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Optimizing Nitrogen Application for Enhanced Barley Resilience: A Comprehensive Study on Drought Stress and Nitrogen Supply for Sustainable Agriculture

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Abstract: Soil water scarcity hinders crop productivity globally, emphasizing the imperative for sustainable agriculture. This study investigated the role of nitrogen in alleviating drought stress in barley. Parameters such as relative water content, photosynthetic rate, stomatal conductance, mesophyll concentration of CO_2, total leaf nitrogen, grain yield, total organic nitrogen content, starch content, and macronutrient concentrations (N, P, K, Ca, Mg) were examined. The optimal grain yield (3.73 t·ha^{-1}) was achieved with 1 g of nitrogen per container (near 200 kg N hectare^{-1}) under ideal moisture conditions. However, under drought stress, nitrogen supply variants (1 g and 2 g per container) exhibited a significant decrease in photosynthetic rate (Pn), NRA activities, and a notable increase in Ci values. Stomatal conductance exhibited a substantial decrease by 84% in the early growth phase, especially with a 2 g dose of nitrogen supply. Nitrogen enhanced crude protein levels, yet both drought stress and nitrogen application reduced grain weight and starch content. Nitrogen effectively improved metabolic processes under drought, particularly in earlier growth stages (e.g., tillering). This research highlights the importance of sustainable agricultural practices related to the growth stage of barley, emphasizing nitrogen optimization to enhance crop resilience in water-scarce environments. The results underscore the intricate interplay between nitrogen fertilization, drought stress, and crop yield, indicating benefits during initial stress exposure but detrimental effects in subsequent growth stages.

Keywords: sustainable agriculture; relative water content; CO_2 assimilation; drought stress; nitrogen use efficiency

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1. Introduction

The emerging impacts of climate change present a growing threat to agricultural crops, especially in tropical and sub-tropical regions. Drought stress stands out as a critical factor negatively influencing crop growth and productivity [1]. Simulation models play a crucial role in predicting the changing scenario of drought conditions due to climate change. The effort to enhance the resilience of strategic crops against the harmful effects of drought stress, inevitably resulting in reduced crop productivity, is essential for more sustainable agricultural practices [2].

The success of the Green Revolution can be primarily attributed to the development of superior plant varieties and the extensive application of fertilizers, particularly synthetic nitrogen fertilizers, to realize the increased yield potential of these new varieties. Approximately 50% of the nitrogen fertilizer applied to fields is assimilated and utilized by the designated crop, with the remainder being lost to the environment [3]. A small percentage is converted to the potent greenhouse gas N_2O, which contributes substantially to the total greenhouse gas emissions from agriculture. By the way, Green Economy thinking aims to promote sustainability through N_2O emission reduction in the context of the wider nitrogen cycle, with an emphasis on improving full-chain nitrogen use efficiency, optimal dose use for each crop plant and region, and exploiting a combination of technical measures in agriculture and other combustion sources [4,5]

Barley, a widely cultivated and economically valuable crop, stands at the forefront of the agricultural landscape [6]. The correlation between agronomic yield and nitrogen supply under well-watered conditions is well established [7]. Nitrogen, when efficiently managed, possesses the potential to alleviate water stress in crops by sustaining metabolic activities even under low tissue water potential. Establishing optimal fertilizer regimes is therefore essential to enhance metabolic and regulatory processes during kernel development in cereal crops [8]. Nitrogen application has been shown to influence starch synthesis and grain quality, particularly under drought stress conditions [9].

In the quest for sustainable solutions, the use of nitrogen supply not only improves water use efficiency but also supports the antioxidant system. This includes crucial enzymes like superoxide dismutase and catalase, which play a role in mitigating the stress associated with deficit irrigation [10]. Nitrogen serves as a regulator influencing the impact of short-term heat, drought, and combined stresses on diverse aspects of wheat physiology, encompassing photosynthesis, yield, nitrogen metabolism, and nitrogen use efficiency [11,12].

Despite extensive global research on plant responses to drought stress, studies on the role of nitrogen in mitigating drought stress in barley compared to other cereals crops are comparatively rare [13]. Barley, a resilient crop cultivated in both highly productive agricultural systems and marginal subsistence environments, holds the position of the fourth most important cereal crop globally, following wheat, maize, and rice [14,15]. Although its direct contribution to human food may be minor, the potential for new applications that leverage the health benefits of whole grains and beta-glucans is significant [16].

Drought stress significantly impacts barley physiology, affecting soluble and insoluble sugar levels, as well as the uptake of grain nitrogen (N), phosphorus (P), and potassium (K) [17]. The pursuit of climate-resilient and high-yielding barley varieties is ongoing, with researchers focusing on understanding the genetic controls influencing morphological and physiological responses to drought at different stages of plant growth [18].

In tandem with breeding strategies, exploring locally sourced barley genotypes becomes crucial to harness the potential of plant biodiversity in mitigating drought stress with the aid of nitrogen supply. Parameters such as relative water content, photosynthetic rate, stomatal conductance, mesophyll concentration of CO_2, total leaf nitrogen, grain yield, total organic nitrogen content, starch content, and macronutrient concentrations (N, P, K, Ca, Mg) are significant for optimizing agricultural practices. The current study aimed to investigate, through a long-term vegetation pot experiment, the above-mentioned parameters in spring barley measured under the influence of drought stress and different dosages of nitrogen supply. A focus on the potential of nitrogen supply in mitigating drought stress is important in developing more sustainable agricultural practices.

2. Materials and Methods

2.1. Plant Experiment

This three-year field experiment involved the cultivation of spring barley (variety Kompakt) plants in cylindrical plastic containers measuring 290 mm in diameter and 260 mm in height. Each container, as detailed in the preceding section, received 15 kg of soil, which was meticulously homogenized through thorough mixing. Soil was introduced into the containers ten days before sowing to facilitate proper settling. Before sowing, any germinating weeds on the soil surface were eliminated, and the top 30 mm of the soil was aerated by loosening.

The experiment aimed to investigate the impact of three different nutrition strategies under two levels of water regimes during three distinct growth phases of spring barley (shooting, stalk elongation, and budding flowering). An optimal water regime was set up and maintained at the level of 50 to 60% of field water capacity. Under drought stress, soil humidity was reduced to 15–20% of field water capacity.

The following fertilization methods were employed:

1st variant—without N fertilization (control);
2nd variant—N fertilization to the level of 1 g per pot + 0.33 g P + 1.1 g K (near 200 kg N hectare^{-1});
3rd variant—N fertilization to the level of 2 g per container + 0.33 g P + 1.1 g K (near 400 kg N hectare^{-1}).

In the context of the presented pot experiment, we consciously opted to maintain the original values of 1 g and 2 g of nitrogen per container, thereby preserving the experiment's initial framework. Simultaneously, we introduced the conversion to kg N hectare^{-1}, acknowledging its dependency on various factors in field experiments. This assumption was made with the intention of fertilizing one hectare of soil to a depth of 0.2 m. Considering an approximate weight of 3,000,000 kg per hectare of land and assuming uniform soil density (1.5 g/cm^3) in the Slovak Republic, this value was employed for calculations.

The nitrogen fertilizer utilized in this study was DAM 390 (containing 30% nitrogen). For phosphorus fertilization, we employed triple superphosphate (containing 20% phosphorus), and for potassium, we used potassium salt with a potassium content of 60% (equivalent to 49.8% K). These fertilizers were incorporated into the soil in predetermined amounts during the filling of the experimental containers. Each combination of experimental factors, defined by the interaction between moisture regime and fertilization, was replicated four times. The biological replication for each variant was n = 10 (10 containers for each variant, 29 plants in one container), and the analytical replication for the studied experimental parameters was n = 3.

The investigation focused on different fertilization variants applied under two distinct soil moisture regimes:

1. Optimal water regime (50–60% PVK);
2. Stress due to water deficit (15–20% PVK).

The growth and development of the spring barley plants was monitored during the growing season, and the onset of the main growth phases were recorded by date. The growth phases were rated using the DC scale:

1st branching (DC 21–DC 29);
2nd stabling end (DC 30–DC 49);
3rd blooming end (DC 50–DC 69).

DC is decimal code for the development stages of cereals (wheat, barley), corresponding to Zadoks et al. (1974) [19].

2.2. Relative Water Content

The relative water content (RWC), expressed as a percentage, reflects the relationship between the water content within a plant organ (e.g., leaf) and its water content under full turgor pressure conditions, as described by Turner (1981) and Olsovska et al. (2016) [20,21]. RWC values were calculated both before initiating the drought treatment (control, representing turgid plants) and after specific periods or stages of water deprivation (drought).

To determine RWC, leaf discs were extracted from the central region of an experimental leaf. Fresh weight (FW) was measured immediately after conducting gas exchange analyses. Turgid weight (TW) was obtained after hydrating the leaf disc in distilled water at 4 °C in the dark for 12 h. Dry weight (DW) was determined after drying the leaf disc at 80 °C for a 24 h period.

$$RWC = FW\text{-}DW/TW\text{-}DW \quad (1)$$

FW—fresh weight of the leaf segment;
TW—weight of the leaf segment in full turgor;
DW—dry weight of the analysed leaf segment.

2.3. Net Assimilation Rate of CO_2

The net assimilation rate of CO_2 (Pn—μmol·m^{-2}·s^{-1}) was determined by measuring the CO_2 consumption in intact photosynthesizing plant leaves enclosed within an assimilation chamber of the CIRAS-3 DC (PP Systems International, Inc., Amesbury, MA, USA) non-dispersive, open-type infra-red gas analyser, which contained, in the Ciras-3 console, four independent gas analysers simultaneously measuring absolute CO_2 and H_2O for both reference and analysis gas streams. All measurements were corrected for temperature and pressure. The external CO_2 concentration was maintained at 370 μL·L^{-1}, and the illumination level was set at 800 μmol·m^{-2}·s^{-1}.

2.4. Stomatal Conductance and Internal CO_2 Concentration

Stomatal conductance, denoted as g_s in mmol·m^{-2}·s^{-1}, and internal CO_2 concentration (Ci) in (μmol.m^{-2}.mol^{-1}) were derived from the same measurements of the CO_2 and H_2O flows in the CIRAS-3 photosynthetic system as Pn, as outlined by Olšovská and Brestic in 2001 [22].

2.5. Monitoring of Crop-Forming Elements

The following crop-forming elements were monitored in the container experiment:

(a) Grain yield per container (g);
(b) Thousand kernel (grain) weight—HTZ (g) (used DIPOS grain counter).

2.6. Assessment of Spring Barley Grain Quality

Following post-harvest maturation, the barley grain underwent evaluation based on the following parameters:

(a) Starch content (%): Determined using the Ewers polarimetric method.
(b) Measurement of total nitrogen: This was accomplished through Kjeldahl analysis, with nitrogen content being multiplied by 6.25 to calculate total nitrogen levels as per the method outlined by Kjeldahl in 1883 [23].

2.7. Agrochemical Analyses of Plants

Plant yield and the concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) were evaluated annually throughout the duration of the experimental study. The spring barley yield was determined by manually harvesting plants from two adjacent central rows at various growth stages: shoot emergence or when the second stem node became visible (DC 29), the second phase of stem elongation (DC 49), and the third phase of flowering (DC 69).

For the analysis of nutrient concentrations, six randomly selected spring barley plants (including both control and drought treatment groups) were divided into stem, leaf, husk, and grain components. These plant samples were dried at 65 °C to a constant weight and subsequently ground for further analyses. Nitrogen concentration in the plant material was determined using the Kjeldahl method [23]. Phosphorus and potassium concentrations were assessed in ground plant material after mineralization at 550 °C for 6 h. The resulting ash was mixed with 2 cm^3 of diluted HNO_3 (a 1:1 mixture of concentrated nitric acid and distilled water). Phosphorus was quantified calorimetrically using vanadium–ammonium molybdate, while potassium and calcium concentrations were determined via flame photometry (SpectrAA-250Plus, Varian, Markham, ON, Canada). Magnesium was quantified using atomic absorption spectrophotometry.

All macronutrient concentrations (N, P, K, Ca, Mg) were expressed as percentages based on their dry weight representation, and macronutrient uptakes by above-ground biomass were recalculated per kilogram per hectare (kg·ha^{-1}). This paper will present the results of the statistical analysis of the investigated agrochemical parameters.

2.8. Statistical Analyses

Statistical analyses were carried out using the Statistica v. 10 software (StatSoft Inc., Tulsa, OK, USA) and the graphics software SigmaPlot version 11.0 (Systat Software Inc., San Jose, CA, USA).

3. Results

Over the span of three experimental years, in conditions of optimal soil moisture, a positive impact of nitrogen fertilization on Nitrate Reductase Activity (NRA) was observed in all growth phases, regardless of the nitrogen dosage. NRA displayed a slight upward trend as the growth phases progressed, signifying an increase as the stand aged. The highest NRA value, reaching 45.8 nmol N-NO^{2-} g^{-1} fresh mass.min^{-1}, was observed during the scallion growth phase, when 2 g N per container was applied (Table 1).

Table 1. The physiological parameters of spring barley, as influenced by the growth phase, water regime, and fertilization treatments, averaged over a three-year period.

A	B	C	D	E	F	G	H	I
Stress in Growth. Phase	**Water Regime**	**g N. Container^{-1}**	**kg N hectare^{-1}**	**NRA (nmol N-NO_2^-.g^{-1} Fresh mass.min^{-1})**	**RWC (%)**	**P_n (μmol. m^{-2}.s^{-1})**	**C_i (μmol. m^{-2}.mol^{-1})**	**g_s (mmol. m^{-2}.s^{-1})**
tillering	control	0 g	0 kg	8.89	93.89	9.35	126.42	73.35
		1 g	200 kg	39.79	94.92	8.37	94.68	53.22
		2 g	400 kg	30.35	94.38	9.02	95.53	59.59
	stress	0 g	0 kg	5.01	83.57	5.82	120.21	34.09
		1 g	200 kg	16.83	65.31	3.52	145.94	18.43
		2 g	400 kg	15.61	69.28	0.98	183.33	9.09
shooting	control	0 g	0 kg	8.66	91.01	12.97	131.42	108.68
		1 g	200 kg	27.78	91.37	10.12	76.59	62.41
		2 g	400 kg	39.09	87.52	9.37	65.95	59.26
	stress	0 g	0 kg	7.50	79.40	10.84	98.54	85.43
		1 g	200 kg	4.68	63.90	2.83	224.48	19.09
		2 g	400 kg	12.91	62.05	3.62	202.00	22.11
blooming	control	0 g	0 kg	18.77	92.77	12.15	216.30	137.37
		1 g	200 kg	31.12	89.99	14.30	217.40	134.04
		2 g	400 kg	45.80	90.69	11.43	178.81	57.49
	stress	0 g	0 kg	9.22	77.60	7.00	213.59	76.57
		1 g	200 kg	7.01	64.95	2.77	438.49	15.17
		2 g	400 kg	19.77	63.14	4.23	382.00	15.36

Under drought conditions, significantly lower NRA values were consistently recorded at all growth stages and under various nutrition levels in comparison to plants grown under optimal soil moisture conditions. Among the stressed plants, NRA values decreased during the seedling phase in fertilized variants but increased during the tillering stage, particularly when a higher fertilization level (2 g N per container) was applied, compared to NRA values recorded during the tillering phase.

When averaged over three years, as well as within individual years, the relative water content (RWC) of the leaf tissues in optimally irrigated plants was found to be statistically significantly higher than that of leaves from plants subjected to soil drought.

In the nitrogen-fertilized treatments, a reduction in relative water content (RWC) was observed in all growth phases except for the tillering stage under optimal moisture conditions when compared to the unfertilized control. Over the average of the experimental years, net assimilation (Pn) in the main stem leaves of barley showed statistically significantly higher values in all monitored growth phases when grown under optimal irrigation conditions compared to the Pn values measured in the barley plants subjected to drought stress conditions. In all experimental variants with different nitrogen supplies (0 g, 1 g, 2 g of N), a significant decrease in Pn values was observed under the effects of drought stress, especially during the shooting phase of barley growth. Drought stress led to decreases in Pn values of 84% in the 0 g N supply variant, 72% in the 1 g nitrogen supply variant, and 61% in the 2 g nitrogen supply variant compared to the control (without N supply).

Under optimal soil moisture conditions, barley leaves displayed the highest intercellular CO_2 concentration (Ci) values on the non-fertilized treatments (ranging from 120 to 216 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) in the individual growth phases, in contrast to the nitrogen-fertilized treatments, where a significant decrease in Ci values was evident (statistically supported). Under stress conditions, the barley plants responded differently to nitrogen fertilization concerning this parameter. The lowest Ci values were recorded in the unfertilized control treatment in all three growth phases. A particularly significant decrease of 25% in Ci value was observed during the shooting growth phase in the variant with 0 g N under drought stress. Nitrogen fertilization significantly increased the intercellular concentration of CO_2 in barley leaves, reaching peak values (438.49 or 382.00 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) during the shooting growth phase, with a greater impact observed at the lower nitrogen dose (1 g N). At the lower N dose (1 g per container), the Ci values increased by 35% during the tillering growth phase, by 66% during the shooting growth phase, and by 50% during the blooming phase. Interestingly, higher increases in Ci values were observed in the variant with a higher nitrogen dose (2 g per container)—by 48% during the tillering growth phase, 67% during the shooting growth phase, and 52% during the blooming phase, respectively.

Overall, the stomatal conductance (g_s) values were higher in the unfertilized, optimally irrigated plants (ranging from 73.35 to 137.37 mmol$\cdot m^{-2} \cdot s^{-1}$) compared to the values obtained for the plants grown under stress conditions (ranging from 34.09 to 85.43 mmol$\cdot m^{-2} \cdot s^{-1}$), indicating that drought reduced stomatal conductance (through stomata closure). However, there was a substantial 84% reduction in stomatal conductance observed in the variant with a 2 g N supply during the early growth phase (tillering). In the variant with a 1 g N supply under drought stress, the barley plants exhibited a 65% decrease in stomatal conductance compared to the control variant without drought stress. As the growth stages progressed, both variants with nitrogen supply demonstrated noteworthy reductions in stomatal conductance values. Notably, the early stage of barley growth proved to be more sensitive to drought stress, displaying a significantly higher difference in the response between the variant with a 1 g N supply and the variant with a 2 g N supply—a 19% difference.

Nitrate reductase activity (NRA) was significantly decreased under drought stress in both experimental variants with N supply. At the tillering growth stage, NRA values were reduced by 58% in the variant with a nitrogen supply dose of 1 g and by 49% in the variant with a N supply dose of 1 g. At further growth stages, the variant with a nitrogen supply dose of 1 g under drought stress showed a significant decrease in NRA by 83% and 78% at the shooting and blooming growth phases, respectively. In the subsequent growth stages, the barley plants in the variant receiving 2 g of N supply under drought stress exhibited a noteworthy reduction in NRA, with decreases of 67% during the shooting growth phase and 57% during the blooming growth phase.

Across an average of three experimental years, all monitored factors, including year, growth phase, and fertilization, had a statistically significant effect on all of the observed physiological characteristics of barley, namely NRA, RWC, net CO_2 assimilation rate (Pn), intercellular CO_2 concentration (Ci), and stomatal conductance (g_s), under both optimal and stress moisture levels.

All experimental variables demonstrated a statistically significant influence on the nutrient concentration (N, P, K, Ca, Mg) within the dry matter of barley's above-ground biomass and the nutrient uptake by the above-ground plant biomass (Table 2). However, there were exceptions to this pattern. Notably, the impact of the year on the concentration and uptake of potassium (K) and magnesium (Mg) during barley cultivation under optimal moisture conditions did not reach statistical significance. In contrast, under drought stress conditions in barley plants, both the year's effect on Mg uptake and the impact of fertilization on the dry matter concentration of Mg were found to be statistically significant.

Table 2. Overview of the influence of experimental factors on the concentration of nutrients in the dry matter of the above-ground biomass and the uptake of nutrients by the above-ground mass of spring barley.

Monitored Parameter	Optimal Water Regime (n = 108)			Water Stress (n = 108)		
	Year	Growth Phase	Fertilization	Year	Growth Phase	Fertilization
Concentration N	++	++	++	++	++	++
Concentration P	++	++	++	++	++	++
Concentration K	-	++	++	++	++	++
Concentration Ca	++	++	++	++	++	++
Concentration Mg	++	++	++	++	++	+
Variant N	++	++	++	++	++	++
Variant P	++	++	++	++	++	++
Variant K	-	++	++	++	++	++
Variant Ca	++	++	++	++	++	++
Variant Mg	-	++	++	+	++	++

Note: ++ statistically highly demonstrable influence; + statistically demonstrable influence; - statistically unproven influence.

Over a three-year period, the nitrogen fertilization of spring barley resulted in a twofold increase in nitrogen (N) concentration within the dry matter of the above-ground biomass, both under optimal soil moisture conditions and in the plants subjected to stress compared to the unfertilized control. As the growth phases progressed, a dilution effect led to a reduction in N concentration in the dry matter for both the fertilized and unfertilized variants (Table 3).

In the final observed growth phase (heading), the N concentration in dry matter was higher for the fertilized variants of the stressed plants (2.19% and 2.71%, respectively) compared to the same fertilized plants grown under optimal conditions (1.85% and 2.41%, respectively). Nitrogen fertilization, whether under optimal or stress conditions, increased N uptake by the above-ground biomass; however, the rate of this increase was notably lower under stress conditions when compared to the plants grown under optimal moisture conditions. The application of 2 g N per container proved to be more effective in all cases than the half-dose of 1 g N per container.

Throughout all three years, the total harvested yield (HTZ) in the unfertilized treatments under stress conditions remained relatively consistent, occasionally even surpassing that of the unfertilized treatments under stress-free conditions (Table 3). Nitrogen fertilization further exacerbated this reduction. Conversely, when stress was applied during the different growth phases, nitrogen fertilization had the opposite effect, decreasing HTZ. At the tillering growth phase in the variants with N supply under drought stress, the HTZ values were mostly at the control level (without N supply). The HTZ values decreased under drought stress in barley plants of both nitrogen treatment variants (1 g and 2 g N) by 18% and 15%, respectively. This decreasing tendency regarding the HTZ level also continued at the shooting and blooming growth phase.

Nitrogen enhanced crude protein levels, yet both drought stress and nitrogen application reduced starch content. A significant increase in crude protein levels was observed in both the shooting and blooming growth phases. In the variant with a dose of 1 g of N supply, it increased by 43% in the shooting growth phase and by 34% in the blooming growth phase. Similar results with an increasing crude protein level were shown for the variant with a dose of N 2 g—by 43% in the shooting growth phase and by 33% in the blooming growth phase.

Table 3. Effect of stress on barley grain yield, HTZ, and quality parameters (average of three years).

A	B	C	D		E	F	G
stress in growth phase	dose N	dose N	Grain harvest		HTZ	Crude protein	Content of starch
	g.conteiner^{-1}	kg hectare^{-1}	(t·ha^{-1})	(g.container^{-1})	(g)	(%)	(%)
tillering	0 g	0 kg	1.01	6.69	32.84	12.17	63.14
	1 g	200 kg	3.14	20.72	30.86	13.99	60.65
	2 g	400 kg	3.07	20.27	34.05	19.50	59.11
shooting	0 g	0 kg	1.21	8.01	36.09	10.25	63.85
	1 g	200 kg	1.10	7.24	29.64	17.79	58.73
	2 g	400 kg	0.97	6.43	30.62	17.71	58.09
blooming	0 g	0 kg	0.95	6.29	29.95	11.72	62.17
	1 g	200 kg	2.32	15.31	21.16	17.63	61.34
	2 g	400 kg	0.98	6.45	21.90	17.58	56.21
optimum	0 g	0 kg	1.116	7.37	31.35	12.72	60.75
	1 g	200 kg	3.73	24.60	40.18	14.08	57.61
	2 g	400 kg	2.24	14.76	37.00	16.25	55.79

4. Discussion

4.1. The Physiological Parameters of Spring Barley, as Influenced by the Growth Phase, Drought Stress, and Nitrogen Fertilization Treatments

A significant increase in the intercellular CO_2 concentration in barley leaves, particularly during the steaming growth phase, attributed to nitrogen fertilization, with a more pronounced effect at a lower dose (1 g per container), was estimated. Concurrently, stomatal conductance (g_s) values displayed a noticeable trend, indicating higher values in the unfertilized, optimally irrigated plants compared to those under stress conditions, highlighting reduced g_s during drought. High levels of internal CO_2 and decreasing trends in stomatal conductance under drought stress are principal responses of C3 plants to help to reduce transpiration water loss by decreasing stomatal conductance and simultaneously increasing assimilation rates [24–26]. The changes in stomatal conductance indicate the adaptive changes of the experimental barley plants under different treatments, among which the dose of 2 g of N supply was evidently more stressful, presumably exposing the plant seedlings to soil osmotic stress.

Nitrogen fertilization had a negative impact on photosynthesis (Pn), resulting in reduced net assimilation values, though this impact was especially minor under irrigated conditions (except for the 1 g dose during the early growth phase). The high N application rate did not improve the net photosynthetic rate of the leaves but was able to inhibit it to some extent [27]. Specifically, this was visible in our experiment at the early stage of barley growth (tillering stage) for the variant with a 2 g dose of N. However, approved markable reductions in the photosynthesis of the plants under water deficit and combined N and water deficit were attributed to an imbalanced ATP/NADPH ratio, linked to the photosynthetic light reactions influencing carbon metabolism in the Calvin cycle [28]. Antagonistic, synergetic, and neutral effects between nitrogen addition and drought on resource use efficiency were found as well [29].

Conversely, under drought stress growing conditions, CO_2 assimilation was greater due to fertilization than in the non-fertilized control, resulting in Pn values dropping by 84% in the 0 g N supply variant. The decrease of 72% in the 1 g N supply variant and the decrease of 61% in the 2 g N supply variant compared to the control variant (without N) was estimated as well. In support of our findings, Guo et al. (2021) demonstrated that coupling a 20% reduced irrigation with traditional nitrogen application significantly enhanced grain yield in medium-density planted maize. This improvement was associated with heightened photosynthesis (Pn) and Y(NPQ) and a reduction in Y(NO) [30].

NRA was significantly decreased under drought stress in both experimental variants with N supply. However, during the early stage of barley growth (tillering), the variants with N supply showed a more moderate decrease in NRA activity compared to the variant without N under drought stress. Drought stress elevated nitrate nitrogen (NO_3-N) while concurrently diminishing the activities of N metabolism enzymes and the transcriptional levels of nitrate reductase, glutamine synthetase, and glutamate synthase [31].

Furthermore, during the early stage of barley growth (tillering), the variants with N supply demonstrated a more moderate decrease in NRA activity compared to the variant without N under drought stress. This may serve as evidence of a possible mitigating effect of nitrogen supply on barley plants under the influence of drought stress. Ru et al. (2022) demonstrated that a moderate application of nitrogen enhanced the activities of nitrate reductase and glutamine synthase in grains under post-anthesis heat and drought stress alone. This provided a basis for the accumulation of nitrogen and protein in the grains at the later stage of growth [11,32]. Chang et al. observed that nitrogen supply significantly enhanced the drought tolerance of grass plants. This improvement was attributed to the promotion of antioxidant metabolism and nitrogen metabolism, thereby safeguarding cell membranes against oxidative damage [33].

4.2. The Influence of Experimental Factors on the Concentration of Nutrients and Their Uptake in the Dry Matter of the Above-Ground Biomass of Barley Plants

The accumulation of minerals in plants during drought stress plays a crucial role in enhancing drought tolerance [34]. Plants employ various strategies to mitigate the deleterious effects of drought stress, including the promotion of phosphorus (P) and potassium (K) accumulation in various organs. They also regulate mineral concentrations in the phloem and xylem to prevent xylem embolism. Additionally, a decrease in magnesium (Mg) uptake was observed under drought stress [35]. All experimental variants with N supply under drought stress demonstrated a statistically significant influence on the nutrient concentration (N, P, K, Ca, Mg) within the dry matter of barley's above-ground biomass and the nutrient uptake by the above-ground plant biomass. Plants adapt to the high N and drought environment by altering their N uptake preference, and mineral uptake may explain changes in biomass with crude protein levels, nitrogen deposition, and drought [36].

The impact of drought stress on barley plants revealed a statistically significant effect on the annual uptake of magnesium (Mg) and the positive influence of nitrogen supply on the dry matter concentration of Mg. Additionally, another study confirmed that under rained conditions, nitrogen (N) fertilization significantly enhances barley productivity through its indirect influence on nitrogen accumulation in grain and straw. This process concurrently leads to an enhancement in grain quality by augmenting the accumulation of micronutrients such as magnesium (Mg) [37].

4.3. Effect of Stress on Barley Grain Yield, HTZ, and Quality Parameters: Average of Three Years

After our experimental assessment, it became clear that early exposure to stress during tillering significantly benefited the barley grain yield in terms of the stands' growth status. Křen et al. (2014) acknowledged that the timely and accurate prediction of grain yield and quality in spring barley is an essential requirement for efficient crop management [38]. To mitigate yield losses in plant objects due to high temperatures, it is crucial to apply the optimal amount of total nitrogen [10,39,40]. However, in the present study, in the later growth phases, starting from tillering, stress had a detrimental effect on grain yields.

Tillering is recognized as a crucial agronomic trait influencing the quality of plant crop populations and grain productivity. Wang et al., in 2017, illustrated that increased N levels enhanced the quantity and productivity of late-emerging tillers in rice, although these tillers demonstrated significantly fewer spikelets per panicle and less efficient grain filling in comparison to the primary stem or early-emerging tillers across varying nitrogen levels [41]. In our research, nitrogen application during the tillering phase in spring barley partially alleviated the detrimental effects of stress on the crop but proved ineffective when applied under stressful conditions or even resulted in a negative impact on grain yield. Abid et al. (2016) demonstrated that nitrogen nutrition enhances the capacity of wheat plant to mitigate the impacts of drought stress during vegetative growth stages [42].

Throughout all three years, the thousand kernel weight (HTZ) in the unfertilized treatments under stress conditions remained relatively consistent, occasionally even surpassing that of the unfertilized treatments under stress-free conditions. In the first year of the field experiment, drought stress consistently led to a reduction in HTZ, regardless of when the stress occurred. Nitrogen fertilization further exacerbated this reduction. In the second and third years of the experiment, HTZ increased in plants exposed to stress during tillering as a result of nitrogen fertilization. Conversely, when stress was applied during the seedling or tillering phases, nitrogen fertilization had the opposite effect, decreasing HTZ.

Utilizing linear fitting analysis, Wang et al. (2023) demonstrated a parabolic trend in grain yield, biomass yield, hundred kernel weight, and the ear grain number of maize plants as the nitrogen fertilization rate increased [43]. Across the three-year average considered, it became apparent that a fertilization rate of 1 g N per container significantly boosted yield. The application of moderate nitrogen levels was demonstrated to mitigate yield loss and ameliorate the decline in maize grain quality caused by post-silking heat stress [40]. In contrast, a double dose was found to be ineffective for optimal crop development, demonstrating a suppressive effect on crop formation, leading to reduced grain yield compared to the lower nitrogen dosage.

The barley plants demonstrated the lowest sensitivity to drought stress with regard to grain yield when the stress coincided with the tillering growth phase. Previous studies have highlighted that drought leads to a reduction in the number of tillers, plant height, and grains per ear in barley [44]. Overall, drought induced alterations in the morphology and architecture of the barley roots, with the roots transmitting stress signals to the caryopses, triggering the expression of various genes associated with protein biosynthesis, ultimately resulting in an increased accumulation of endosperm protein [45]. In this scenario, nitrogen fertilization demonstrated a significant stress-mitigating effect, maintaining grain yields at a high level. Conversely, the plants displayed the greatest sensitivity to stress in conjunction with fertilization when subjected to stress during transplanting. In this case, neither basal nor double nitrogen fertilization countered the impact of drought stress. When stress was induced during heading, a 1 g N per pot dose exhibited relatively effective stress mitigation, while the double dose failed to demonstrate any stress-mitigating effect, with yields remaining comparable to the unfertilized control.

Nitrogen enhanced crude protein levels, yet both drought stress and nitrogen application reduced starch content. A significant increase in crude protein levels was observed in the shooting and blooming growth phases. In the variant with a dose of 1 g of nitrogen supply, it increased by 43% in the shooting growth phase and by 34% in the blooming growth phases. Li and Wang (2023) reported a reduction in protein synthesis in barley leaves under drought stress conditions with a deficient nitrogen supply. The degradation of proteins in leaves was intensified in the absence of nitrogen [46]. The application of nitrogen amplifies the impact of pre-drought priming by modulating starch and protein biosynthesis in wheat [47]. Therefore, based on our results, we suppose that additional nitrogen supply may have a mitigating effect on barley plants under drought stress.

An investigation into the combined effect of drought stress and nitrogen fertilization on soybean plants revealed that a high nitrogen (N) rate is not advisable in the absence of drought conditions. In comparison to a low rate, it led to a reduction in the number of flowers and pods per plant, plant height, and seed yield. Conversely, under drought stress conditions, a high N rate positively influenced most traits, with thousand kernel weight showing the strongest correlation with yield [48].

Simultaneously, a modest reduction in nitrogen fertilizer application leads to enhanced plant quality through the modulation of starch properties without inducing any discernible yield loss. This was confirmed by our results derived from the use of 1 g of N supply as well. However, the augmentation of nitrogen levels induces alterations in the structure and characteristics of starch [49].

Nitrogen supply has been reported to be especially important under abiotic stresses [50,51]. When considering the three-year average data, it is evident that lower harvest total yield (HTZ) consistently occurred under stress conditions in the nitrogen-fertilized treatments compared to their counterparts, with analogous fertilization under optimal water conditions. Notably, nitrogen supply led to an increase in crude protein level and the maintenance of a moderate level of total yield compared to the unfertilized control.

5. Conclusions

In summary, our study reveals the complexity of nitrogen's role in sustainable barley cultivation. The additional nitrogen supply during drought stress heightened CO_2 concentration throughout the barley growth period, affecting stomatal conductance. Positive effects on magnesium uptake enhanced barley productivity, but the temporal dynamics varied across growth phases.

The thousand kernel weight (HTZ) responded inconsistently over three years, influenced by nitrogen and stress timing. While nitrogen boosted HTZ during tillering under stress, the complexities in the outcomes highlight the challenge of balancing nitrogen, water stress, and barley yield. Further research is essential for refined, sustainable crop management strategies.

Our findings underscore the intricate interplay between nitrogen fertilization, drought stress, and crop yield. The benefits observed during the early exposure of the plants to stress contrasted with the adverse effects in the later growth phases, emphasizing the need for a nuanced approach in sustainable crop management. These insights contribute to evolving strategies for effective and sustainable barley cultivation in changing environmental conditions.

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