed yield or N removed to the amount of nitrogen applied:

$$NUE\ (\mathrm{kg\ N\ per\ kg}\ applied\ N) = \frac{Seed\ N\ removed\ (\mathrm{kg/ha})}{Applied\ N\ (\mathrm{kg/ha})}$$

2.6. Statistical Analysis

Data were analyzed using Microsoft Excel. For each year of the study, a one-way analysis of variance (ANOVA) was performed. When the ANOVA revealed a statistically

significant effect, post hoc pairwise comparisons were conducted using Fisher's least significant difference (LSD) test.

To explore the relationship between nitrogen fertilization and selected seed quality traits across seasons, a simple linear regression was performed using data pooled from both growing seasons. The response variables were (i) starch yield (kg ha^{-1}) and (ii) seed protein content (%), while the explanatory variable was the applied nitrogen rate (kg N ha^{-1}) for each treatment. Regression models were fitted using the ordinary least squares method. Model fit was evaluated by the coefficient of determination (R^2), and the significance of the regression was tested using the overall F-test ($\alpha = 0.05$). The Chaddock scale was used for interpreting R^2, where 0 represents no relationship, 0–0.25 represents a weak relationship, 0.25–0.65 represents a moderate relationship, and 0.65–1.00 represents a strong relationship [39].

Pairwise correlations were calculated using the Pearson correlation coefficient, and the correlation structure was visualized using a heat map generated with the ChiPlot online platform [40].

3. Results

3.1. Statistical Evaluation

Across both growing seasons, most seed quality parameters (moisture, hectoliter weight, starch, protein, gluten, and sedimentation value) had low R^2 values, and the corresponding ANOVA F-tests were not statistically significant ($p > 0.05$), indicating that the applied treatments explained only a small proportion of the observed variability (Table 5).

Table 5. Analysis of variance (ANOVA) statistics for seed quality, yield, and fertilization efficiency parameters across two growing seasons.

Parameter	R-Square	Coefficient of Variation	F Value	*p* Value
Season 2021/2022				
Moisture	0.15	7.31	0.60	ns
Seed yield	0.03	12.10	0.12	ns
Hectoliter	0.21	6.42	0.92	ns
Starch	0.12	3.59	0.49	ns
Protein	0.11	15.89	0.43	ns
Gluten	0.12	17.68	0.47	ns
Sediment value	0.12	27.63	0.49	ns
Starch yield	0.16	9.13	0.65	ns
PFP	0.62	11.69	5.76	***
NUE	0.55	9.46	4.37	***
Season 2022/2023				
Moisture	0.26	5.34	1.22	*
Seed yield	0.50	9.77	3.47	**
Hectoliter	0.31	2.09	1.56	*
Starch	0.04	1.49	0.16	ns
Protein	0.16	3.97	0.65	ns
Gluten	0.36	4.03	2.01	*
Sediment value	0.29	6.99	1.40	ns
Starch yield	0.53	9.54	3.87	**
PFP	0.30	9.54	1.51	*
NUE	0.23	9.44	1.04	ns

PFP—partial factor productivity; *NUE*—nitrogen use efficiency; * as $p \leq 0.05$; ** as $p \leq 0.01$, *** $p \leq 0.001$, or "ns" (nonsignificant).

In the 2021/2022 season, none of the quality or yield components showed statistical significance, confirming a weak treatment response under the prevailing environmen-

tal conditions. However, both *PFP* and *NUE* exhibited very highly significant F values ($p < 0.001$), demonstrating the strong sensitivity of these efficiency indicators to the applied fertilization levels (Table 5).

In contrast, during the 2022/2023 season, several parameters did show significant responses (Table 5). Seed yield, hectoliter weight, gluten content, and starch yield displayed statistically significant F values ($p < 0.05$ and 0.01), indicating a clearer treatment effect compared with the first season.

3.2. Wheat Seed Yield and Quality

In the 2021/2022 growing season, across all treatments, no statistically significant differences were observed for any of the measured parameters, as indicated by the non-significant influence of different nitrogen fertilization (Table 6). Moisture content, seed yield, hectoliter weight, starch, protein, gluten, and sedimentation value remained within a relatively narrow range, suggesting that the applied treatments had only minimal effects on seed quality and yield components.

Table 6. Mean values and standard deviation (SD) of the wheat yield and quality in the 2021/2022 season.

Treatment	Moisture (%)	Yield (t/ha)	Hectoliter (kg/hl)	Starch (%)	Protein (%)	Gluten (%)	Sediment Value (mL)
Control	10.2 ± 0.9	6.9 ± 0.5	70.75 ± 5.0	70.92 ± 2.2	12.2 ± 2.2	26.8 ± 5.6	42.6 ± 13.3
TSE_1	10.2 ± 0.6	6.9 ± 1.1	71.50 ± 4.4	69.45 ± 2.3	13.3 ± 2.3	29.9 ± 5.7	49.7 ± 14.9
TSE_2	9.9 ± 0.5	6.9 ± 0.7	70.92 ± 4.5	69.40 ± 1.8	13.3 ± 1.8	29.8 ± 4.2	49.1 ± 12.1
TSEH_1	9.6 ± 0.9	6.7 ± 0.7	65.25 ± 4.9	68.27 ± 2.0	14.3 ± 2.0	32.2 ± 5.2	56.7 ± 13.2
TSEH_2	9.6 ± 1.1	6.5 ± 0.9	68.85 ± 3.5	68.60 ± 2.2	14.1 ± 2.1	31.7 ± 5.3	55.4 ± 13.7
TSEH_3	10.0 ± 0.4	6.9 ± 0.9	70.78 ± 4.4	69.85 ± 2.6	13.6 ± 2.6	29.1 ± 6.2	49.0 ± 17.2
TSH	10.2 ± 0.5	6.9 ± 0.9	69.68 ± 4.4	69.83 ± 1.7	13.1 ± 1.7	29.1 ± 4.4	47.0 ± 11.4
Mean	9.9	6.8	69.68	69.48	13.3	29.8	49.9
$LSD_{0.05}$	ns	ns	ns	ns	ns	ns	ns

Treatment naming convention: First letter indicates growth stage where initial application occurs, T—tillering stage; SE—stem elongation phase; H—heading stage; ns—not significant. Where significant differences existed ($p \leq 0.05$), treatments sharing the same letter were not significantly different according to the LSD test.

The Control and TSE-based treatments (TSE_1, TSE_2) showed very similar values for moisture, yield, starch, and protein content. Treatments combining TSE with higher nitrogen levels (TSEH_1, TSEH_2, TSEH_3) tended to slightly increase protein and gluten percentages as well as sedimentation values, indicating a modest trend toward improved seed technological quality. Treatment TSEH_1 showed the highest gluten content (32.5%) and 14.6% higher relative protein content compared to the Control (Table 6). However, these increases were not statistically significant.

In the second season (2022/2023), moisture content and gluten percentage showed statistically significant differences among treatments (Table 7). Moisture varied slightly, with TSEH_3 showing a higher value (11.6%), likely reflecting environmental or treatment-driven effects on seed drying. Gluten content demonstrated a clearer treatment response: the TSEH_1 and TSH treatments exhibited the highest gluten percentages (32.5% and 31.5%, respectively), suggesting that higher nutrient availability promoted improved gluten formation.

Table 7. Mean values and standard deviation (SD) of the wheat yield and quality in the 2022/2023 season.

Treatment	Moisture (%)	Yield (t/ha)	Hectoliter (kg/hl)	Starch (%)	Protein (%)	Gluten (%)	Sediment Value (mL)
Control	10.8 ± 0.3	4.1 ± 0.4	77.3 ± 1.2	66.4 ± 1.2	14.7 ± 0.9	29.9 ± 2.1	54.6 ± 6.5
TSE_1	10.8 ± 0.2	5.2 ± 0.3	77.4 ± 3.4	66.5 ± 1.22	15.0 ± 0.6	30.7 ± 1.0	57.6 ± 3.4
TSE_2	10.8 ± 0.1	5.6 ± 0.4	78.9 ± 1.1	66.7 ± 0.2	14.7 ± 0.1	30.4 ± 0.7	54.2 ± 0.93
TSEH_1	10.8 ± 0.2	5.3 ± 0.3	79.9 ± 0.8	66.6 ± 0.9	15.2 ± 0.5	32.5 ± 0.9	59.4 ± 2.5
TSEH_2	10.7 ± 0.1	5.2 ± 0.6	79.3 ± 1.5	66.3 ± 1.1	15.1 ± 0.5	31.3 ± 0.9	58.0 ± 2.4
TSEH_3	11.6 ± 1.4	5.4 ± 0.1	79.5 ± 1.3	66.9 ± 1.3	14.7 ± 0.9	30.3 ± 1.8	54.6 ± 6.5
TSH	10.8 ± 0.3	5.2 ± 0.9	79.4 ± 0.6	66.5 ± 0.6	15.2 ± 0.2	31.5 ± 0.6	59.6 ± 1.1
Mean	10.9	5.1	78.8	66.6	14.9	30.9	56.8
$LSD_{0.05}$	0.86	ns	2.42	ns	ns	1.83	ns

Treatment naming convention: First letter indicates growth stage where initial application occurs, T—tillering stage; SE—stem elongation phase; H—heading stage; ns—not significant. Where significant differences existed ($p \leq 0.05$), treatments sharing the same letter were not significantly different according to the LSD test.

Seed yield in the 2022/2023 season showed consistent numerical increases across all fertilized treatments compared with the Control (4.1 t/ha), particularly in TSE_2 (5.6 t/ha) and TSEH_3 (5.4 t/ha), although these differences were not statistically significant. Hectoliter weight showed a modest but significant increase, with treated variants reaching up to 79.9 kg/hl, indicating denser and potentially higher-quality seed.

Despite the nonsignificant statistical outcomes for other seed quality parameters, slight numerical trends were observed: treatments with enhanced fertilization (TSEH variants) tended to produce marginally higher protein and sedimentation values, indicating a tendency toward improved technological quality.

The highest hectoliter weight (Table 7) was observed in TSEH_1 (79.9 kg/hl), indicating the best seed quality among the treatments. Treatments TSEH_2 (79.3), TSEH_3 (79.5), and TSH (79.4) also showed high values, all higher than the overall mean. TSE_2 (78.9) performed moderately well and is close to the grand mean. The lowest values were recorded for the Control (77.3) and TSE_1 (77.4). Thus, TSEH_1, TSEH_2, TSEH_3, TSH, and the lowest values were found for the Control and TSE_1.

Another parameter of seed quality that showed statistically significant differences between the treatments is gluten content (Table 7). The highest gluten content was recorded in TSEH_1 (32.5%), clearly above the overall mean. Treatments TSH (31.5%) and TSEH_2 (31.3%) also showed elevated gluten levels. TSE_1 (30.7%) and TSE_2 (30.4%) had moderate values, close to the grand mean. The lowest gluten contents were observed for the Control (29.9%) and TSEH_3 (30.3%).

3.3. Correlation Analysis

According to the correlation analysis, a strong positive correlation among quality parameters was found (Figure 3). Protein, gluten, and sediment value show very strong positive correlations (0.919 to 0.982, ***). These results indicate that samples with higher protein content generally also have higher gluten content and higher sedimentation values. Starch shows strong negative correlations with protein content (−0.968 ***), gluten (−0.801 ***), and sediment value (−0.914 ***). A higher starch content is consistently associated with lower protein and gluten, which is expected in winter wheat. Yield showed a strong positive correlation with hectoliter mass (0.833 ***), indicating that denser seed tends to be associated with higher yield. Seed moisture at the harvest correlated only weakly with most traits, and many of these correlations were not significant (ns). Seed yield was negatively correlated with protein and gluten (−0.734 *** and −0.475 **, respectively), as confirmed in other studies.

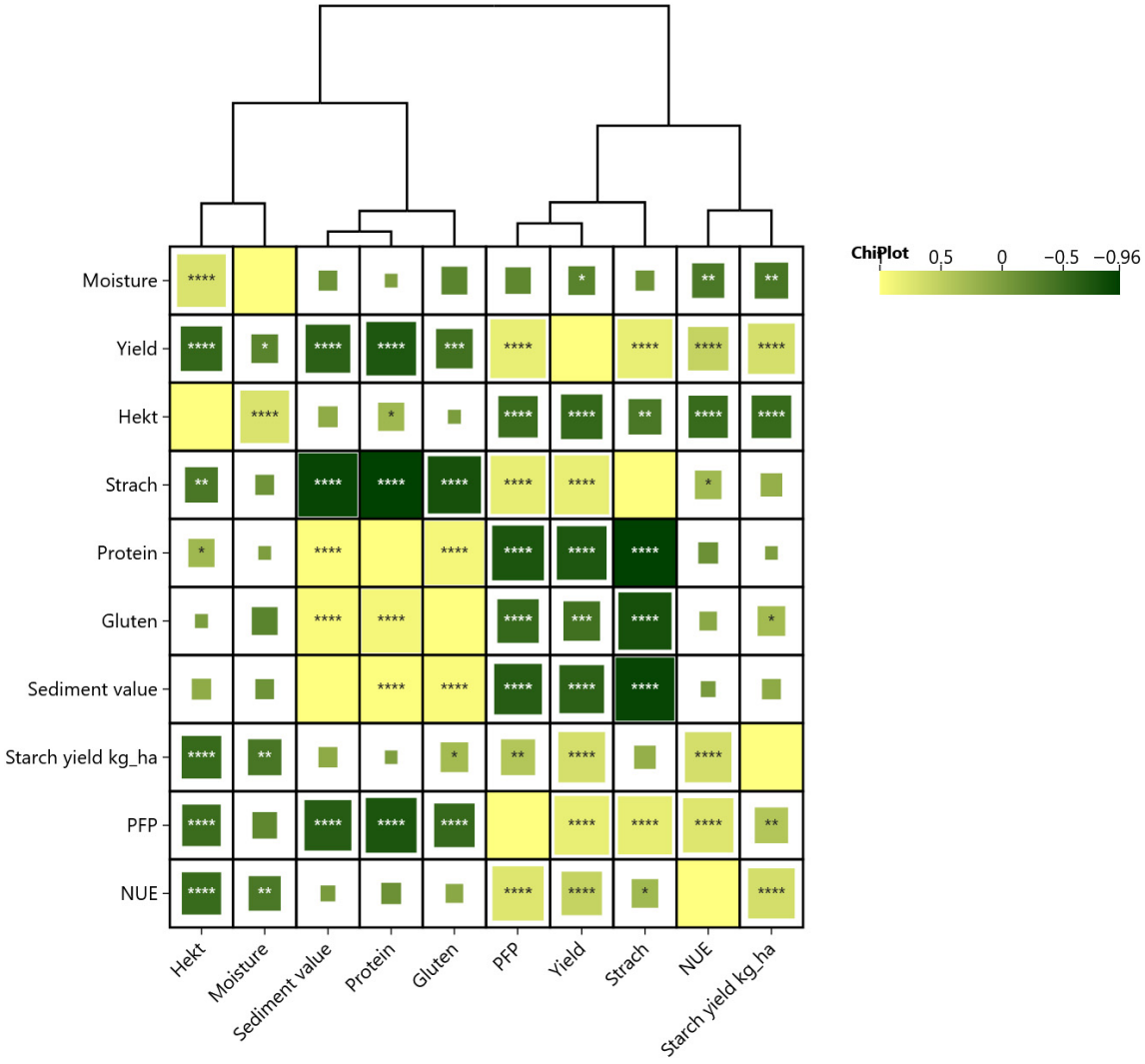


Figure 3. Pearson correlation matrix illustrating relationships among key seed quality and yield-related traits. Correlation strength and direction are indicated by color intensity. Statistical significance is denoted as * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, and **** $p \leq 0.0001$. Hierarchical clustering (dendrogram) groups variables with similar correlation patterns; shorter branch lengths indicate greater similarity, whereas longer branches indicate lower similarity. Exact 95% confidence intervals for Pearson's r (Fisher's z-transformation) are provided in Table S1, while the exact Pearson correlation (r) coefficient is presented in Table S2.

3.4. Partial Factor Productivity and Nitrogen Use Efficiency Regard Nitrogen Use for Winter Wheat

The *PFP* had a similar trend in both vegetation periods (Figure 2). In the 2021/2022 season (Figure 4a), the Control treatment showed the highest *PFP* (46), significantly exceeding all tested extracts. Treatments TSE_2, TSEH_3, and TSH formed a statistically homogeneous group with intermediate productivity (36, 34, and 34, respectively), while TSE_1 and TSEH_1 exhibited the lowest values (23 and 20, respectively).

In contrast, during the 2022/2023 season (Figure 4b), the Control, TSE_2, and TSH treatments showed similarly high productivity, forming the top statistical group (28). The TSE_1 and TSEH_3 treatments resulted in moderate productivity, whereas TSEH_1 again had the lowest *PFP*. Overall, differences among treatments were consistent between seasons, though absolute productivity values were lower in 2022/2023.

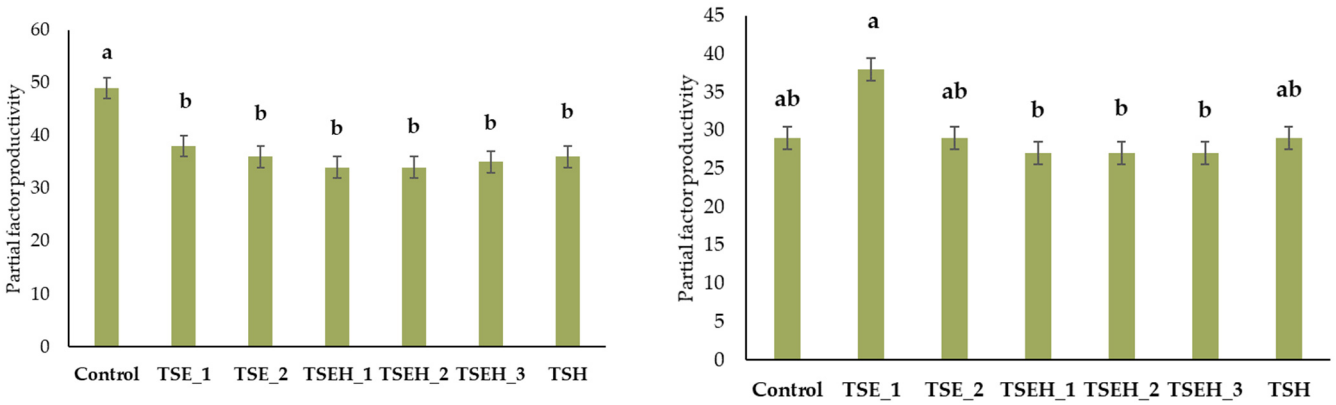


Figure 4. Partial factor productivity of winter wheat regarding fertilization management. (**a**) Season 2021/2022; (**b**) season 2022/2023. Treatment naming convention: First letter indicates growth stage where initial application occurs, T—tillering stage; SE—stem elongation phase; H—heading stage. Vertical bars indicate standard error at 5%. Treatments sharing the same letter were not significantly different according to the LSD test.

3.5. Regression Analysis

The regression analysis illustrates the relationship between nitrogen application (kg/ha) and starch yield (kg/ha) as well as protein content (%) across two growing seasons—2021/2022 and 2022/2023 (Figure 5).

The relationship in 2021/2022 for starch yield (Figure 5a) and added N was not significant (ns). The very low R^2 value (R^2 = 0.0538) indicates that nitrogen application explains only about 5% of the variability in starch yield. The slope is positive but extremely small, showing that increased nitrogen has almost no effect on starch yield in this season.

The regression equation (y = 0.57x + 633.1, with R^2 = 0.3261) for starch yield in relation to N applied in 2022/2023 was statistically significant ($p \leq 0.01$). The higher R^2 indicates that nitrogen application explains about 33% of the variation in starch yield, much stronger than in the previous season. The slope is more pronounced, suggesting that increasing nitrogen led to a meaningful improvement in starch yield in this year.

On the opposite of starch yield, the protein content of the wheat seed was very significant in relation to the nitrogen applied (Figure 5b). The regression equation y = 0.0077x + 11.597, with factor R^2 = 0.6354, indicates an increase in protein content of 0.0077% for each kg of N applied, but a nonsignificant relationship of protein content (%) and N applied was found in the 2022/2023 season (Figure 5b).

Seasonal conditions play a crucial role in determining nitrogen use efficiency (*NUE*) in wheat (Figure 5c). Figure 5c illustrates the relationship between nitrogen application rate and nitrogen use efficiency (*NUE*) across two growing seasons. A clear seasonal contrast is visible. In 2021/2022, *NUE* values were generally higher and showed a significant negative linear trend with increasing nitrogen rates ($p \leq 0.01$). This indicates that, in a favorable season, wheat is able to use nitrogen more efficiently at lower application levels, but efficiency progressively declines as nitrogen inputs increase. The relatively high R^2 value (0.3937) suggests that nitrogen rate explained a meaningful portion of the variation in *NUE* during this season. In contrast, the 2022/2023 season exhibited consistently lower *NUE* values and a much flatter regression slope. The weak relationship (R^2 = 0.0797) and nonsignificant trend (ns) indicate that nitrogen application rate had little influence on *NUE*. This suggests that environmental conditions during 2022/2023 likely limited the crop's capacity to utilize nitrogen effectively, regardless of the applied amount. Such conditions

may include drought, excessive rainfall, or temperature stress, all of which can impair nitrogen uptake and assimilation.

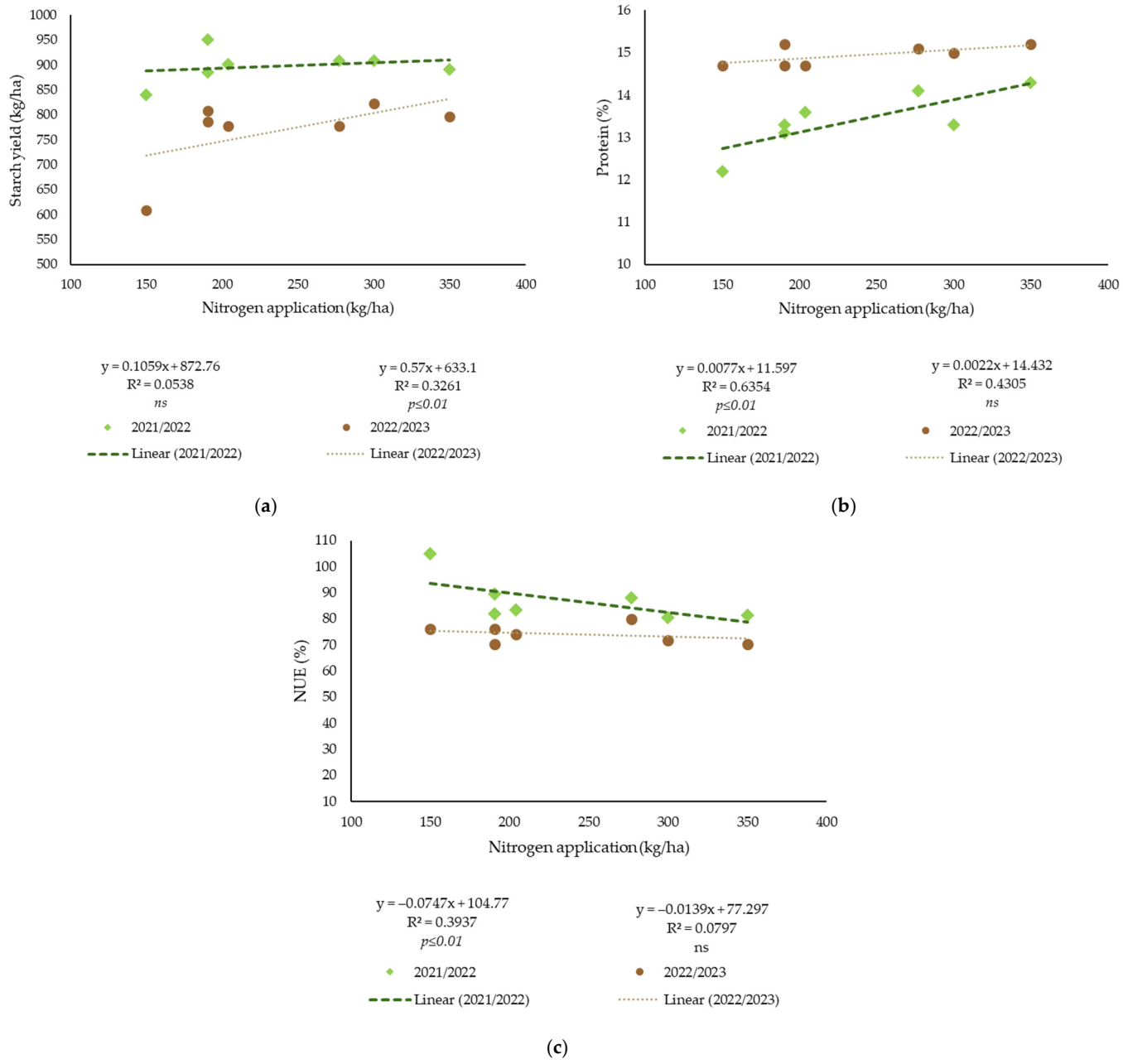


Figure 5. Regression analyses for both seasons (2021/2022 and 2022/2023) showing the relationships between nitrogen fertilization rate (kg ha^{-1}) and (**a**) starch yield (kg ha^{-1}), (**b**) protein content (%), and (**c**) nitrogen use efficiency (*NUE*, %) in winter wheat seed. Regression lines are shown with 95% confidence limits, and 95% prediction limits are presented in Supplementary Figure S1a–f.

4. Discussion

Wheat production is a key branch of plant production because it provides an irreplaceable raw material needed for human nutrition, various industries, and domestic animal nutrition in Croatia and the world [41]. Today's intensive wheat production involves the use of large amounts of mineral fertilizers [42]. Heffer and Prud'homme [43] reported that nitrogen fertilizers play a leading role in improving yield and seed quality. They emphasized that nitrogen is typically applied in relatively large amounts and should be split across different growth stages to maximize uptake efficiency and reduce losses. However,

in agricultural systems with high investments, such as those practiced in developed countries, about 50% of N is lost to the environment, polluting the soil, water, and atmosphere, according to Farnworth et al. [44]. About 2/3 of excessive levels of N and phosphorus (P) in waters come from mineral fertilizers in agriculture. According to data from the European Union (EU), in Croatia, the loss of N to the environment for 2015 was 54.7, which places us at the very top of the scale [45].

Nitrogen is globally the most important and currently irreplaceable nutrient in agricultural production for both plants and animals. Biradar et al. [46] state that N is a key nutrient limiting wheat yields, especially in low-fertility and dry soils, and many smallholder farmers in South Asia have limited capacity to invest in fertilizers, highlighting the need for nitrogen-efficient wheat lines. Evaluation of 50 diverse genotypes under reduced (50%) and full (100%) N revealed significant genetic variation, identified low-N tolerant genotypes with high yield and nitrogen use efficiency, and provided valuable trait data for breeding N-efficient wheat suited to marginal agro-ecologies. In our study, the differences in N application rates are shown in the treatments, ranging from about 140 to 195 kg N/ha (Table 2). These amounts are typical of fertilization practices for wheat, both globally and in Croatia. Worldwide, intensive wheat production systems commonly apply between 120 and 200 kg N/ha, with higher rates used in high-yielding environments and where water availability supports greater biomass production [47–50]. Therefore, the total N amounts presented here are realistic and practically applicable, and the different combinations of granular and foliar N reflect modern efforts to optimize nitrogen timing and efficiency. Such strategies, especially foliar applications, are increasingly adopted to reduce nitrogen losses, improve nitrogen use efficiency, and better synchronize fertilizer supply with crop demand, which is important both economically and environmentally, particularly within EU agricultural regulations [51,52]. According to EU data, the total costs of all forms of N losses in Europe (estimated at 70–320 billion € per year at current rates) outweigh the direct economic benefits of nitrogen in agriculture. The EU is, therefore, planning to reduce nutrient losses through the European Green Deal (EGD) and various strategies (Biodiversity strategy, Farm to Fork, and Zero Pollution Action Plan). Generally, the plan is to reduce fertilizer consumption by at least 20%. The Common Agricultural Policy (CAP) is a key tool to support the sustainable use of fertilizers in agriculture, ensuring that farmers can maintain productivity while reducing adverse environmental impacts.

Soil–climatic conditions, particularly precipitation and temperature, also played a major role in determining high yields under non-irrigated maize production. Our results link nitrogen management not only to agronomic efficiency (*PFP*/*NUE*) but also to commercially relevant seed quality outcomes, as shifts in protein–starch balance and gluten can affect seed lot value through compliance with quality specifications and consistency demanded by the seed and processing industry. According to Cui et al. [32], different N forms can alter plant N status through uptake kinetics and soil N transformations (hydrolysis, nitrification) and through loss pathways (e.g., volatilization risk with surface-applied urea under warm and dry conditions as well as leaching and denitrification risks when large rainfall occurs after application). These differences influence how well N supply is synchronized with crop demand at specific stages, ultimately affecting *NUE*, yield, and quality. In field conditions, N form (urea vs. nitrate/ammonium combinations) can produce different outcomes in yield and seed quality, consistent with a physiology-based explanation (crop N status during critical windows). Nitrogen use efficiency (*NUE*) is crucial in forage harvester crops because it maximizes biomass production while minimizing nitrogen losses and environmental impact [53–58]. Jarecki et al. [59] stated that mineral nitrogen–phosphorus fertilization significantly increased maize yields in a four-year crop rotation, explaining up to 97% of yield variation depending on soil type, and more than doubled productivity

compared to unfertilized treatments. In the 2021/2022 growing season, the lack of significant differences suggests that environmental variability and year effects likely had a stronger influence on wheat seed properties than the treatments themselves. The similar mean values across treatments further support the conclusion that the tested fertilization strategies did not substantially alter seed quality or yield in this dataset. This highlights that seasonal conditions strongly influence *NUE*, both in terms of absolute values and in the responsiveness of wheat to nitrogen inputs. While higher *NUE* is achievable under favorable conditions (2021/2022), adverse seasonal factors can substantially reduce efficiency and weaken the relationship between fertilization rate and plant nitrogen use (2022/2023). In contrast, seasons characterized by drought, excessive rainfall, or heat stress often reduce *NUE*. Water limitations restrict nitrogen mobility in the soil and reduce root activity, while excessive rainfall can promote nitrogen losses through leaching or denitrification. Heat stress during stem elongation or heading accelerates phenological development, shortening the nitrogen uptake period and lowering the efficiency of late applications.

In the present study, in 2021/2022, seed yield showed minimal responsiveness to fertilization, suggesting that non-nitrogen limitations dominated crop performance. The climatic pattern (very low precipitation during January–March 2022 and strong seasonal temperature variability) likely restricted crop growth and N uptake, thereby limiting the capacity of the crop to translate additional N supply into higher yield. Under such yield-constrained conditions, increasing N rate typically leads to diminishing marginal yield gains, while N efficiency indices remain responsive because they are ratio-based (e.g., *PFP* as yield/applied N; *NUE* as seed N removal/applied N). Consequently, even when yield differences are small, higher N inputs reduce *PFP* and *NUE*, reflecting a larger fraction of applied N not recovered in harvested seed (remaining in soil, lost, or used for non-seed sinks).

Congreves et al. [60] stated that, despite extensive research, the genetic basis of nitrogen use efficiency (*NUE*) remains poorly understood due to complex interactions among genotype, environment, and management. Authors in their study used a ^{15}N tracer approach to accurately quantify fertilizer nitrogen recovery in diverse wheat genotypes and to assess how background soil nitrogen levels influence that recovery. According to a one-year study in Shandong, China, Liu et al. [61] quantified how manure compost, urea fertilizer, and maize root residues contribute to wheat nitrogen uptake, showing that urea provided the highest contribution and recovery efficiency. While manure and maize roots supplied smaller portions of plant-absorbed N, the results highlight clear differences among N sources and their roles in supporting sustainable wheat production. Chen et al. 2025 [62] studied N fertilizer partial factor productivity (N-*PFP*) rice yield and found that N-*PFP* was strongly influenced by how much and how often nitrogen was applied as well as by soil clay content, and higher precipitation further increased the effect size on N-*PFP*. In contrast, while N_2O emissions showed clear links to climate and soil properties, NH_3 volatilization, N runoff, and N leaching showed no relationship with environmental conditions or nitrogen management. In the present study, the heatmap reveals clear internal structure within seed quality traits, highlighting two contrasting clusters: (i) protein–gluten–sedimentation value cluster and (ii) starch, which is inversely related to the protein/gluten group. Yield and hectoliter mass form a separate cluster with moderate connections to quality traits. Moisture is mostly independent. Generally, the results indicate that, while traditional seed quality traits were only weakly influenced by treatment, yield efficiency parameters (*PFP* and *NUE*) consistently demonstrated strong and highly significant responses, making them reliable indicators for assessing the effectiveness of fertilization strategies across contrasting seasons. Based on the long-term experiments across four sites in India, Yadav [63] showed that continuous rice–wheat cultivation greatly reduced rice yields, especially without fer-

tilizer, and wheat yields only declined in unfertilized plots and even increased where N, P, or K was applied. Over 16 years, nitrogen use efficiency and profitability generally rose with balanced fertilization, while soil nutrient trends varied by location, with some sites experiencing declines in organic carbon and available N and P and others showing improvements in soil fertility. In the study by Rawal et al. [64], a two-year average wheat seed yield reached 6516 kg ha^{-1} with 150 kg N ha^{-1}, which was statistically similar to the 6316 kg ha^{-1} obtained with 125 kg N ha^{-1}. In contrast, the plots without nitrogen fertilizer produced the lowest dry matter (3307 kg ha^{-1}) and seed yield (2556 kg ha^{-1}).

Starch serves as the primary energy source for germination and early seedling growth, while storage proteins supply nitrogen and amino acids essential for enzymatic activity and tissue differentiation [65]. An increased protein proportion may enhance seedling vigor under stress conditions but can reduce starch availability, potentially limiting early biomass accumulation. Differences in wheat seed protein and starch among N treatments of the present study can be explained by how N management changes canopy development and sink size before anthesis and post-anthesis N supply/remobilization during seed filling. According to Kong et al. [66], wheat obtains seed N from two main sources as remobilization of N stored in vegetative organs accumulated before anthesis and post-anthesis N uptake. The relative contribution of these sources strongly affects both seed yield and seed protein concentration (GPC). Bogard et al. [67] stated that an early N top-dressing in tillering–stem elongation primarily increases leaf area, photosynthetic capacity, tiller survival, and spike number, thereby increasing yield potential (sink size). If additional N is not available later, higher carbohydrate deposition (starch) can dilute seed N, lowering GPC despite higher yield ("protein dilution" concept). Triboï et al. [68] explained that, at the biochemical level, improved N availability during seed filling of wheat increases amino acid flux to the seed, supporting gliadin and glutenin accumulation and potentially shifting protein composition relevant to end-use quality. In the present study, fertilization had a generally positive but mostly modest effect on wheat yield and technological quality: treatments with enhanced nitrogen supply (TSEH/TSH) tended to increase protein, gluten percentage, and sedimentation value, while in 2022/2023, hectoliter weight and gluten content increased significantly. At the same time, higher nitrogen rates were often associated with lower nitrogen use efficiency (*NUE*) and a negative relationship between yield and protein/gluten, indicating a trade-off between maximizing yield and improving seed protein quality. In the study by Svećnjak et al. [69], in the middle of Croatia, a 2-year study found that, in 2002, more favorable weather conditions were associated with higher seed yield and higher seed nitrogen (N) compared with 2001, indicating that seasonal climate strongly influenced N uptake and accumulation in the seed. Across both years, the high N rate increased seed N content (e.g., from 166 to 210 g kg^{-1} in 2001 and from 176 to 232 g kg^{-1} in 2002) and also raised yield, showing that the crop's response to N was enhanced under better growing conditions. Another experiment in Eastern Croatia by Horvat et al. [70] found that late-season foliar urea (35 kg N ha^{-1}) significantly increased seed yield and key physical quality traits (1000-kernel weight and hectoliter weight) while also raising total seed protein by 17.5% compared with the Control. The authors found that, for six wheat cultivars (El Nino, Ficko, Katarina, Vulkan, Silvija, Kraljica, Tika taka, Renata, Srpanjka, and Os Olimpija), the protein-fraction level and urea tended to higher total gliadins and a higher GLI/GLU ratio, while total glutenins (including HMW-GS) often decreased, which indicates cultivar-dependent changes in bread-making quality. In another study by Horvat et al. [71], field trials were conducted on 16 wheat cultivars (two years, three locations). The results of their study showed that raising nitrogen from 0 to 100 kg ha^{-1} increased seed protein by about 11.3% (11.5% to 12.8%) and that a higher N slightly shifted protein composition toward more total gliadins (especially α- and γ-gliadins) and HMW glutenin subunits while reducing

albumins/globulins, ω-gliadins, total glutenins, and LMW glutenin subunits. Responses were cultivar-specific: historical cultivars (Libellula, San Pastore, U-1) reached higher protein and a higher gliadin/glutenin ratio (1.68–1.92), whereas most modern cultivars maintained a more balanced ratio (1.08–1.50), except MV Nemere, which remained high at both N levels. A three-year field study by Maričević et al. [72] in Staro Topolje evaluated 12 winter wheat varieties under three nitrogen fertilization levels and showed that protein content was significantly influenced by year, variety, and nitrogen treatment, with the year having the strongest effect, followed by nitrogen fertilization and variety. Average protein content increased consistently with nitrogen rate, from 11.08% at the lowest to 11.95% at the medium and 12.89% at the highest nitrogen level, while all varieties responded similarly to nitrogen application. From an economic perspective, Ravnjak et al. [73] emphasized that careful management of production inputs is essential to maintain profitability, particularly under rising and volatile input prices. Nitrogen fertilization is one of the largest and most cost-sensitive components of cereal production, so optimizing N rates and timing is critical to achieve high performance while avoiding unnecessary costs. Under highly variable seasonal conditions, the economic viability of intensified nitrogen strategies for seed producers depends on whether added N generates a high yield and quality. In unresponsive years, higher inputs mainly reduce *PFP*/*NUE* and increase production costs without proportional benefits. In the present study, according to regression analysis, among the seed quality parameters evaluated, seed protein content shows the strongest and most consistent responsiveness to nitrogen management. Seed yield is moderately responsive depending on seasonal conditions, while *NUE* declines with higher nitrogen inputs. This indicates that improvements in certain seed quality traits through nitrogen fertilization may come at the cost of reduced nitrogen use efficiency, highlighting a trade-off between seed quality enhancement and resource efficiency.

Nitrogen management shifted carbon–nitrogen allocation during seed filling, affecting the protein–starch balance that determines seed reserves: starch supports germination energy, while protein provides N for early seedling growth [74–77]. According to the correlations analysis of Wen et al. [28], increased absolute protein content (ACP) was associated with higher seedling dry weight, suggesting improved seedling vigor when more N is stored in the seed, whereas changes in relative protein concentration (RCP) may reflect dilution effects linked to seed size rather than the total N reserve.

In seed production systems, optimized partial *PFP* and *NUE* contribute not primarily to seed yield maximization but to the stabilization of seed quality traits. Improved *PFP* and *NUE* enable adequate protein deposition without excessive nitrogen input, reducing variability in seed composition, enhancing reserve utilization efficiency, and improving seed storability and longevity. *PFP* and *NUE* were emphasized in the present study because they describe the efficiency with which nitrogen inputs are converted into biologically functional biomass and seed components rather than reflecting yield alone. Generally, in the present study, *PFP* expresses seed yield per unit of applied nitrogen (kg seed kg^{-1} N) and, therefore, represents an overall input productivity indicator rather than a direct measure of plant N recovery. This distinction is particularly relevant in seasons with weak treatment effects on yield (e.g., 2021/2022), when similar yields across treatments combined with different N inputs can inflate *PFP*, mainly due to a smaller denominator. Because this study did not quantify N uptake, fertilizer N recovery, soil residual N, or loss pathways, *PFP* should be interpreted primarily as a management-oriented metric indicating marginal returns to N inputs, while conclusions about physiological N use efficiency should be made cautiously and supported by direct N balance measurements.

5. Conclusions and Future Perspectives

Variability in temperature, rainfall distribution, and radiation strongly influences both crop growth dynamics and the plant's ability to take up and utilize applied nitrogen. In favorable seasons, when soil moisture is adequate and temperatures support steady vegetative growth, wheat typically shows higher *NUE* because nitrogen uptake is well synchronized with crop demand. Under such conditions, the crop can respond more effectively to top-dressing applications, leading to greater biomass accumulation and improved seed protein content.

Across both seasons, the fertilization treatments had limited and inconsistent effects on most seed yield and seed quality traits, whereas *PFP* and *NUE* were highly responsive, indicating that optimizing N rate should prioritize efficiency rather than maximizing inputs. In the season with clearer treatment responses (2022/2023), fertilized variants improved hectoliter weight and gluten content and showed numerical yield gains, suggesting that moderate N intensification and/or late-season N availability can be justified when crop potential is high. However, because *NUE* tended to decline or become insensitive at higher N rates and yield was negatively associated with protein and gluten, N management should be adjusted to seasonal conditions using split applications (tillering + stem extension with optional late correction) to balance yield goals with technological seed quality while avoiding unnecessary N losses.

Based on the results of the present study, *PFP* in wheat is highly season-dependent. Optimizing application timing (e.g., splitting doses, foliar feeding under dry conditions), matching nitrogen rate to seasonal yield potential, and adjusting fertilization strategies to actual weather patterns can significantly improve nitrogen utilization across different growing seasons. In practice, this approach turns *PFP* into a quick economic signal and *NUE* (based on seed N removal) into a seed quality-relevant efficiency metric, helping producers optimize N to secure both seed yield and seed quality.

Given the strong seasonal dependence of responses, future research should test these strategies across more environments and years and include direct seed-performance traits (germination, vigor, seedling growth) together with refined *NUE*/*PFP* metrics. For practical use, we recommend adopting split N applications aligned with key growth stages, adjusting total N to seasonal yield potential, and prioritizing strategies that maintain seed quality targets (protein/gluten) while preserving high *PFP*/*NUE*, particularly under variable weather conditions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/seeds5010012/s1, Table S1: Pearson Correlation Coefficients, N = 56; Table S2: Pearson Correlation Statistics (Fisher's z Transformation) with 95% Confidence limits among key grain-quality and yield-related traits; Figure S1: Linear regression of starch yield (a–f).

Author Contributions: Conceptualization, L.D. and I.V. (Ivana Varga); methodology, L.D., I.V. (Ivan Varnica), and G.J.; software, I.V. (Ivana Varga) and D.I.; validation, D.I., I.V. (Ivan Varnica), and L.D.; formal analysis, L.D. and I.V. (Ivan Varnica); investigation, L.D. and G.J.; resources, G.J.; data curation, L.D. and I.V. (Ivan Varnica); writing—original draft preparation, L.D., I.V. (Ivana Varga) and D.I.; writing—review and editing, L.D., I.V. (Ivana Varga), G.J., I.V. (Ivan Varnica), and D.I.; visualization, I.V. (Ivana Varga); supervision, G.J.; project administration, G.J. All authors have read and agreed to the published version of the manuscript.

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