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Nitrogen inhibitors as a tool for improving soil nitrogen retention and maize yield under unstable moisture

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ABSTRACT

Nitrogen losses from agricultural soils represent a major environmental challenge, particularly under unstable moisture, where intensified nitrification and leaching reduce nitrogen use efficiency and increase the risk of environmental contamination. This study assessed the effects of nitrogen inhibitors and nitrogen-stabilizing agents on soil mineral nitrogen dynamics (NH_4^+-N and NO_3^--N) and maize grain yield under rainfed conditions in the left-bank forest-steppe of Ukraine during 2023–2025. Field experiments included four maize hybrids and five treatments: a humic substances-based stabilizer (T1), two DMPP-based nitrification inhibitor formulations (T2–T3), an Azotobacter-based biological product (T4), and a nitrapyrin-based inhibitor (T5), compared with a control (T0). Application of the tested products increased the soil pool of mineral nitrogen, indicating improved mineral N retention in the root zone under unstable moisture. The strongest effect was observed for T1, which increased NO_3^--N by 7.48 mg kg^{-1} (≈46% vs. T0) and NH_4^+-N by 6.18 mg kg^{-1} (≈38%). Overall, NO_3^--N increased by 14–46% and NH_4^+-N by 12–38% relative to T0; the smallest increases were observed for T3. Improved soil N retention was associated with increased maize grain yield, with the highest average increase for T1 (1.18 t ha^{-1}; ≈13%), followed by T5 (≈10%). Treatment effectiveness depended on inhibitor type, maize hybrid, and interannual climatic variability, emphasizing the need for environmentally adapted nitrogen management strategies.

Keywords: maize, nitrogen inhibitors, nitrogen stabilization, soil mineral nitrogen, nitrate nitrogen, ammonium nitrogen, unstable moisture.

INTRODUCTION

Nitrogen nutrition plays a key role in maize productivity; however, the inefficient use of nitrogen fertilizers remains one of the major environmental challenges in modern agriculture. Excess nitrogen input is associated with gaseous losses (N_2O and NH_3 emissions), nitrate leaching, and reduced nitrogen use efficiency, which together increase environmental pressure on agroecosystems. Beyond direct yield penalties, nitrogen losses translate into off-site environmental impacts, including nitrate contamination of water bodies and increased greenhouse gas emissions. Field evidence shows that management factors such as fertilizer source, placement, and additive use can substantially modify NH_3 volatilization and N_2O fluxes in maize systems, thereby affecting both nitrogen use efficiency and environmental performance (Woodley et al., 2018; Woodley et al., 2020). Therefore, optimizing nitrogen management strategies is essential to improve fertilizer efficiency while minimizing adverse environmental impacts (Muller et al., 2023; Alonso-Ayuso et al., 2016).

One of the widely discussed approaches to improving nitrogen retention in soils is the use of nitrification inhibitors and nitrogen conservation products. These compounds slow down the microbial transformation of ammonium into nitrate, thereby reducing nitrogen losses and potentially enhancing crop uptake. For example, Muller et al. (2023) demonstrated that urea coated with 3,4-dimethylpyrazole phosphate (DMPP) applied at a 20% reduced nitrogen rate decreased N_2O emissions by 51% without reducing yield. Similarly, Alonso-Ayuso et al. (2016) reported that the repeated use of DMPP allowed a reduction in nitrogen fertilizer rates by up to 23%, indicating its potential for environmentally efficient fertilization. Under practical farming conditions, additive performance is additionally shaped by the fertilizer form and application technique. On-farm and field evaluations have shown that different nitrogen fertilizer additives may produce contrasting outcomes depending on soil type and management, ranging from clear agronomic benefits to mainly environmental effects without consistent yield gains (Barker & Sawyer, 2017; Jackson, 2019).

However, the effectiveness of nitrification inhibitors is not always consistent. Wang et al. (2020) showed that DMPP application reduced nitrification rates in the rhizosphere but negatively affected maize growth and water use efficiency under drought stress, while simultaneously reducing NH_3 emissions and increasing N_2O emissions. These findings suggest that the environmental and agronomic outcomes of inhibitor application may depend strongly on soil conditions, climatic factors, and crop physiological responses (Lasisi et al., 2021).

Several studies indicate that yield responses to nitrification inhibitors are often modest and highly variable. Yield increases of approximately 4–7% have been reported under high-temperature conditions, while more pronounced effects were observed only under favorable environmental conditions (Lucas et al., 2019; Zhao et al., 2017). Comparable results were obtained for other compounds based on dimethylpyrazole derivatives, where nitrogen leaching was reduced by about 25% but no significant differences in plant growth were observed (Allende-Montalbán et al., 2022). In contrast, some studies reported substantial increases in root biomass (up to 64%) without a clear yield response, highlighting the complexity of nitrogen–plant–soil interactions (Guardia et al., 2018a; Guardia et al., 2018b).

Nitrapyrin- and pronitridine-based inhibitors have also been extensively studied as tools for mitigating environmental nitrogen losses. These compounds inhibit the activity of *Nitrosomonas* bacteria, thereby reducing nitrification rates and associated nitrogen emissions. Yield increases ranging from 3.4 to 19% have been reported, depending on application timing and environmental conditions (Ren et al., 2022; Dawar et al., 2021). Nevertheless, concerns remain regarding their long-term environmental performance, as reductions in one nitrogen loss pathway may be offset by increases in another (Woodward et al., 2021; Omonode and Vyn, 2019).

The effectiveness of nitrogen inhibitors is strongly governed by weather conditions and soil properties. Similar patterns have been reported for maize production systems under unstable moisture regimes, where nitrogen availability and uptake efficiency are closely linked to water supply during critical growth stages. In field experiments conducted in the Forest-Steppe zone of Ukraine, fertigation with urea–ammonium nitrate significantly affected maize seed yield; however, yield formation remained highly sensitive to interannual climatic variability and water availability (Marenych et al., 2024). These findings indicate that even technologically advanced nitrogen application methods cannot fully compensate for moisture-related limitations, thereby emphasizing the relevance of nitrogen stabilization strategies under rainfed conditions.

Under such conditions, nitrogen inhibitors may serve as a complementary tool for stabilizing soil nitrogen transformations and improving nitrogen retention under variable moisture availability. Late application of nitrapyrin has been shown to enhance certain yield components without affecting thousand-kernel weight (Rácz et al., 2021), whereas under rainy conditions inhibitors may not increase yield but can still alter soil nitrogen dynamics (Guardia et al., 2018a). Positive environmental effects have also been reported in chernozem and poorly drained clay soils, where the use of inhibitors reduced NH_3 volatilization and N_2O emissions (Hao et al., 2023; Martins et al., 2017). In addition, inhibitor-based approaches have been reported to delay nitrification and reduce nitrogen losses when combined with different nitrogen carriers (urea, UAN, or anhydrous ammonia), which may improve the retention of mineral N in the root zone under rainfed maize production (Degenhardt et al., 2016; Drury et al., 2017).

The environmental rationale for inhibitor use is further supported by studies demonstrating shifts in soil nitrogen species and nutrient uptake patterns in maize in response to nitrification suppression, including changes in mineral N pools and associated plant nutrient acquisition (Vogel et al., 2020). Despite extensive research, the agronomic and environmental benefits of nitrogen inhibitors remain controversial. Meta-analyses indicate both positive and neutral yield responses, while highlighting strong dependence on soil–climatic conditions, fertilizer type, and management practices (Cook et al., 2015; Thapa et al., 2016). In some cases, nitrogen inhibitors improved nitrogen retention without increasing yield, which raises questions regarding their overall agronomic effectiveness (Pawlick et al., 2019; Santos et al., 2023).

Consequently, effective nitrogen management remains a global challenge. Although nitrogen inhibitors are theoretically well grounded as tools for reducing nitrogen losses and improving fertilizer efficiency, field results are often inconsistent and difficult to interpret. This uncertainty is particularly relevant for regions with unstable moisture regimes, where water stress can strongly modify nitrogen transformations in soil. In Ukraine, the application of nitrogen inhibitors is still relatively new, and systematic field-based evaluations under local soil and climatic conditions remain limited.

Working hypothesis and objectives

We hypothesized that, under rainfed maize production in an unstable moisture regime, nitrogen inhibitors and nitrogen-stabilizing agents can reduce nitrogen losses by slowing nitrification and/or modifying microbially mediated N transformations, thereby increasing the retention of mineral N forms (NH_4^+ and NO_3^-) in the root zone and improving grain yield. The objectives were: (i) to determine the significance of inhibitor effects on soil nitrate and ammonium nitrogen contents; (ii) to quantify maize yield response to inhibitor application across contrasting years (2023–2025); and (iii) to evaluate whether yield response depends on hybrid genotype under the conditions of the Left-Bank Forest-Steppe of Ukraine.

MATERIALS AND METHODS

Plant material and experimental conditions. Field experiments were conducted in production-field conditions in the left-bank forest-steppe of Ukraine during 2023–2025 under rainfed management characterized by unstable moisture supply. The plant material included maize hybrids widely represented on the Ukrainian market: Pioneer 9241 (FAO 360), DKC4964 (FAO 380), EC Sensor (FAO 370), and MAS 30.K (FAO 280).

Experimental design. A two-factor field experiment was established in a randomized complete block design with three replications. Factor A was maize hybrid, and factor B was nitrogen inhibitor/stabilizer treatment. The accounting plot area was 100 m². During the growing season, visual crop observations were conducted to record general plant status. Background fertilization and agronomic management were kept identical across treatments to ensure comparability.

Nitrogen inhibitor/stabilizer treatments and active agents. The study used nitrogen inhibitors/stabilizers with different modes of action on soil biota and nitrogen transformation processes. To improve clarity and avoid reliance on local commercial names, treatments are described primarily by their active agents:

1. A humic substances-based nitrogen stabilizer containing humic, fulvic, and ulmic acids (16%, 3%, and 1%), soluble calcium (9%) and micronutrients (6%), positioned as a nutrient source for nitrogen-consuming microorganisms and a crop-residue decomposer.
2. |Two nitrification inhibitors based on 3,4-dimethylpyrazole phosphate (DMPP; 1H-pyrazole, 3,4-dimethyl-, phosphate), described as suppressing ammonia-oxidizing bacteria (e.g., *Nitrosomonas* spp.) and thus slowing nitrification.
3. A biological product based on free-living nitrogen-fixing bacteria (*Azotobacter chroococcum* and *Azotobacter vinelandii*) with accompanying metabolites (amino acids, vitamins, sugars, polysaccharides, phytohormones, enzymes and other components of the nutrient medium).
4. A nitrapyrin-based inhibitor (nitrapyrin, 300 g L^{-1}), acting via inhibition/deactivation of ammonia-oxidizing bacteria.

Treatment coding and product identification

Treatments were coded and referred to throughout the manuscript primarily by mode of action and/or active ingredient to reduce reliance on local commercial names. The correspondence between treatment codes, active ingredients, and product identifiers was as follows: T0, control (no inhibitor); T1, humic substances-based nitrogen stabilizer (commercial name: *Ultra Boost*; rate: 1.5 L ha^{-1}); T2, DMPP-based nitrification inhibitor, formulation A (commercial name: NovaTec One; rate: 1.35 L t^{-1}); T3, DMPP-based nitrification inhibitor, formulation B (active ingredient: 3,4-dimethylpyrazole phosphate, DMPP; rate: 25 g ha^{-1}; formulation details provided by the manufacturer); T4, Azotobacter-based biological product (commercial name: *Azotolife*; rate: 1.5 L ha^{-1}); T5, nitrapyrin-based inhibitor (commercial name: *N-Lock*; rate: 1.7 L ha^{-1}).

Soil samples were collected from the 0–20 cm layer at two time points: before fertilizer/inhibitor application and one month after joint application with fertilizers. Mineral nitrogen forms (NO_3^--N and NH_4^+-N) were determined in distilled-water extracts (soil:solution 1:5, w/v) after shaking for 30 min and filtration, using potentiometry with nitrate- and ammonium-selective electrodes. Results were expressed as mg kg^{-1} dry soil.

Yield measurement. Grain yield was determined by full-plot harvesting using combine harvesting. Yield values were recalculated to standard grain moisture content (14%) and expressed in t ha^{-1}.

Statistical analysis. Statistical processing was performed using two-way analysis of variance (two-way ANOVA) for the factorial design (hybrid × inhibitor/stabilizer), including the interaction term. Mean separation was performed using the least significant difference (LSD) test at $p \leq 0.05$. Yield stability across years was characterized using the coefficient of variation (CV, %). Correlation and regression analyses were used to explore relationships between soil NO_3^- content and grain yield, including year-specific analyses due to strong interannual climatic variability.

RESULTS AND DISCUSSION

Ammonium (NH_4^+) and nitrate (NO_3^-) represent the primary mineral nitrogen forms available for plant uptake and simultaneously constitute the main pathways of nitrogen loss from soil systems. Both forms are subject to transformation and migration processes controlled by physical, chemical, and biological soil properties. In the present study, a strong positive linear relationship was observed between ammonium and nitrate nitrogen contents across all treatments, indicating a coupled response of soil nitrogen forms to inhibitor application (Figure 1). This finding confirms that changes in nitrification dynamics directly influence the overall pool of mineral nitrogen retained in soil.

Application of nitrogen-stabilizing products significantly increased the contents of both mineral nitrogen forms compared with the control (T0) (Figure 2). The most pronounced effect was observed for T1 (humic substances-based stabilizer), which increased NO_3^--N by 7.48 mg kg^{-1} (≈46% relative to T0) and NH_4^+-N by 6.18 mg kg^{-1} (≈38%).

Other treatments also enhanced soil nitrogen retention, although to a lesser extent. T2 (DMPP-based formulation A) increased NO_3^--N by 24%

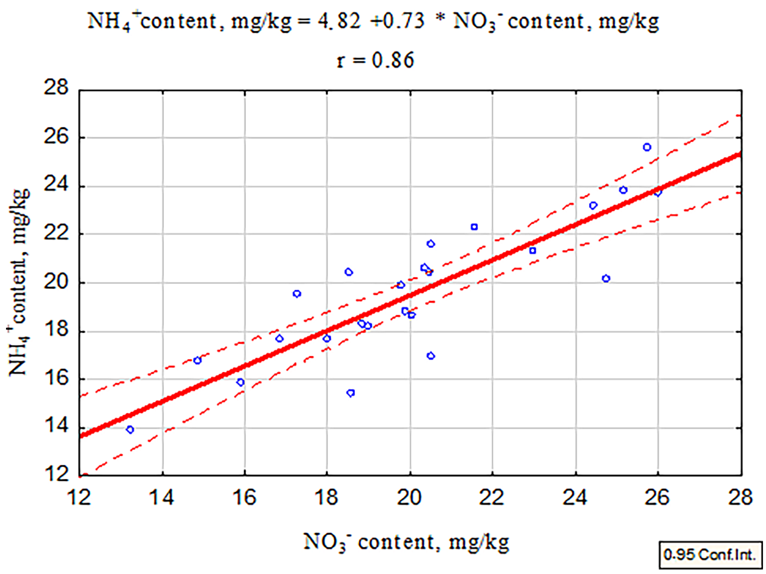

Figure 1. Relationship between soil NH_4^+-N and NO_3^--N under different treatments

and NH_4^+-N by 38%. T4 (Azotobacter-based biological product) resulted in increases of 28% for nitrate and 23% for ammonium nitrogen. T5 (nitrapyrin-based inhibitor) increased NO_3^--N by 31% and NH_4^+-N by 29%.

The lowest accumulation of mineral nitrogen was recorded for T3 (DMPP-based formulation B), where NO_3^--N and NH_4^+-N increased by 14% and 12%, respectively. This effect was noticeably weaker than that obtained with T2 despite the shared active ingredient (DMPP), suggesting formulation-specific differences and possible interactions with soil biota. Similar context dependence has been reported in previous field studies, where agronomic performance of enhanced-efficiency fertilizers varied depending on soil and environmental conditions (Lasisi et al., 2021; Zhao et al., 2017; Cassim et al., 2022).

Overall, across treatments, NO_3^--N increased by 14–46% and NH_4^+-N by 12–38% relative to T0.

Increased soil mineral nitrogen availability under inhibitor application was accompanied by improved maize grain yield (Figure 3). The highest yield response was recorded for T1, with an average increase of 1.18 t ha^{-1} over three years (≈13% relative to T0). The second most effective treatment was T5, which increased yield by approximately 10%.

The biological treatment T4 increased grain yield by 6.4%. Among the DMPP-based treatments, T2 increased yield by 8.6%, whereas T3 resulted in a relatively modest increase of 3.7%.

A more detailed analysis revealed a genotype-dependent response to nitrogen inhibitor application (Table 1). The highest and statistically significant yield increases were observed for the hybrids Pioneer 9241 and EC Sensor, where most treatments exceeded the $LSD_{0.05}$ value of 0.46 t ha^{-1}. In contrast, DKC4964 exhibited a weaker response, while for MAS 30.K statistically significant yield increases were detected only under T1.

These findings indicate that the effectiveness of nitrogen inhibitors depends not only on active agent type but also on hybrid genotype, confirming the importance of genotype × environment interactions reported in previous studies (Lucas et al., 2019; Ren et al., 2022).

A positive correlation was established between nitrate nitrogen content and grain yield (Figure 4), supporting the concept that improved soil nitrogen retention contributes to enhanced crop productivity. Under the favorable conditions of the 2023 growing season, when maize yields exceeded 10 t ha^{-1}, the correlation coefficient reached $r = 0.86$, indicating a strong relationship between nitrate availability and yield formation.

Across pooled data from all years, the correlation was moderate ($r \approx 0.53$). Therefore, the linear fit was used primarily to visualize the direction of association rather than for predictive modeling. Year-specific analysis demonstrated that under drier conditions the strength of association weakened, highlighting the dominant role of climatic variability in yield formation.

The coefficient of variation (CV) was used as an additional indicator of yield stability (Table 2). In the favorable year (2023), control plots (T0) exhibited higher variability compared with

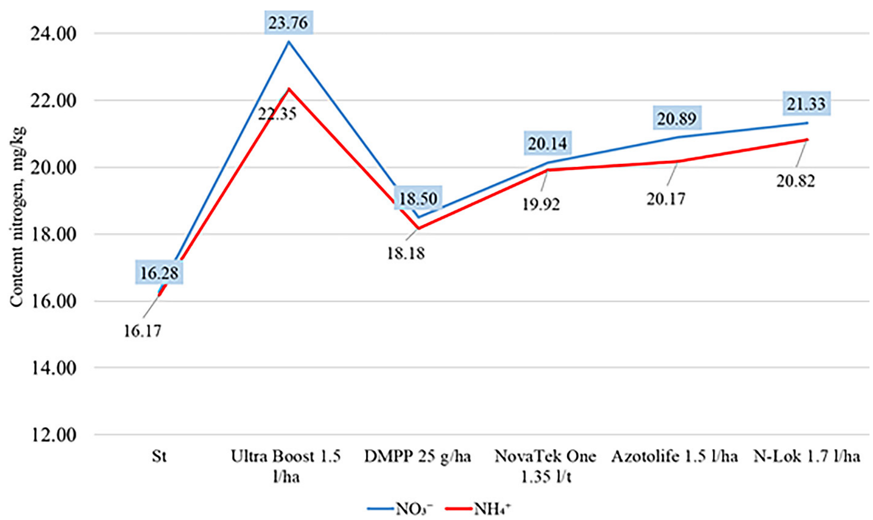

Figure 2. Dynamics of soil nitrogen forms (NH_4^+ and NO_3^-) under different treatments (T0–T5)

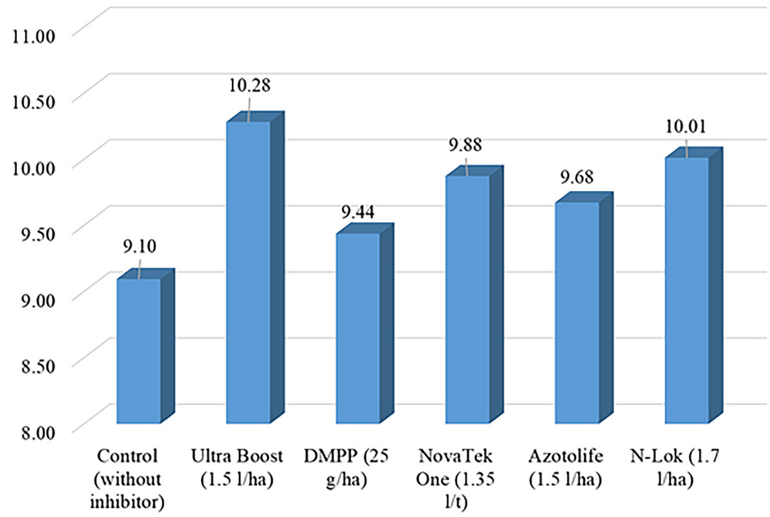

Figure 3. Average maize grain yield depending on treatment (2023–2025)

Table 1. Maize grain yield depending on treatment (T0–T5), 2023–2025

Hybrid	Nitrogen inhibitor	Grain yield (t/ha)	Δ vs. control (t/ha)	Increase (%)
Pioneer 9241	T0 (Control)	9.46	–	–
	T1 (humic-based stabilizer)	10.48	1.02	10.8
	T2 (DMPP-based inhibitor A)	10.25	0.79	8.4
	T3 (DMPP-based inhibitor B)	9.96	0.50	5.3
	T4 (biological treatment)	10.41	0.95	10.0
	T5 (nitrapyrin-based inhibitor)	10.55	1.09	11.5
DKC4964	T0 (Control)	9.48	–	–
	T1 (humic-based stabilizer)	10.31	0.83	8.8
	T2 (DMPP-based inhibitor A)	10.23	0.75	7.9
	T3 (DMPP-based inhibitor B)	9.92	0.44	4.6
	T4 (biological treatment)	10.05	0.57	6.0
	T5 (nitrapyrin-based inhibitor)	9.87	0.39	4.1
EC Sensor	T0 (Control)	8.91	–	–
	T1 (humic-based stabilizer)	10.86	1.95	21.9
	T2 (DMPP-based inhibitor A)	10.19	1.28	14.4
	T3 (DMPP-based inhibitor B)	9.29	0.38	4.3
	T4 (biological treatment)	9.55	0.64	7.2
	T5 (nitrapyrin-based inhibitor)	10.70	1.79	20.1
MAS 30.K	T0 (Control)	8.53	–	–
	T1 (humic-based stabilizer)	9.49	0.96	11.3
	T2 (DMPP-based inhibitor A)	8.82	0.29	3.4
	T3 (DMPP-based inhibitor B)	8.59	0.06	0.7
	T4 (biological treatment)	8.69	0.16	1.9
	T5 (nitrapyrin-based inhibitor)	8.93	0.40	4.7

Note: $LSD_{0.05}$ = 0.46 t/ha; increase (%) calculated relative to the hybrid-specific control (T0); treatment codes are defined in Materials and Methods.

inhibitor-treated plots, suggesting a stabilizing effect of nitrogen inhibitors under optimal moisture conditions. However, in the less favorable years (2024–2025), CV values indicated moderate variability across all treatments, regardless of inhibitor application.

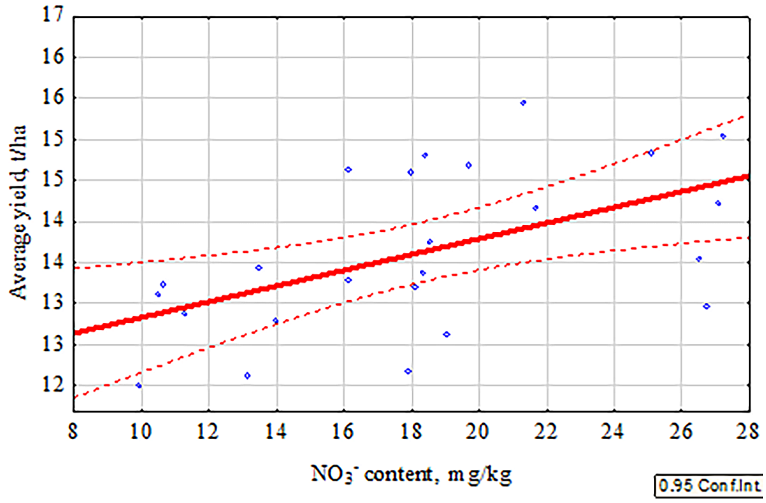

Figure 4. Relationship between maize grain yield and soil nitrate nitrogen content (NO_3^--N)

This pattern demonstrates that under pronounced water stress, the buffering effect of nitrogen inhibitors on yield variability becomes limited. Similar observations have been reported in high-frequency emission studies, where environmental benefits occurred even in the absence of consistent yield gains (Machado et al., 2020).

The dominant influence of climatic variability observed in the present study aligns with previous findings from maize production systems in Ukraine, where optimized nitrogen application strategies did not eliminate yield instability under fluctuating moisture conditions (Marenych et al., 2024). These results confirm that nitrogen supply optimization alone is insufficient without stabilization of soil nitrogen transformations under water-limited conditions.

Overall, the results confirm that nitrogen inhibitors can improve soil mineral nitrogen retention and maize yield under unstable moisture conditions; however, the magnitude of the effect is strongly dependent on interannual climatic variability.

The yield increases observed for humic-based stabilizers (9–18%, occasionally up to 33%) are consistent with reports on combined applications of urea and humic substances (Swify et al., 2022; Drulis et al., 2022a, Drulis et al., 2022b). Similarly, yield responses to DMPP-based inhibitors (4–12.6%) correspond well with previously reported values under comparable climatic conditions (Lucas et al., 2019; Zhao et al., 2017).

The effectiveness of nitrapyrin-based treatments observed in this study, with yield increases reaching up to 17%, aligns with literature data demonstrating the potential of nitrapyrin-based inhibitors to enhance nitrogen retention and productivity, particularly under stress-prone conditions (Nelson, 2018; Ren et al., 2022). Nevertheless, the strong dependence of treatment effectiveness on weather conditions supports earlier conclusions that nitrogen inhibitors do not guarantee yield increases in all environments and may exhibit neutral or contradictory effects depending on soil and climatic context (Guardia et al., 2018b; Pawlick et al., 2019).

The substantial variability in yield response across years and hybrids confirms that nitrogen inhibitor performance is strongly site-specific. This variability likely explains the contradictory conclusions reported in meta-analyses, which indicate both positive and neutral agronomic effects of nitrogen inhibitors despite their recognized role in reducing nitrogen losses (Cook et al., 2015; Thapa et al., 2016). In agreement with Fan et al. (2022), the present results support the concept of a probabilistic response to nitrogen inhibitor application, governed primarily by weather conditions and soil properties.

Table 2. Coefficient of variation (CV, %) of maize grain yield depending on nitrogen inhibitor application (2023–2025)

Nitrogen inhibitor	2023	2024	2025
T0 (Control)	10	16	19
T1 (humic-based stabilizer)	7	11	16
T2 (DMPP-based inhibitor A)	5	15	16
T3 (DMPP-based inhibitor B)	5	14	17
T4 (biological treatment)	5	15	20
T5 (nitrapyrin-based inhibitor)	8	13	19

CONCLUSIONS

A strong positive relationship between soil NH_4^+-N and NO_3^--N was observed under unstable moisture conditions, confirming their coupled behavior and sensitivity to nitrogen transformation processes in soil. In addition, a positive association between soil NO_3^--N and maize grain yield was detected. The relationship was strongest in the favorable year 2023 (r = 0.86) and weakened under drier conditions, indicating the dominant influence of climatic variability on yield formation.

Among the tested treatments, T1 (humic substances-based stabilizer) demonstrated the greatest effect on soil mineral nitrogen retention, increasing NO_3^--N by 7.48 mg kg^{-1} (≈46% relative to the control, T0) and NH_4^+-N by 6.18 mg kg^{-1} (≈38%). The nitrapyrin-based treatment (T5), the DMPP-based formulation A (T2), and the biological treatment based on free-living nitrogen-fixing bacteria (T4) also increased mineral nitrogen contents to varying degrees, whereas the second DMPP-based formulation (T3) showed comparatively weaker effects under the studied conditions.

Improved soil mineral nitrogen retention was associated with increased maize grain yield. The highest average yield increase over three years was obtained for T1 (1.18 t ha^{-1}; ≈13%), followed by T5 (≈10%). Yield response varied among hybrids, indicating a genotype-dependent reaction to nitrogen stabilization strategies.

Overall, nitrification inhibitors and nitrogen-stabilizing agents enhanced mineral nitrogen conservation in soil and supported maize productivity under rainfed conditions in a zone of unstable moisture. However, treatment effectiveness strongly depended on inhibitor type, hybrid genotype, and interannual climatic variability. These findings highlight the importance of site-specific and environmentally adapted nitrogen management strategies rather than assuming a universal yield response to inhibitor application.

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