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Unlocking Nitrogen Use Efficiency in Tritordeum: A Holistic Evaluation of Enhanced-Efficiency Fertilisers Under Mediterranean Conditions

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Abstract: Improving nitrogen use efficiency (NUE) is critical to advancing sustainable cereal production, particularly under Mediterranean conditions where environmental pressures challenge input-intensive practises. This study evaluates NUE in Tritordeum, a climate-resilient wheat–barley hybrid, using a holistic experimental approach that integrates pre- and post-harvest soil analyses, including an electrical conductivity (EC) assessment, plant and seed nutrient profiling, and an evaluation of yield performance and nitrogen ratio dynamics. Four treatments were tested: conventional urea (T1), urea with an urease inhibitor (NBPT) (T2), urea with a nitrification inhibitor (DCD) (T3), and an unfertilised control (C). While conventional urea achieved the highest yield (1366 kg ha^{-1}), enhanced-efficiency fertilisers (EEFs) improved nutrient synchronisation and seed nutritional quality. Specifically, EEFs increased seed zinc (T2: 34.93 mg/kg), iron (T1: 33.77 mg/kg), and plant potassium (T2: 1.66%; T3: 1.61%) content, and also improved nitrogen remobilisation (elevated Nplant/Nseed ratios). EEFs also influenced soil properties, increasing organic matter (T3: 2.75%) and EC (T3: 290.78 μS/cm). These findings suggest that while EEFs may not always boost yield in the short term, they contribute to long-term soil fertility and nutrient density in grain. This study underscores the importance of synchronising nitrogen availability with Tritordeum's phenological stages and highlights the crop's suitability for sustainable, low-input agriculture under climate variability.

Keywords: nitrogen use efficiency (NUE); enhanced-efficiency fertilisers (EEFs); urease inhibitor (NBPT); nitrification inhibitor (DCD); Tritordeum; Mediterranean agriculture; sustainable fertilisation; nutrient remobilisation; soil fertility; climate-resilient crops

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1. Introduction

N fertilisation plays a pivotal role in sustainable agricultural systems, influencing both crop productivity and environmental integrity. As an essential macronutrient, N supports plant functions such as chlorophyll synthesis, enzymatic activity, and protein formation [1–3]. However, N fertilisation, particularly in the form of conventional urea,

has been associated with considerable environmental drawbacks [4,5], including N losses through volatilisation, leaching, and denitrification, contributing to water and air pollution and N_2O emissions [6–8]. A recent review by Swify et al. (2023) [9] highlighted that up to 35% of applied urea is lost due to poor synchronisation with plant uptake, while Zhang et al. (2022) [10] added that excessive N application also accelerates soil acidification and disrupts its microbial balance. These environmental impacts underscore the urgency to improve NUE through innovative fertilisation strategies that align with the principles of sustainable agriculture and climate resilience.

In response to these concerns, enhanced-efficiency fertilisers (EEFs), such as urea-based formulations incorporating inhibitors, have been developed to improve NUE and mitigate environmental losses by slowing N release and improving its synchronisation with plant uptake [11]. Urease inhibitors such as N-(n-butyl) thiophosphoric triamide (NBPT) delay urea hydrolysis, improving N retention and reducing ammonia volatilisation losses, which can reach up to 40% of the applied N, particularly in hot and humid conditions [12,13]. Field studies and a meta-analysis have shown that NBPT application increases yields, reduces N losses, and decreases ammonia emissions by 53.2%, particularly in cereals like wheat [14–16]. Similarly, NIs such as dicyandiamide (DCD) and nitrapyrin slow the conversion of ammonium to nitrate, reducing N leaching and N_2O emissions [17,18]. Several studies have shown that NIs improve NUE, increase yields, and reduce environmental impacts in wheat, with Dawar et al. (2022) [19] reporting that nitrapyrin alongside urea enhanced both yield and NUE, while DCD reduced leaching and N_2O emissions, in intensive agricultural systems [20].

Numerous studies have examined NUE in cereals such as wheat, maize, and barley, using various approaches to evaluate the impact of urea-based fertilisers. These include pre- and post-harvest soil sampling to track N availability and transformations, as in Cowan et al. (2019) [21], who assessed N losses in intensively managed grasslands, and Xu et al. (2024) [22], who evaluated N uptake and translocation in wheat using stage-specific soil tests. The leaf chlorophyll index and N content in plant tissues are also widely used to assess N status [23], while grain yield and protein content often serve as indirect NUE indicators [24]. Other studies have focused on N losses through leaching or volatilisation [25–27], or have compared conventional urea with specific types of EEFs, such as urease or nitrification inhibitors [28–30]. Although these approaches offer valuable insights, they usually address isolated components, whether soil, leaves, or grain, without capturing the system as a whole. As a result, there is a clear gap in holistic approaches that simultaneously examine the soil–plant–seed continuum. To address this, the present study applies a comprehensive design combining baseline and post-harvest soil analyses, including EC assessment; macro- and micronutrient profiling in soil, plant tissues, and seeds; yield evaluation; and N ratio assessments. This holistic approach provides a multidimensional view into N flow and uptake dynamics, contributing practical evidence toward more sustainable N management practises.

Tritordeum (× Tritordeum Ascherson et Graebner), a hybrid of durum wheat (Triticum turgidum) and wild barley (Hordeum chilense), has emerged as a novel resilient cereal crop well suited to Mediterranean and semi-arid regions [31,32]. In response to increasingly frequent droughts, higher temperatures, and the need to reduce agriculture's environmental footprint, particularly in countries such as Greece, Spain, and Italy, interest in low-input, adaptable crops like Tritordeum has grown [33]. Tritordeum combines favourable traits from its parent lines, including a strong adaptability to harsh climates, good yields under limited irrigation, and promising nutritional qualities [34–37]. Under Mediterranean conditions, it has shown superior performance compared to durum wheat, particularly in drought-prone environments and nutrient-limited soils, where it maintains stable yields [35,38]. Its potential as a sustainable crop is further supported by its low input

requirements, resistance to common pests and diseases like yellow rust and aphids [39], and its suitability for organic systems due to its favourable rhizosphere microbiome [40]. Nutritionally, Tritordeum is rich in protein and bioactive compounds, including antioxidants, and contains lower levels of gluten than traditional wheat, making it a suitable alternative for individuals with gluten sensitivities [41,42]. These attributes make Tritordeum a promising candidate for meeting sustainability goals in food production, particularly within the European Union's Green Deal framework, which prioritises reductions in fertiliser and pesticide use by 2030 [33,43].

Despite its potential, Tritordeum remains underexplored in terms of optimising N management practises, especially under the use of EEFs such as urease and nitrification inhibitors. While traditional cereals like wheat and maize have been the focus of extensive research on NUE [14,15,17,18], Tritordeum has received far less attention in this context. A study by Aranjuelo et al. (2013) [44] reported that Tritordeum exhibits high nitrate reductase activity and efficient N remobilisation, suggesting its potential for improved NUE, yet little is known about how EEFs affect its yield, soil fertility, or grain nutritional quality. To address this gap, the present study compares the effects of conventional urea, urea combined with a urease inhibitor, and urea combined with a nitrification inhibitor, alongside an unfertilised control. A holistic experimental design is employed, integrating baseline and post-harvest soil analyses, including EC assessment; macro- and micronutrient quantification in soil, plant tissues, and seeds; crop yield measurement; and N ratio assessments. This comprehensive approach offers valuable insights into how stabilised N sources influence nutrient dynamics, crop performance, and NUE in a climate-resilient cereal under Mediterranean conditions. To our knowledge, this is the first study to comprehensively evaluate NUE-related parameters across the soil–plant–seed system in Tritordeum. Overall, this research aims to support the development of sustainable N management practises and reinforce Tritordeum's suitability for low-input, environmentally conscious farming systems.

2. Materials and Methods

2.1. Experimental Design

A field experiment was conducted at the experimental site of the Laboratory of Agronomy, located at the Agricultural University of Athens (37°59′01.83″ N, 23°42′07.37″ E, altitude: 30 m) from November 2023 to May 2024. To document the management history of the experimental area, it is noted that the field was cultivated with Tritordeum in the previous season, following earlier cultivations with wheat and barley in preceding years. According to the weather data provided by the National Observatory of Athens (2024, "Athens-Votanikos/Gazi region weather station data"—Link: http://meteo.gr/stations/athens/, accessed on 15 January 2025), the average temperature and precipitation are presented in Figure 1.

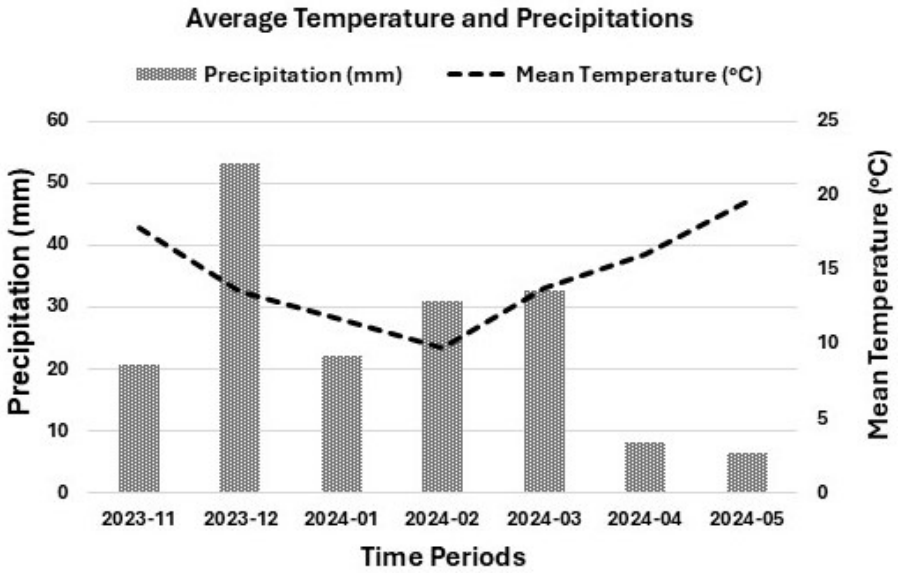

Figure 1. Average temperatures and precipitations throughout the duration of the experiment.

The experiment was conducted using a Randomised Complete Block Design, incorporating three distinct urea-based fertilisation treatments, each replicated four times. An unfertilised plot was used as the control treatment (C). The three urea-based fertilisation treatments were as follows: (T1) urea (46-0-0), (T2) urea (46-0-0) with a urease inhibitor [NBPT(N-(n-butyl) thiophosphoric triamide) 0.06%], and (T3) urea (46-0-0) with a nitrification inhibitor [3,4-dimethyl-1H-pyrazole phosphate (DMPP; 0.276%)]. Figure 2 provides a visual representation of the experimental design overlaid on a photo of the experimental field. The experimental field covered an area of 420 m^2, with each respective plot measuring 20 m^2. The sowing took place on November 27th (2023), with the seeds being sown at a depth of 2–3 cm and a spacing of 20 cm. Based on the planting layout, the seeding density was estimated at approximately 250 seeds per m^2. The fertiliser treatments were applied in two stages and the total rate was 175 kg N ha^{-1}. Basic application took place with the fertilisers being broadcasted at a rate of 115 kg N ha^{-1} and incorporated into the soil, and a second application took place 40 days after sowing, during the stem elongation stage, at a rate of 60 kg N ha^{-1}. Weeds were managed weekly by hand weeding, and the crop was harvested on 20 May 2024. Harvesting was executed by hand, harvesting the whole plot. Plant tissues and seeds from each treatment were collected for further analysis. Seed extraction was performed using a Wintersteger LD350.

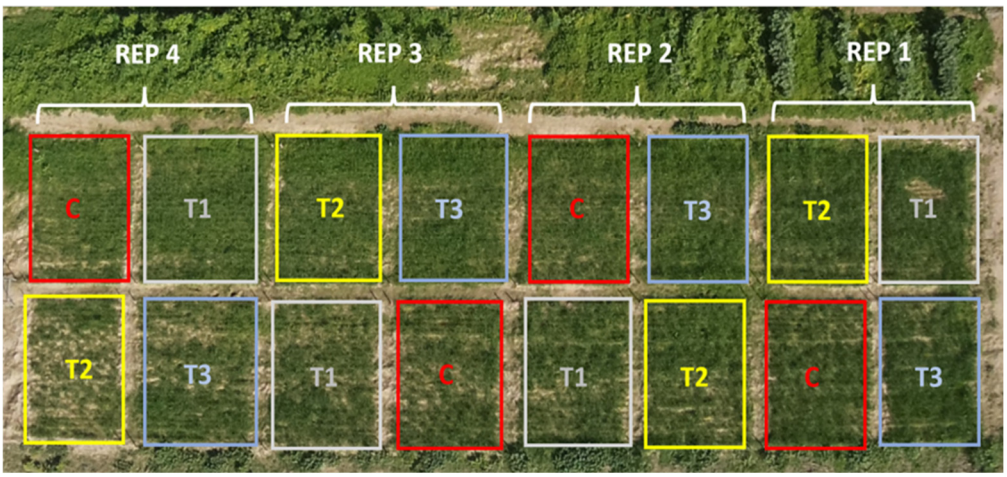

Figure 2. Experimental design.

2.2. Soil Sampling, Analysis, and Electrical Conductivity Assessment

Soil sampling was carried out in 2 phases. The first sampling took place prior to the installation of the experiment to determine the background values of the soil properties, providing a reference for assessing the impact of the treatments. A complete set of soil analyses was conducted to assess its baseline conditions, after which the field was divided into 16 experimental plots and seeding was carried out as planned. The second sampling was carried out, per plot, combined with the harvesting. For each plot, three soil samples were collected to evaluate any changes in soil nutrient content and other properties resulting from the fertilisation treatments and plant uptake over the growing season.

During both phases, the samples were collected from the experimental field and transferred in sterile plastic bags to the laboratory, where they were air-dried, passed through a 2 mm sieve, and kept for further analysis. Particle size distribution was determined following the hydrometer method according to Bouyoucos [45]. The resulting data were used to classify soil texture according to the USDA soil texture triangle, as described by Shirazi and Boersma, 1984 [46]. Total organic matter was analysed utilising the Walkley–Black dichromate oxidation method [47]. In addition, the soil pH and EC were measured electrometrically in a 1:1 (*w*/*v*) soil/water suspension using an automated pH-metre and conductivity-metre (Selecta 2000, J.P. Selecta S.A. Barcelona, Spain), according to Klute and Page (1982) [48]. To estimate available phosphorus, the Olsen method [49] was followed, with the measurements taken at 882 nm using a Shimadzu UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan). The total N concentration was determined using the Kjeldahl method according to Bremner (1960) [50], whereas exchangeable cations were extracted using ammonium acetate following the method described by Thomas (1982) [51]. The concentrations of copper (Cu), manganese (Mn), iron (Fe), and zinc (Zn) were measured using the DTPA extraction method in a soil/ammonium acetate suspension with a 1:2 (*w*/*v*) ratio. The extracted elements were determined using an atomic adsorption spectrometer (AA240FS, Varian, Middelburg, The Netherlands) [52].

2.3. Sampling and Analysis of Plant Tissues and Seeds

In parallel to soil sampling at the final stage, three plant samples were also collected randomly from each plot on harvest day. The plant samples were divided into leaves and seeds. Late-stage sampling was chosen to capture the redistribution of nutrients from the vegetative tissues, such as leaves and stems, to the reproductive tissues, including seeds. This redistribution was critical to better understand the nutrient mobility inside the plants, especially for key nutrients like N and phosphorus, which are commonly remobilised from leaves to seeds. Sampling at this stage also minimised temporary fluctuations that may have occurred earlier due to external factors, such as changes in weather or soil conditions, resulting in more stable and reliable data on overall nutrient uptake.

Following the analysis phase, the plant samples were dried in an oven (DHG-9203A) at 60 °C until a constant weight was obtained. Subsequently, the dried plant samples were ground to <0.5 mm utilising a stainless-steel mill. Macro- and micronutrient concentrations were determined using the wet digestion procedure described by Jones and Case (1990) [53]. Briefly, 0.5 g of dried, ground plant material was placed in a digestion tube and treated with ultra-pure concentrated HNO_3. The samples were covered and left to stand overnight at room temperature. Digestion was then carried out on a hot plate (125 °C) for 1 h. After slight cooling, 30% (*v*/*w*) H_2O_2 was added in drops until the solution became colourless, indicating complete digestion. The resulting digest was diluted to a volume of 25 mL with deionised water. This solution was then used for phosphorus determination following the molybdenum blue method of Murphy and Riley (1962) using a Shimadzu UV-1700 spectrophotometer [54]. The total concentrations of copper (Cu), manganese (Mn), iron (Fe), and zinc (Zn) in the extracted solutions were determined using an

atomic adsorption spectrometer (AA240FS, Varian, Middelburg, The Netherlands). The total N concentration in the plant tissues was quantified using the Kjeldahl procedure as mentioned by Nelson and Sommers (1980) [47].

2.4. Statistical Analysis

Statistical analysis was conducted with Statistica (version 10.0) to evaluate the effects of different fertilisation treatments on Tritordeum growth, nutrient uptake, and yield. One-way ANOVA was used to identify the differences among the treatments, while comparisons among the means were performed via Tukey's HSD test at a 5% probability.

3. Results

3.1. Baseline Soil Properties and EC

The background physicochemical parameters of the soil are illustrated in Table 1. Regarding soil texture, the soil was classified as a clay loam, with a mean pH of 7.96 and a mean EC of 219 μS/cm. The baseline soil properties were characterised by its available phosphorus of 30.65 mg/kg, exchangeable potassium of 285 mg/kg, and total nitrogen content of 0.19%. The soil organic matter (SOM) content was measured at 2.54%. Additionally, the micronutrient concentrations were within acceptable ranges, with Fe measured at 4.56 mg/kg, Cu at 8.94 mg/kg, Mn at 12.77 mg/kg, and Zn at 8.41 mg/kg.

Table 1. Basiline soil properties of the experimental site.

pH	EC	SOM	K	N	P	Fe	Cu	Mn	Zn
7.96	219	2.54	285.00	0.19	30.65	4.56	8.94	12.77	8.41

Details of Table 1: pH (1:1): Soil pH measured in a 1:1 soil/water ratio; EC: measured in microsiemens per centimetre (μS/cm); SOM: measured as a percentage of the soil composition; exchangeable K: potassium in the soil, measured in milligrammes per kilogramme (mg/kg); Total-N: total nitrogen content of the soil, measured as a percentage (%); P: measured in milligrammes per kilogramme (mg/kg); Fe, Cu, Mn, Zn: concentrations of iron, copper, manganese, and zinc, respectively, measured in milligrammes per kilogramme (mg/kg).

3.2. Post-Harvest Soil Properties and EC

At the end of the experiment, a soil analysis was conducted to determine the effects of N fertilisation on the soil properties, and the results are summarised in Tables 2 and 3. As expected, the pH ranged from 7.87 to 8.04, with a significantly lower value determined when the fertiliser added was urea, while no significant differences were identified among the other treatments. The lowest value of EC was measured in the control treatment, at 240.00 μS/cm, whereas the highest value was in the T3 treatment. Fertilisation significantly affected the EC, with the T2 and T3 treatments exhibiting significantly higher values compared to the control. According to the SOM, the range varied from 2.57 to 2.75%, with the minimum value obtained in the T2 treatment and the maximum value measured in T3.

Table 2. Physicochemical properties of the soil in all treatments at the final stage.

Treatments	pH	EC	SOM	P	K	N
C	8.03 ± 0.03 b	240.00 ± 18.07 a	2.61 ± 0.05 a	30.34 ± 1.71 b	253.33 ± 7.64 ab	0.18 ± 0.01 ns
T1	7.87 ± 0.04 a	272.44 ± 7.33 ab	2.66 ± 0.01 ab	25.09 ± 0.92 a	260.89 ± 4.64 ab	0.21 ± 0.01 ns
T2	8.04 ± 0.04 b	289.56 ± 1.35 b	2.57 ± 0.01 a	28.25 ± 1.47 ab	240.67 ± 12.69 a	0.20 ± 0.01 ns
T3	8.02 ± 0.04 b	290.78 ± 16.21 b	2.75 ± 0.04 b	26.35 ± 0.87 ab	270.44 ± 6.14 b	0.21 ± 0.01 ns
F-fertilisation	4.37 *	3.46 *	6.10 *	3.14 *	2.38 *	1.11 ns

Details of Table 2: pH (1:1); Soil pH measured in a 1:1 soil-to-water ratio; EC: measured in microsiemens per centimetre (μS/cm); SOM: measured as a percentage of the soil composition; P:

phosphorus available in the soil as determined by the Olsen extraction method, measured in milligrammes per kilogramme (mg/kg); exchangeable K: potassium in the soil, measured in milligrammes per kilogramme (mg/kg); Total-N: total N content of the soil, measured as a percentage (%); ns: not significant; *: significant at the 1% probability level; a, b, ab: letters indicate significant differences between the treatments within a column, where different letters denote statistically significant differences and similar letters or combinations denote no significant difference at the 5% level; F-fertilisation: represents the F-statistics from ANOVA tests evaluating the effects of the fertilisation treatments on the soil properties, and an asterisk (*) indicates significance at the 1% level; C: unfertilised control; T1: urea treatment; T2: urea with a urease inhibitor treatment; T3: urea with a nitrification inhibitor treatment.

The concentration of available phosphorus (Table 2) ranged from 25.09 to 30.34 mg/kg; the lowest value was shown when the fertiliser added was urea, which was significantly different from the control. The potassium concentrations in all treatments remained above 200 mg/kg, with the minimum value presented in the T2 treatment (240.67 mg/kg), while the maximum concentration was 270.44 mg/kg in the T3 treatment. Finally, there were no significant differences in total N content, ranging from 0.18% to 0.21%. Regarding micronutrient concentrations (Table 3), iron (Fe) ranged from 4.16 to 4.30 mg/kg and copper (Cu) from 8.51 to 8.64 mg/kg. The zinc (Zn) concentration was between 8.38 and 8.56 mg/kg, while the manganese concentration showed its highest value in the T3 treatment and its lowest concentration in the control, 12.66 to 12.79 mg/kg. Subsequently, there were no significant differences among the treatments.

Table 3. Micronutrient concentrations (Fe, Mn, Cu, Zn) in soil across all treatments at final stage.

Treatments	Fe	Cu	Mn	Zn
C	4.30 ± 0.14 ns	8.64 ± 0.21 ns	12.66 ± 0.07 ns	8.52 ± 0.14 ns
T1	4.16 ± 0.06 ns	8.51 ± 0.08 ns	12.71 ± 0.04 ns	8.63 ± 0.08 ns
T2	4.23 ± 0.04 ns	8.59 ± 0.06 ns	12.72 ± 0.01 ns	8.38 ± 0.07 ns
T3	4.29 ± 0.09 ns	8.63 ± 0.04 ns	12.79 ± 0.07 ns	8.56 ± 0.09 ns
F-fertilisation	0.59 ns	0.20 ns	1.00 ns	1.13 ns

Details of Table 3: Fe, Cu, Mn, Zn: Concentrations of iron, copper, manganese, and zinc, respectively, measured in milligrammes per kilogramme (mg/kg); ns: not significant; F-fertilisation: represents F-statistics from ANOVA tests evaluating the effects of the fertilisation treatments on soil properties; C: unfertilised control; T1: urea treatment; T2: urea with a urease inhibitor treatment; T3: urea with a nitrification inhibitor treatment.

3.3. Macro- and Micronutrient Quantification in Plant Tissues and Seeds

A plant tissue analysis was conducted after harvest to evaluate the effects of fertilisation on plant growth as well as macro- and micronutrient content. The results, presented in Table 4, indicate that fertilisation only significantly affected the potassium content, which varied between 0.99% and 1.66%. The treatments in which the fertilisation method included added inhibitors resulted in a significantly higher potassium content compared to the control. The total N content in plant tissues was between 0.65% and 0.86%, while the phosphorus concentrations varied between 0.40% and 0.50% without any significant differences among the four treatments.

Table 4. Macronutrient concentrations in plant tissue samples (dry weight) among treatments.

Treatments	N	P	K
C	0.67 ± 0.11 ns	0.50 ± 0.07 ns	0.99 ± 0.23 a
T1	0.68 ± 0.10 ns	0.40 ± 0.07 ns	0.98 ± 0.14 a
T2	0.86 ± 0.05 ns	0.40 ± 0.05 ns	1.66 ± 0.18 b
T3	0.65 ± 0.09 ns	0.46 ± 0.05 ns	1.61 ± 0.01 b
F-fertilisation	1.23 ns	0.915 ns	5.26 *

Details of Table 4: Fe, Cu, Mn, Zn: Concentrations of iron, copper, manganese, and zinc, respectively, measured in milligrammes per kilogramme (mg/kg); ns: not significant; F-fertilisation: Represents the F-statistics from ANOVA tests evaluating the effects of the fertilisation treatments on soil properties, where an asterisk (*) indicates significance at the 1% level; a, b: letters indicate significant differences between the treatments within a column, where different letters denote statistically significant differences and similar letters or combinations denote no significant difference at the 5% level; C: unfertilised control; T1: urea treatment; T2: urea with a urease inhibitor treatment; T3: urea with a nitrification inhibitor treatment.

In the case of micronutrients, the concentrations of copper and iron in the plant tissues ranged from 3.75 to 4.68 mg/kg and 18.76 to 29.60 mg/kg, respectively, with no significant differences among the treatments. Manganese concentrations were from 14.31 mg/kg in the dry plant tissue (in the fertilisation with urea) to 21.36 mg/kg in the control, showing a significant difference between these two treatments. Regarding Zn, the lowest concentration (27.36 mg/kg) was observed in the T3 treatment, which differed significantly from the T1 and T2 treatments. The highest concentration, 35.49 mg/kg, was found in the T2 treatment (Table 5).

Table 5. Micronutrient concentrations in plant tissue samples (dry weight) among the treatments.

Treatments	Fe	Cu	Mn	Zn
C	25.74 ± 3.85 ns	3.90 ± 0.56 ns	21.36 ± 0.50 b	30.88 ± 3.57 ab
T1	18.76 ± 2.28 ns	3.75 ± 0.24 ns	14.31 ± 2.41 a	35.28 ± 0.66 b
T2	26.63 ± 4.81 ns	4.68 ± 0.22 ns	18.78 ± 2.35 ab	35.49 ± 2.15 b
T3	29.60 ± 1.05 ns	4.07 ± 0.27 ns	16.69 ± 0.99 ab	27.36 ± 1.44 a
F-fertilisation	1.90 ns	1.31 ns	2.87 *	3.05 *

Details of Table 5: Fe, Cu, Mn, Zn: Concentrations of iron, copper, manganese, and zinc, respectively, measured in milligrammes per kilogramme (mg/kg); ns: not significant; *: significant at the 1% probability level; a, b, ab: letters indicate significant differences between the treatments within a column, where different letters denote statistically significant differences and similar letters or combinations denote no significant difference at the 5% level; F-fertilisation: represents the F-statistics from ANOVA tests evaluating the effects of the fertilisation treatments on soil properties, where an asterisk (*) indicates significance at the 1% level; C: unfertilised control; T1: urea treatment; T2: urea with a urease inhibitor treatment, T3: urea with a nitrification inhibitor treatment.

The results of the seed analysis are summarised in Table 6. As shown, fertilisation only significantly affected the N content among the macronutrients in the seeds. The highest N content (3.57%) was observed in the urea treatment, while the lowest content, 3.25%, was recorded when the added fertiliser was the urea with a nitrification inhibitor, showing a significant difference between these two treatments. Phosphorus (P) and potassium (K) concentrations showed no significant differences between the treatments, ranging from 0.62% to 0.73% and from 0.267% to 0.274%, respectively.

Table 6. Macronutrient concentrations in seeds (dry weight) among treatments.

Treatments	N	P	K
C	3.44 ± 0.120 ab	0.70 ± 0.060 ns	0.267 ± 0.003 ns
T1	3.57 ± 0.061 b	0.62 ± 0.030 ns	0.267 ± 0.014 ns
T2	3.41 ± 0.020 ab	0.65 ± 0.061 ns	0.274 ± 0.010 ns
T3	3.25 ± 0.091 a	0.73 ± 0.022 ns	0.267 ± 0.003 ns
F-fertilisation	2.44 *	0.520 ns	0.14 ns

Details of Table 6: N, P, K: Nitrogen, phosphorus, and potassium concentrations, measured as a percentage (%); ns: not significant; *: significant at the 1% probability level; a, b, ab: letters indicate significant differences between the treatments within a column, where different letters denote statistically significant differences and similar letters or combinations denote no significant difference at the 5% level; F-fertilisation: represents the F-statistics from ANOVA tests evaluating the effects of the fertilisation treatments on soil properties, where an asterisk (*) indicates significance at the 1% level; C: unfertilised control; T1: urea treatment; T2: urea with a urease inhibitor treatment; T3: urea with a nitrification inhibitor treatment.

For the micronutrients in the seeds, the results are illustrated in Table 7. Fe concentration showed significant differences, with the lowest concentration (25.29 mg/kg) obtained in the control, while the highest concentration (33.77 mg/kg) was measured in the urea treatment. Copper concentrations ranged from 3.98 mg/kg (control) to 4.18 mg/kg (T3 treatment), and manganese concentrations ranged between 17.75 mg/kg and 19.65 mg/kg. In terms of the Zn concentration in the seeds, the lowest concentration (32.41 mg/kg) was found in the urea treatment, while the highest value was 34.93 mg/kg in the T2 treatment.

Table 7. Micronutrient concentrations in seeds (dry weight) among treatments.

Treatments	Fe	Cu	Mn	Zn
C	25.29 ± 0.56 a	3.98 ± 0.10 ns	17.75 ± 0.95 ns	33.49 ± 1.72 ns
T1	33.77 ± 3.53 b	4.11 ± 0.17 ns	17.91 ± 0.82 ns	32.41 ± 1.43 ns
T2	23.92 ± 0.88 a	4.18 ± 0.19 ns	19.33 ± 0.26 ns	34.93 ± 0.33 ns
T3	27.19 ± 1.94 ab	4.16 ± 0.05 ns	19.65 ± 0.59 ns	34.15 ± 0.37 ns
F-fertilisation	4.40 *	0.41 ns	1.88 ns	0.86 ns

Details of Table 7: Fe, Cu, Mn, Zn: Concentrations of iron, copper, manganese, and zinc, respectively, measured in milligrammes per kilogramme (mg/kg); ns: not significant; *: significant at the 1% probability level; a, b, ab: letters indicate significant differences between the treatments within a column, where different letters denote statistically significant differences and similar letters or combinations denote no significant difference at the 5% level; F-fertilisation: represents the F-statistics from ANOVA tests evaluating the effects of the fertilisation treatments on soil properties, where an asterisk (*) indicates significance at the 1% level; C: unfertilised control; T1: urea treatment; T2: urea with a urease inhibitor treatment; T3: urea with a nitrification inhibitor treatment.

3.4. Yield Performance

As expected, fertilisation significantly affected Tritordeum's yield (Figure 3). Yields ranged from 1006 kg ha^{-1} in the unfertilised control (C) to 1366 kg ha^{-1} in the urea treatment (T1), which recorded the highest value. The yield in the treatment with the urease inhibitor (T2) was 1079 kg ha^{-1}, while the nitrification inhibitor treatment (T3) yielded 1209 kg ha^{-1}.

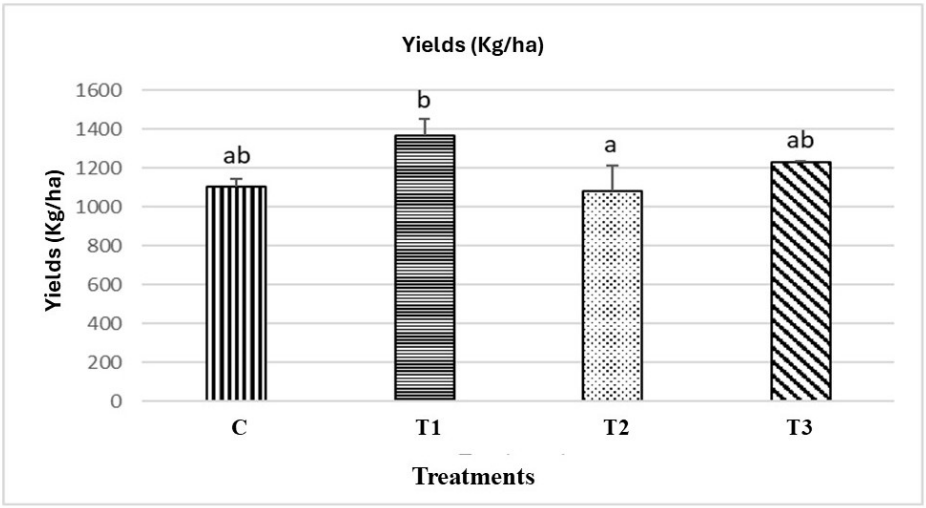

Figure 3. Mean yields among the treatments: C: unfertilised control; T1: urea treatment; T2: urea with a urease inhibitor treatment, T3: urea with a nitrification inhibitor treatment. The letters a, b indicate statistically significant differences among treatments.

3.5. Ratios of Nitrogen

The nitrogen ratios presented in Figure 4 provide insights into the distribution and remobilisation of nitrogen across the soil–plant–seed continuum under the different fertilisation treatments. The Nsoil (%)/Nplant (%) ratio was highest in the conventional urea treatment (T1), reaching 0.29, while the lowest value for this ratio was observed in the urease inhibitor treatment (T2), at 0.26. Regarding the Nplant (%)/Nseed (%) ratio, values ranged from 0.21 in T2 to 0.25 in the nitrification inhibitor treatment (T3), indicating slight variations in nitrogen remobilisation efficiency from the vegetative tissues to the seeds. These patterns reflect treatment-specific differences in nitrogen dynamics and uptake efficiency.

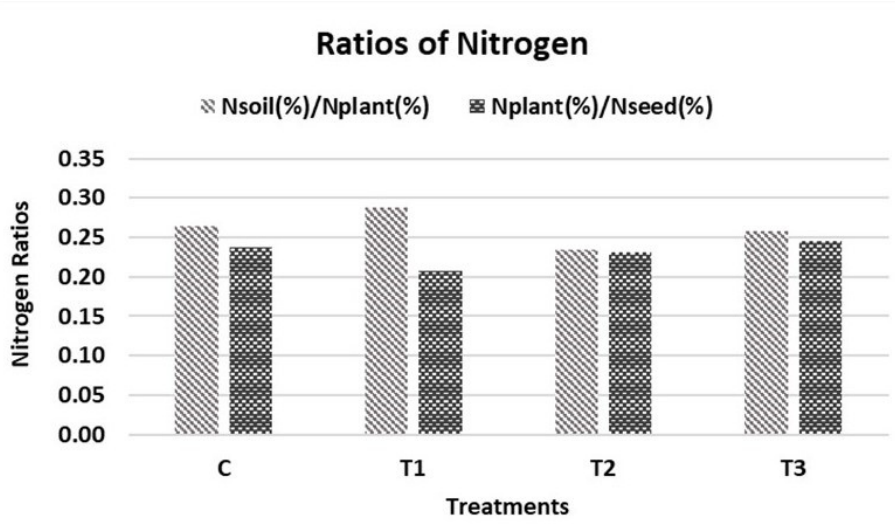

Figure 4. Ratios of nitrogen (Nsoil/Nplant and Nplant/Nseed): C: unfertilised control; T1: urea treatment; T2: urea with a urease inhibitor treatment, T3: urea with a nitrification inhibitor treatment.

3.6. Correlation Matrix Analysis

The correlation matrix presented in Figure 5 summarises the statistically significant relationships among the soil properties, plant and grain nutrient concentrations, and yield. At the treatment level, significant positive correlations were observed between 'Treat' and soil electrical conductivity (SEC) (r = 0.63 *), plant potassium (PK) (r = 0.65 *), and grain manganese (GMn) (r = 0.58 *). Soil pH (SpH) was positively correlated with grain zinc (GZn) (r = 0.65 *) and negatively correlated with grain iron (GFe) (r = −0.70). Soil potassium (SK) showed a negative correlation with plant zinc (PZn) (r = −0.64), while soil nitrogen (SN) was positively correlated with yield (r = 0.58 *) and negatively correlated with plant manganese (PMn) (r = −0.68 *). Among the soil micronutrients, soil iron (SFe) correlated positively with soil copper (SCu) (r = 0.71 *), which in turn showed a positive correlation with plant iron (PFe) (r = 0.57). In the plant and grain tissue variables, plant nitrogen (PN) showed positive correlations with grain manganese (GMn) (r = 0.64 *) and grain zinc (GZn) (r = 0.68 *). Similarly, plant phosphorus (PP) correlated positively with GMn (r = 0.58 *) and GZn (r = 0.72 *). Plant potassium (PK) showed strong positive correlations with grain manganese (GMn) (r = 0.84) and grain zinc (GZn) (r = 0.60 *). Negative correlations were observed between plant iron (PFe) and grain nitrogen (GN) (r = −0.68 *), as well as between plant manganese (PMn) and yield (r = −0.82 *). Lastly, a significant positive correlation was observed between grain manganese (GMn) and grain zinc (GZn) (r = 0.68).

	Treat	SpH	SEC	SOM	SP	SK	SN	SFe	SCu	SMn	SZn	PN	PP	PK	PFe	PCu	PMn	PZn	GN	GP	GK	GFe	GCu	GMn	GZn	Yield
Treat	**1.00**	0.17	**0.63***	0.53	0.34	0.36	0.50	0.04	0.11	0.52	0.04	0.01	0.16	**0.65***	0.38	0.18	0.27	0.36	0.55	0.28	0.22	0.08	0.28	**0.58***	0.24	0.08
SpH		**1.00**	0.15	0.13	0.46	0.10	0.26	0.46	0.41	0.13	0.10	0.45	0.49	0.32	0.52	0.12	0.55	0.28	0.44	0.16	0.23	**0.70****	0.12	0.55	**0.65****	0.55
SEC			**1.00**	0.21	0.26	0.05	0.52	0.40	0.35	0.43	0.39	0.06	0.05	0.40	0.15	0.45	0.24	0.04	0.13	0.25	0.11	0.10	0.25	0.20	0.01	0.06
SOM				**1.00**	0.18	0.49	0.47	0.31	0.32	0.18	0.34	0.31	0.13	0.02	0.02	0.02	0.22	0.32	0.24	0.53	0.46	0.20	0.18	0.01	0.01	0.24
SP					**1.00**	0.55	0.18	0.13	0.29	0.44	0.17	0.42	0.01	0.03	0.03	0.31	0.42	0.16	0.09	0.01	0.06	0.43	0.01	0.06	0.35	0.24
SK						**1.00**	0.24	0.23	0.44	0.19	0.44	0.26	0.30	0.09	0.10	0.40	0.12	**0.64***	0.26	0.26	0.54	0.07	0.21	0.13	0.12	0.20
SN							**1.00**	0.04	0.09	0.55	0.07	0.07	0.20	0.11	0.01	0.01	**0.68***	0.27	0.11	0.27	0.25	0.19	0.34	0.19	0.17	**0.58***
SFe								**1.00**	**0.71****	0.03	0.14	0.17	0.01	0.16	0.53	0.29	0.07	0.53	0.26	0.06	0.07	0.30	0.31	0.46	0.01	0.09
SCu									**1.00**	0.36	0.17	0.05	0.16	0.13	**0.57***	0.48	0.01	0.45	0.52	0.26	0.23	0.27	0.12	0.36	0.18	0.03
SMn										**1.00**	0.08	0.40	0.22	0.13	0.28	0.03	0.34	0.40	0.23	0.39	0.13	0.14	0.43	0.04	0.08	0.21
SZn											**1.00**	0.08	0.46	0.05	0.22	0.60*	0.29	0.11	0.34	0.56	0.57	0.29	0.16	0.07	0.32	0.49
PN												**1.00**	0.39	0.54	0.07	0.05	0.14	0.48	0.14	0.01	0.20	0.40	0.07	**0.64***	**0.68***	0.07
PP													**1.00**	0.40	0.08	0.31	0.13	0.17	0.25	0.32	0.18	0.09	0.09	**0.58***	**0.72****	0.13
PK														**1.00**	0.18	0.15	0.18	0.15	0.46	0.11	0.06	0.06	0.55	**0.84*****	**0.60***	0.10
PFe															**1.00**	0.15	0.06	0.36	**0.68***	0.40	0.12	0.50	0.04	0.45	0.32	0.27
PCu																**1.00**	0.38	0.01	0.23	0.30	0.26	0.21	0.22	0.13	0.08	0.36
PMn																	**1.00**	0.22	0.03	0.26	0.28	0.33	0.24	0.20	0.06	**0.82****
PZn																		**1.00**	0.26	0.16	0.20	0.20	0.05	0.01	0.32	0.05
GN																			**1.00**	0.54	0.46	0.44	0.07	0.51	0.54	0.06
GP																				**1.00**	0.53	0.08	0.07	0.24	0.45	0.34
GK																					**1.00**	0.37	0.46	0.11	0.34	0.22
GFe																						**1.00**	0.40	0.23	0.35	0.51
GCu																							**1.00**	0.34	0.04	0.43
GMn																								**1.00**	**0.68***	0.08
GZn																									**1.00**	0.19
Yield																										**1.00**

Figure 5. Correlation matrix for variables: pH: soil pH; SEC: soil electrical conductivity; SOM: soil organic matter; SP: soil phosphorus; SK: soil potassium; SN: soil nitrogen; SFe: soil iron; SCu: soil copper; SMn: soil manganese; SZn: soil zinc; PN: plant nitrogen; PP: plant phosphorus; PK: plant potassium; PFe: plant iron; PCu: plant copper; PMn: plant manganese; PZn: plant zinc; GN: grain nitrogen; GP: grain phosphorus; GK: grain potassium; GFe: grain iron; GCu: grain copper; GMn: grain manganese; GZn: grain zinc; ns: not significant; *: significant at the 1% probability level; **: significant at the 0.1% probability level; ***: significant at the 0.01% probability level.

4. Discussion

4.1. Effects of EEFs on Soil Properties and EC

The application of EEFs influenced key soil physicochemical properties, with the extent of change varying according to the type of inhibitor used. Soil pH values after harvest ranged from 7.87 to 8.04 (Table 2), compared to an initial baseline of 7.96 (Table 1), which did not reflect a substantial variation in soil ecosystems. The lowest pH was recorded in the urea-only treatment (T1: 7.87 ± 0.04), which may reflect a slight acidification likely linked to the hydrolysis of urea and the associated release of ammonium (NH_4^+), which can temporarily reduce soil pH [12,13]. Conversely, treatments incorporating inhibitors (T2 and T3) maintained pH levels close to the baseline, suggesting that EEFs helped buffer acidification by moderating N transformation rates. Soil EC also showed a clear treatment effect ($F = 3.46$; $p < 0.05$), increasing from the baseline of 219 μS/cm (Table 1) to as high as 290.78 ± 16.21 μS/cm in T3 and 289.56 ± 1.35 μS/cm in T2 (Table 2). In contrast, the unfertilised control remained lower at 240.00 ± 18.07 μS/cm. This increase in EC may indicate a higher ionic concentration in soils treated with inhibitors, likely due to a slower N transformation and the prolonged presence of NH_4^+ in the rhizosphere [18]. It also suggests a better synchronisation of nutrient availability with plant uptake, as similarly demonstrated in dryland maize using slow-release N strategies [55].

The SOM content increased slightly in most treatments, rising from the baseline value of 2.54% (Table 1) to 2.75% in T3 (Table 2) ($F = 6.10$; $p < 0.05$). This increase under the nitrification inhibitor treatment could reflect enhanced microbial activity or reduced organic matter mineralisation, processes supported by more stable N cycling [17]. The lowest SOM was recorded in T2 (2.57%), while the control and T1 treatments showed intermediate values, indicating that EEFs may have differential effects on SOM dynamics depending on the type of inhibitor used. While the total soil N content did not significantly differ among the treatments ($p > 0.05$) and was close to the baseline level of 0.19% (Table 1), the levels were marginally higher in the fertilised plots (T1 and T3: 0.21%) compared to the control (0.18%) (Table 2). This suggests improved N retention, though the short duration of the trial may limit observable accumulation. These findings are consistent with the hypothesis that EEFs primarily affect N transformation and availability rather than total N input [18].

Phosphorus (P) and potassium (K) availability also responded to fertilisation. The available P declined in all fertilised plots compared to the baseline of 30.65 mg/kg (Table 1), with the lowest value observed in T1 (25.09 ± 0.92), significantly lower than the control (30.34 ± 1.71) (Table 2). This reduction may be explained by competitive interactions between NH_4^+ and phosphate ions, as reported by Silva et al. (2017) [16] and Rietra et al. (2017) [56]. On the other hand, the highest K concentration was observed in T3 (270.44 ± 6.14), which may result from improved nutrient synchrony under the stabilised N regime. Similar trends have been reported in cereals under slow-release fertiliser applications [55], supporting the view that stabilised N inputs can enhance K availability. The micronutrient concentrations in the soil (Fe, Cu, Mn, Zn) did not show statistically significant differences among the treatments (Table 3) and baseline values (Table 1), indicating that the fertilisers used did not alter micronutrient bioavailability within the timeframe of this study. This stability aligns with expectations, as none of the treatments included micronutrient inputs, and transformations of trace elements are generally slower and more buffered in mineral soils [56].

Together, these findings confirm that EEFs, particularly those containing nitrification inhibitors, can positively influence soil chemical dynamics by enhancing nutrient retention, moderating acidification, and promoting SOM maintenance. While the short-term effects on total N and micronutrients were limited, the observed shifts in EC, P, K, and

SOM suggest that EEFs may support longer-term improvements in soil fertility and sustainability when applied under appropriate conditions.

4.2. Effect of EEFs on Tritordeum's Plant Tissue Nutrients

Fertilisation with EEFs significantly influenced potassium uptake in plant tissues, with both the urease inhibitor (T2) and nitrification inhibitor (T3) treatments resulting in marked increases of 67% and 68%, respectively, compared to the control (T2: 1.66 ± 0.18, T3: 1.61 ± 0.01%, C: 0.99 ± 0.23) (Table 4). This enhanced K accumulation may be attributed to reduced ammonium antagonism and improved root activity under stabilised N release, as suggested by Matczuk and Siczek (2021) [57] and Rietra et al. (2017) [56]. In contrast, potassium content was lowest under conventional urea (T1: 0.98 ± 0.14), which may be due to the transient accumulation of ammonium from rapid urea hydrolysis, known to interfere with K uptake [57]. Although the total N concentration in the plant tissues did not differ significantly between the treatments ($p > 0.05$), T2 showed the highest mean N value (0.86 ± 0.05) (Table 4), representing a 28% increase relative to the unfertilised control (0.67 ± 0.11). This trend aligns with the previous research by Hube et al. (2017) [58], who reported up to a 33% greater N uptake in crops treated with NBPT-stabilised urea, indicating improved N assimilation through delayed urea hydrolysis and reduced losses.

The micronutrient uptake patterns further revealed important interactions between N dynamics and trace element availability. Manganese concentration was significantly higher in the control (21.36 ± 0.50) and in T2 (18.78 ± 2.35) compared to the urea-only treatment (T1: 14.31 ± 2.41) (Table 5). This suggests that EEFs, particularly the urease inhibitor, may sustain favourable rhizosphere conditions, such as mild acidification through ammonium retention, that enhance Mn solubility and uptake [56]. Conversely, higher nitrate levels in urea-only plots may have limited Mn bioavailability due to less acidic root zone conditions [16]. Zinc concentrations were also significantly affected by the treatments. The highest Zn content in plant tissues was found in T2 (35.49 ± 2.15), while the lowest was observed in the T3 treatment (27.36 ± 1.44) (Table 5). The enhanced Zn uptake under the NBPT treatment may stem from improved root morphology or microbial-mediated nutrient mobilisation, as previously reported by Matczuk and Siczek (2021) [57]. While the nitrification inhibitor in T3 sustained higher soil K levels (Table 2), it may have altered rhizosphere microbial activity or pH in a way that reduced Zn availability.

In contrast, copper and iron concentrations did not show statistically significant differences among the treatments (Table 5), indicating that the short-term use of EEFs did not strongly affect the uptake of these elements, consistent with the soil micronutrient data (Table 3), which showed no significant changes in Cu and Fe availability post-harvest. This reinforces the idea that the observed variations in nutrient concentrations were primarily driven by N-related interactions rather than direct micronutrient supply from the fertilisers.

Overall, these findings demonstrate that while EEFs may not drastically alter total N uptake in above-ground tissues, they significantly influence potassium, manganese, and zinc dynamics, nutrients critical for enzymatic function, stress resilience, and grain quality in Tritordeum. This highlights the importance of considering macronutrient–micronutrient interactions when designing N fertilisation strategies for sustainable cropping systems.

4.3. Effect of EEFs in Tritordeum's Seed Nutrients

Among the seed macronutrients, N was the only element significantly affected by fertilisation (Table 6). Contrary to expectations, the highest seed N concentration was observed in the conventional urea treatment (T1: 3.57 ± 0.06), while the lowest was in the nitrification inhibitor treatment (T3: 3.25 ± 0.09) (Table 6). This finding contrasts with

previous studies in wheat, which often report enhanced grain N content under EEFs [19]. One possible explanation lies in Tritordeum's physiological traits: Aranjuelo et al. (2013) [44] highlighted the species' elevated nitrate reductase activity and efficient N remobilisation, suggesting that early N availability may be more important than sustained release. Since urease and nitrification inhibitors delay N availability, they may have failed to meet the crop's N demands during critical early developmental stages, ultimately limiting N accumulation in seeds.

The phosphorus (P) and potassium (K) concentrations in the seeds did not differ significantly across the treatments (P range: 0.62–0.73%; K range: 0.267–0.274%) (Table 6). This stability reflects the general trend observed in the soil and plant tissue data, where macronutrient responses to EEFs were more pronounced for N and potassium but less so for phosphorus. Regarding micronutrient accumulation (Table 7), the seed iron (Fe) concentration was significantly higher in T1 (33.77 ± 3.53) compared to the control (25.29 ± 0.56) and T2 (23.92 ± 0.88), while zinc (Zn) content peaked in T2 (34.93 ± 0.33). These results highlight that the fertilisation strategy not only affects macronutrient uptake but also alters the internal allocation of trace elements to seeds. The enhanced Zn accumulation in the urease-inhibited treatment aligns with the pattern observed in plant tissue (Table 5), suggesting that NBPT-stabilised N may improve root function and micronutrient uptake, as supported by Matczuk and Siczek (2021) [57]. Conversely, the elevated Fe levels in the conventional urea treatment could be attributed to early N availability enhancing root oxidation capacity, which would facilitate Fe uptake and translocation.

These observations echo findings by Ghafoor et al. (2022) [59], who reported that synchronised and balanced N regimes improved seed nutritional quality in wheat. The relatively stable Mn and Cu concentrations across the treatments (Mn: 17.75–19.65 mg/kg; Cu: 3.98–4.18 mg/kg) (Table 7) further confirm that short-term use of EEFs has selective effects on seed micronutrient profiles, likely mediated by N availability and its influence on plant physiological and biochemical pathways. Overall, the results emphasise the importance of N timing over total N application when aiming to enhance seed quality in Tritordeum. The differential accumulation of N, Fe, and Zn in seeds across treatments underscores the complex relationship between fertilisation strategy, nutrient remobilisation, and seed nutrient composition in this climate-resilient crop.

4.4. Nitrogen Ratios, Yield Performance, and Implications for NUE

The N ratios (Figure 4) calculated in this study provide valuable insights into the dynamics of N distribution within the soil–plant–seed system under different fertilisation treatments. The ratios of Nsoil/Nplant and Nplant/Nseed reflect the efficiency of N uptake and remobilisation, both of which are critical for understanding NUE in Tritordeum. The results showed that the highest Nsoil/Nplant ratio was observed in the urea treatment (T1 = 0.29), suggesting that a greater proportion of N remained in the soil rather than being taken up by the plant compared to the urease (T2 = 0.23) and nitrification inhibitor treatments (T3 = 0.26). On the contrary, the inhibitor treatments implied that less N remained in the soil, likely due to the inhibitors' ability to gradually release N to the crops. However, in Tritordeum, this gradual release of N did not translate into higher yields, as the highest value was shown in the urea treatment (T1). This is evidence that direct urea application may provide better N availability during critical growth stages.

Interestingly, the Nplant/Nseed ratio remained relatively stable across the treatments. However, the slightly higher Nplant/Nseed ratios in the treatments with inhibitors (T2 = 0.23 and T3 = 0.25) suggest that slow-release fertilisers may facilitate greater N accumulation in seeds compared to urea-based fertilisers (T1 = 0.21). Similar findings were reported by Ghafoor et al. (2022) [59] in wheat, where optimal N application and slow-release fertilisers enhanced seed nutritional quality, whereas monotypic urea application

resulted in lower N concentrations. These findings underscore the importance of optimising N partitioning within the soil–plant–seed continuum to enhance NUE in Tritordeum.

While N ratios provide insight into nutrient assimilation and uptake, it is crucial to assess how these dynamics translate into the final yield (Figure 3). Our findings indicate that N fertilisation significantly influenced Tritordeum yield, with the highest yield observed in the urea treatment (T1), while the treatments incorporating urease (T2) and nitrification inhibitors (T3) did not outperform the unfertilised control (C). This unexpected outcome suggests that for winter cereals like Tritordeum, the effectiveness of EEFs may be limited under Mediterranean conditions, or that the fertilisation strategy employed in this study (i.e., split application with a basic and a secondary dose) may require optimisation. As an example, Guo et al. (2021) [55] concluded that using a blend of urea and slow-release N fertiliser was the best strategy for sustainable dryland maize production, as it enhanced yield, improved N use efficiency, and reduced ammonia volatilisation and nitrate leaching.

A possible reason why EEFs might not enhance yield in Tritordeum could be related to the timing and rate of N availability. Winter cereals exhibit specific N uptake dynamics, where early-season N availability is crucial for tillering and later applications primarily support grain filling [44,60]. Urease inhibitors, such as NBPT, delay the hydrolysis of urea into ammonium, reducing ammonia volatilisation losses but potentially limiting N availability during the critical early growth stages. Similarly, nitrification inhibitors slow the conversion of ammonium to nitrate, which can be beneficial in minimising N leaching and nitrous oxide emissions but may delay nitrate availability when plants require rapid N uptake [16,19].

Our findings align with previous studies that have reported limited yield benefits from EEFs in winter cereals under Mediterranean conditions. Landolfi et al. (2021) [42] found that while N fertilisation increased grain protein content in Tritordeum, it had no significant effect on grain yield, suggesting that N availability at critical growth stages may be more influential than total N supply. Similarly, research in wheat has shown that the yield response to inhibitors is highly dependent on soil type, climate conditions, and application strategy [6,12]. For instance, studies in Mediterranean environments have suggested that inhibitors may be more effective in sandy soils prone to N leaching, while in clay loam soils, such as those in our experiment, the natural retention of ammonium may reduce their impact [17].

Another important consideration is the fertilisation regime. In this study, N was applied in two stages: a basal application of 115 kg N ha^{-1} and a second dose of 60 kg N ha^{-1} during stem elongation. While this strategy is commonly used in winter cereals cultivated under Mediterranean conditions, alternative application methods, such as a more gradual or mixed fertilisation N supply, may improve NUE and yield [61]. For example, studies in wheat have suggested that post-anthesis N applications can enhance grain filling and yield, particularly in crops with a high N remobilisation capacity, such as Tritordeum [44]. Additionally, recent research in Mediterranean wheat systems has indicated that split applications beyond two doses, or the use of controlled-release fertilisers, may optimise N availability across key phenological stages [34].

Overall, the integration of N ratios and yield performance highlights the complexity of NUE in Tritordeum under Mediterranean conditions. While EEFs demonstrated potential in improving N remobilisation (as indicated by the slightly higher Nplant/Nseed ratios in T2 and T3), this did not translate into yield benefits. The superior performance of conventional urea suggests that timely N availability remains more critical than prolonged N release for optimising NUE in this crop. These findings reinforce the importance of tailoring fertilisation strategies to Tritordeum's phenological needs in order to enhance both productivity and N efficiency.

4.5. Correlation Matrix Analysis

The correlation matrix (Figure 5) provided additional insights into the relationships between the soil, plant, and grain parameters, supporting a system-level understanding of nutrient dynamics under different nitrogen fertilisation strategies. The significant positive correlation between soil nitrogen (SN) and yield (r = 0.58 *) supports the view that N availability remains a critical driver of crop productivity, in line with earlier studies [42,44]. Interestingly, grain nitrogen (GN) was not significantly correlated with yield, suggesting that the final N translocation to the seeds may not directly influence productivity under the tested fertilisation regimes. Meanwhile, the significant negative correlation between SN and plant manganese (PMn) (r = −0.68 *), alongside the strong negative correlation between PMn and yield (r = −0.82 *), may reflect antagonistic interactions or nutrient imbalances limiting productivity. Treatment-level effects were also evident, with 'Treat' showing positive correlations with soil electrical conductivity (SEC) (r = 0.63), plant potassium (PK) (r = 0.65 *), and grain manganese (GMn) (r = 0.58 *), indicating nutrient availability shifts under different fertiliser types.

Furthermore, strong associations were observed between the macronutrients and micronutrients in vegetative tissues and grains. PK was significantly correlated with both GMn (r = 0.84 *) and grain zinc (GZn) (r = 0.60 *), highlighting efficient nutrient remobilisation during reproductive growth. Similar relationships were found for plant nitrogen (PN) and plant phosphorus (PP), which both correlated positively with GZn (r = 0.72 *) and GMn (r = 0.58 *). Lastly, GMn and GZn were positively correlated (r = 0.68 *), suggesting potential co-mobilisation or coordinated deposition in the seed during grain filling. These findings, along with the negative correlation between plant Fe and grain N (r = −0.68 *), underscore that NUE in Tritordeum is governed not only by nitrogen availability but also by the balance of micronutrient flows within the soil–plant–seed continuum, as previously suggested by Ghafoor et al. (2022) [59] and Aranjuelo et al. (2013) [44].

5. Conclusions

This study provides a comprehensive evaluation of NUE in Tritordeum under Mediterranean conditions, employing a holistic experimental approach that integrates pre- and post-harvest soil analyses, soil EC, plant tissue and seed nutrient profiling, yield performance, and N ratio dynamics. By simultaneously assessing multiple compartments of the soil–plant–seed continuum, this study offers valuable insights into how different N fertilisation strategies, namely with conventional urea, a urease inhibitor (NBPT), or a nitrification inhibitor (DCD), influence nutrient uptake, remobilisation, and crop productivity.

The results revealed that conventional urea application led to the highest grain yield (1366 kg ha^{-1}), demonstrating the importance of immediate N availability during early crop development. In contrast, EEFs such as urease and nitrification inhibitors, despite not boosting yields (T2: 1079 kg ha^{-1}; T3: 1209 kg ha^{-1}), showed clear benefits in nutrient partitioning and synchronisation. The treatments with inhibitors facilitated greater potassium uptake in the plants (T2: 1.66%; T3: 1.61%; C: 0.99%) and improved N remobilisation to the seeds, as indicated by higher Nplant/Nseed ratios (T2 and T3 compared to T1 and C). Moreover, the seed micronutrient profiles were favourably influenced by EEFs, with notable increases in zinc (T2: 34.93 mg/kg) and iron content (T1: 33.77 mg/kg), suggesting that fertilisation strategies also impact nutritional quality.

Beyond plant-level responses, this study highlighted significant interactions among soil properties, such as organic matter content (T3: 2.75%; baseline: 2.54%), EC (T3: 290.78 μS/cm; C: 240.00 μS/cm), and macronutrient retention, particularly in treatments with EEFs. While short-term yield gains were not observed with the EEFs, these findings point toward their potential role in promoting long-term soil health and nutrient stability. Such

contributions are essential for developing resilient cropping systems that maintain productivity while preserving the agroecosystem, especially in regions prone to climatic stress and nutrient depletion. Targeted N strategies combining fast- and slow-release fertilisers, timed to Tritordeum's phenology, hold promise for maximising both productivity and sustainability.

In conclusion, while EEFs in Tritordeum under Mediterranean conditions may not universally outperform conventional urea in terms of yield, their role in enhancing nutrient synchrony, maintaining soil fertility (e.g., SOM increase in T3: 2.75%), and promoting more nutrient-dense grain should not be overlooked. Future research should explore site-specific, phenology-aligned application strategies, as well as multi-seasonal trials, to better understand the long-term benefits of EEFs on soil carbon, microbial health, and agroecosystem resilience. These findings contribute practical evidence to support sustainable N management and strengthen the case for Tritordeum as a low-input, nutrient-efficient cereal crop, particularly suited for the environmentally and climatically vulnerable Mediterranean region.

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Abbreviations

N	Nitrogen
NUE	Nitrogen Use Efficiency
EC	Electrical Conductivity
NBPT	N-(n-butyl) thiophosphoric triamide
EEFs	Enhanced-Efficiency Fertilisers
DCD	Dicyandiamide
NIs	Nitrification Inhibitors
SOM	Soil Organic Matter
N_2O	Nitrous Oxide
DTPA	Diethylenetriaminepentaacetic Acid
HNO_3	Nitric Acid
H_2O_2	Hydrogen Peroxide
C	Control Treatment
T1	Urea (46-0-0)

T2	Urea with a urease inhibitor
T3	Urea with a nitrification inhibitor

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