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Article

Responses of Soil Enzymes Activities to Sprinkler Irrigation and Differentiated Nitrogen Fertilization in Barley Cultivation

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Abstract: Our study aimed to assess sprinkler irrigation's impact on the activity of selected soil enzymes concerning nitrogen metabolism and oxidation-reduction processes in the soil with different doses of inorganic nitrogen fertilizers. The soil Alfisol was sampled from the experimental field of spring barley in the University Research Center in the central part of Poland in the moderate transitory climate during the growing seasons 2015–2017. The index resistance (RS) was derived to recognise the resistance enzymes of drought. In the maturity phase, nitrate reductase activity was at an 18% higher level in irrigated soils and the activity of other enzymes was higher in no-irrigated treatments by 25% in dehydrogenase, 22% in peroxydase 33% in catalase and 17% in urease case. The development stage of barley influenced nitrate reductase activity. Enzymatic activity has been changing in the examined years of the research, depending on the weather conditions. During the maturity stage, the soil's lower ammonium nitrogen content resulted from higher spring barley uptake due to drought stress. Irrigation probably contributes to increased leaching of nitrate in soil. The highest index of resilience was presented in the soil catalase activity.

Keywords: urease; nitrate reductase; dehydrogenase; peroxidase; catalase; moderate transitory climate; soil; index of resilience,

1. Introduction

The mineral and organic nitrogen (N) forms undergo several transformations throughout the N cycle. This element is easily transformed from the reduced to the oxidized form, which results in the free migration of nitrogen in hydrological and atmospheric processes. The amount of nitrogen available to plants is positively correlated with the process of mineralization of organic matter in the soil, biological nitrogen fixation, fertilization and the sum and distribution of atmospheric precipitation [1]. However, due to such processes as immobilization, harvesting and removal, denitrification, volatilization, leaching, runoff and erosion, the nitrogen loss from the soil takes place. The intensity of these processes is influenced by environmental features such as soil pH, soil texture, its density, aeration, water content, and thermal conditions, but also, the management of crop residues, the method and timing of fertilization, agricultural treatments such as irrigation and changes in land use. It is assumed that in most cases less than five percent of the nitrogen in the soil is directly available to plants from the total nitrogen content. It is mainly nitrogen in the form of nitrates NO_3-N and ammonium NH_4+-N, and organic N being the residue, which gradually becomes available due to the mineralization process [2,3]. A characteristic feature of arable soils is the exceptionally high dynamics of mineral forms of nitrogen during the growing season, which results from the microbiological nature of nitrogen transformations in the soil. Nitrogen occurs in many forms covering the range of valence states from –3 (in NH_4+) to +5 (in NO_3-) in both, agricultural and natural ecosystems. The change of one valence state into another is mainly biologically mediated and depends primarily on environmental conditions [4]. Soil oxidoreductase enzymes take part in these oxidoreductive processes. Dehydrogenases (E.C.1.1.) are extracellular enzymes that can be

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considered a helpful indicator of microbial activity and oxidative metabolism in soil [5]. Another intracellular enzyme from the oxidoreductase class is catalase (EC 1.11.1.6), which manages oxidative stress in the soil by catalyzing the decomposition of hydrogen peroxide into water and oxygen [6]. Peroxidases (EC 1.11.1) use H_2O_2 as an electron acceptor, and their activity in soil results in the depolymerization of lignin [7]. As an effect of urease activity (EC 3.5.1.5) is an increase in soil pH and loss of nitrogen to the atmosphere due to the release of NH_3 as a result of the hydrolysis of urea to CO_2 and NH_3. [8]. The activity of this enzyme can be viewed as a desirable indicator of soil quality due to its role in regulating plant nitrogen supply. In turn, the enzyme responsible for catalyzing the reduction of NO_3^- to NO_2^- in anaerobic conditions in soil is nitroreductase (EC 1.7.99.4) [9]. It has been proven that changes in soil use and management affect soil enzymes that actively participate in metabolic processes [10,11]. Enzymes indicate the metabolic level of the microbial community in the soil and catalyze specific reactions in the carbon and nutrient metabolism cycle [12,13]. Free enzymes excreted by plants and animals and associated mainly with or within cellular structures are called exoenzymes. Afterwards, they are released into the soil after cell lysis and death [14]. Therefore, if soil use and management influences its microbial environment, changes in the activity of soil enzymes may also be observed [15]. The biochemical properties of the soil, which are indicators of its quality, are highly variable depending on climatic, weather and geographical conditions, pedogenic factors, fertilization and irrigation. Microorganisms living in the soil are important factors that determine the nutrient metabolism cycle. Moreover, they interact intricately with plant organisms. Land-use systems that improve soil microbiological properties can result in higher yields with better raw material quality while reducing production costs. Moreover, by limiting the use of mineral fertilizers and plant protection products, these systems support the sustainable development of agricultural areas. Therefore, to improve the condition of the soil, it is necessary to constantly monitor and evaluate the physicochemical and biological processes taking place in the soil and examine changes in its physicochemical properties. Diverse soil use in agricultural systems regarding crop rotation and plant protection treatments results in changes in soil properties, both physical and chemical, but above all affects biological activity. This, in turn, affects both productivity and environmental quality and thus the health of humans and animals. Multi-annual studies on the impact of agriculture on the biology and biochemical properties of soil bring valuable information on the transformation of nutrients in soils [16,17]. The definition of soil quality indicates the ability of soil to operate within an ecosystem, the ability to support biological productivity, maintain the quality of the environment, and encourage the sanitary of plants and animals [16].

The stability (resistance and action) of the soil system is a consequence of the influence of microorganisms on the properties and processes occurring in the ecosystem. To define different systems, it is important to select appropriate indicators that will quantify the relative value of how the system will respond to specific soil use scenarios. In our paper, we compare our indices with previously published stability indices and test their performance against a real dataset. One of the indicators that quantifies the relative value of the microbiological response in a given situation is the resistance index according to Orwin and Wardle [18].

In this study, we aimed to evaluate the response of N-related properties of Alfisol soil such as some forms of N in the soil and the activity of enzymes involved in the metabolism of nitrogen in the soil. The reaction of enzyme activity related to the transformation of soil nitrogen depending on soil moisture under the influence of sprinkler irrigation during the growing season of spring barley in a warm temperate climate zone has been investigated. Moreover, the research aims to estimate the impact of irrigation on the activity of enzymes related to nitrogen metabolism and oxidation-reduction processes in the soil at varied stages of growth with various doses of inorganic nitrogen fertilizers. We also investigate whether the calculated ratios (RS) can be used as an effective solution to enzymatic stress.

2. Materials and Methods

2.1. Study Area and Soil Sampling

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A strict field experiment was conducted at the Research Center of the Bydgoszcz University of Science and Technology located in the village of Mochełek (53°130N, 17°510E). The research site is located in the Kuyavian-Pomeranian Voivodeship, which represents the area of the central Poland. The tested plant in the experiment was spring barley cv. 'Signora' cultivated in three consecutive growing seasons, 2015–2017.

The tested soil, according to the USDA soil taxonomy, was defined as a typical Alfisol soil made of sandy loam (clay 6%, sand 79% loam 15%) [19]. It was found that the reaction of the topsoil layer is slightly acidic: pH in 1M KCl 5.7–6.1. This layer is characterized by a relatively low content of total organic carbon (TOC) (7.60–7.70 g·kg^{-1}) and total nitrogen (TN) (0.70–0.76 g·kg^{-1}). The content of other available elements' were as follows: phosphorus P (64.0 mg kg^{-1}) and sulfur S (13 mg S kg^{-1}) represented an average content, and potassium K content was high (126.0 mg^{-1}). The subsoil is light loamy sand on shallow medium loam. The properties of the soil were determined before the experiment and are presented below (in Table 1). The water properties of the soil reflected in the water content in one meter of the soil layer at the water capacity of the field is 215 mm.

Table 1. Properties of the soil of the experimental field.

Soil properties	Content
TOC	7.60-7.70 g·kg^{-1}
TN	0.70-0.76 g·kg^{-1}
pH KCL	5.8-6.2
P available	64.0 mg·kg^{-1}
K available	125.0 mg·kg^{-1}
SO_4^{2-}	12 mg·kg^{-1}

2.2. Experimental Design and Weather Conditions

The layout of the experiment was a two-factor dependent split-plot design with four replications. The first factor (i) was sprinkler irrigation (where W0 meant no irrigation, and W1—optimal irrigation, which ensures 100% coverage of the water needs of the plants in the period of high water needs). The second factor (ii) was a differentiated level of nitrogen fertilizer application in the form of ammonium nitrate (three doses assigned as N1, N2, and N3 are detailed in Table 2). The second factor was static and constant throughout the whole experiment. However, the first factor, which was the treatment of irrigation, was dynamic and it was scheduled according to the weather conditions. The spring barley was irrigated optimally. This means that during the entire period of high water needs of plants in the root zone, there was constant reserve of readily available water (RAW). The number of single irrigation doses and the total seasonal doses (Table 3) were established based on the amount and distribution of atmospheric precipitation according to Żarski et al. [20].

Table 2. Description of experimental factors.

Irrigation factor	Fertigation factor	Nitrogen fertigation level
W_0—no irrigation W_1—optimal irrigation	N_0	control
	N_1	pre-sowing 30 kg·ha^{-1}
	N_2	pre-sowing 60 kg·ha^{-1}
	N_3	90 kg·ha^{-1} (pre-sowing 60 kg·ha^{-1} and top dressing 30 kg·ha^{-1} in shooting)

Table 3. Characteristics of weather conditions and irrigation doses applied in the growing seasons 2015–2017.

Growing season	t (°C)	P (mm)	Date	Irrigation dose (mm)
2015	13.8	193.3	26 May	30

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			3 June	30
			10 June	25
			1 July	30
			6 July	20
			in total	135
2016	14.3	386.7	24 May	35
			8 June	32
			in total	77
2017	13.1	474.8	29 May	20
			9 June	20
			28 June	15
			in total	55
Average 1991–2020	14.8	324.5	–	–

The climate conditions of this study area represent a temperate transitory zone in Central Europe. The mean annual thermal and rainfall conditions for the growing season from April to September are 14.8°C and 324.5 mm respectively. In the growing season of 2015 classified as dry, as much as 135 mm was applied in 4 single doses. In the other two seasons, classified as moist, a total of 77 mm was applied in two doses in 2016 and only 55 mm at three doses in 2017. For the whole period of the experiment of 2015–2017 the thermal conditions of the area, were similar to the climate norm of 1991-2020 (Table 3)(Figure 1). However, the atmospheric precipitation totals from April to September were considerably higher in 2016 and 2017 compared to the long-term average (Table 3) (Figure 1). The term od barley sowing was as follow: 23 March 2015, 1 April 2016, and 31 March 2017. The barley was grown according to recommendations of the State Plant Health and Seed Inspection Service, regarding optimization of phosphorus and potassium fertilization and chemical plant protection. The harvesting area was 10 m². Grain harvesting took place on the following dates: 3 August 2015, 23 July 2016, and 8 July 2017.

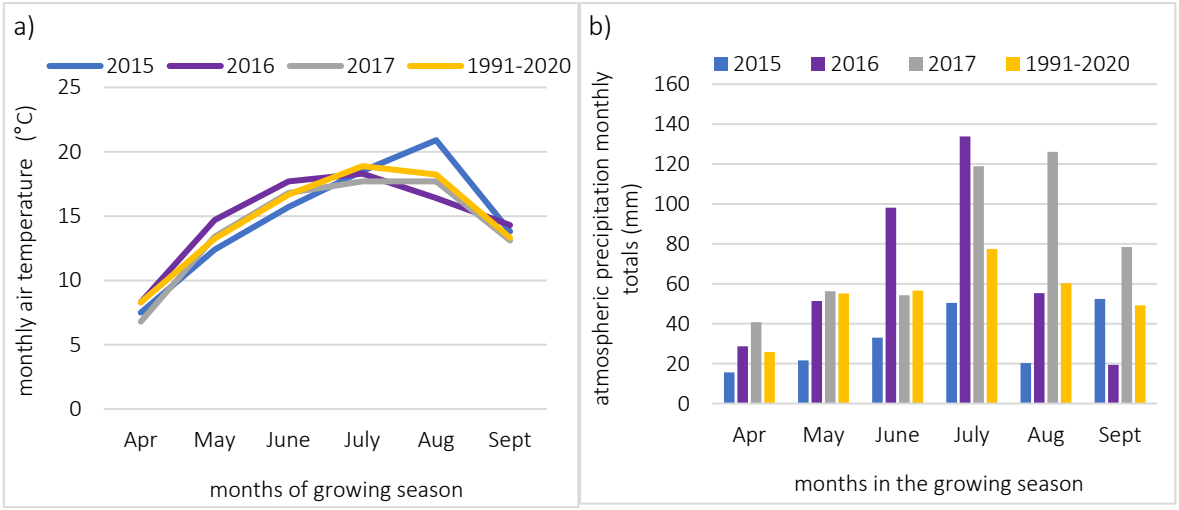

Figure 1. The courses of monthly air temperature and the distribution of monthly atmospheric precipitation totals in the growing seasons 2015–2017 compared to climate normal 1991–2020.

2.3. Irrigation System and Schedule

For the irrigation, a portable sprinkler irrigation system equipped with low-pressure Nelson-type sector sprinkler heads was used. The unit efficiency was 200 $dm^3 \cdot h^{-1}$. The irrigation system was connected to the municipal waterworks network.

We scheduled the dates of irrigation treatments based on meteorological monitoring from an automatic weather station set in the vicinity of the experimental field. Daily atmospheric precipitation

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and the content of readily available water (RAW) in the soil were established. The soil water storage from one metre to the depth of the soil profile is 215 mm at field water capacity. Constant monitoring of root zone moisture was achieved based on the method of readily available water balance commonly used for irrigation scheduling [20]. Moreover, direct measurements of the soil water content were conducted by the TDR method using the Fieldscout TDR 300 Soil Moisture Meter (Spectrum Technologies, Inc.). The coverage of barley water needs resulted from maintaining soil moisture in the range of RAW in the root zone of plants. In barley cultivation on irrigated plots, soil moisture in the root zone of plants was maintained in the range of RAW from 0 to 30 mm of field water capacity.

2.4. Chemical and Biochemical Analysis

Soil samples were collected from 0 to 20 cm of the topsoil three times at the following developmental stages: I – in spring germination (BBCH 9-19). II after fertilization –ripening (BBCH 71–78) and III – before harvest -maturity (BBCH 86-87). At each development stage were gathered the soil samples in four replications of all treatments. Material from field sampled soils were sieved (2-mm mesh) and keep in a plastic box at 4 ◦C. After two days, then stabilize the microbial activity soils were explored enzymes activity.

$N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ contents were extracted from moist field soil using KCl and K_2SO_4, respectively. The nitrate nitrogen content was determined using the phenol disulfonic acid method and the ammonium nitrogen content using the indophenol blue method [21].

Urease activity (UR. EC 3.5.1.5) in soil was determined according to Kandeler and Gerber [22]. The 1 g of soil was incubated with 4 ml of borate buffer (pH 10.0) and 0.5 ml solution of urea at 37°C for 2 hours. Later, filtered after adding 6 ml of 1 M KCl and the solution and then diluted with water. Spectrophotometric evaluate the activity was after 30 min of adding NaOH salicylate and acid dichloroisocyanide at 690 nm. The UR activity was presented in mg $N\text{-}NH_4^+$ $kg^{-1}\cdot h^{-1}$. Nitrate reductase activity (NR, EC. 1.7.99.4) was evaluated as described by Kandeler [23]. Soil samples with KNO_3 (substrate) and solution of 2,4-DNP were incubated at 25° C for 24 hours. The samples were added KCl solution and filtered and to 5 ml of solution 3 ml of ammonium chloride buffer and reagent for staining were added, after mixed then were measured at 520 nm. The unit of NR activity was mg $N\text{-}NO_2^-$ $kg^{-1}\cdot 24\ h^{-1}$. Activity of dehydrogenase (DH. EC 1.1.) was presented in mg TPF $g^{-1}\ h^{-1}$ according to Thalmann [24]. Soil samples mixed with a buffered tetrazolium salts (TTC) and glucose were incubation at 30◦C for 24 h. The activity of that oxidoreductase were spectofotometic estimate at 546 nm. Activity of catalase (CAT. EC 1.11.1.6) was determining by Johnson and Temple's [25]. The investigated soils were incubating with 20 min with hydrogen peroxide and then in an acid environment titrated with potassium permanganate. The catalase activity were calculated used results of performing and control samples in µmol $H_2O_2\cdot g^{-1}\cdot min^{-1}$. Peroxidase activity (PER. EC 1.11.1.7) was quantified in accordance with Ladd [26]. The substrates were pyrogallol and hydrogen peroxide and the unit of catalase was presented as mmol of purpurogaline $g^{-1}\cdot h^{-1}$.

2.5. Data Analyses

The index resistance (RS) was derived from the formulas suggested by Orwin and Wardle [18]:

$$RS(t_0) = 1 - \frac{2|D_0|}{(C_0 + |D_0|)} \quad (1)$$

Where $|D_0|$ is the difference between control (C_0) and performing soil (P_0) at the end of irrigation (t_0).

The enzyme activity results of enzymes' activities and chemical analysis were submittedsubjected to analysis of variance and Tukey'sTukey's test at 5%with a probability, with the aid of the5% using a statistical softwareprogram. The receivedobtained results were analysed by statisticssubjected to statistical analysis using the statistical program Analysis of variance for orthogonal experiments byof the Bydgoszcz University of Science and Technology, Poland. The differences. Differences between the values were examined with Tukey'stested using the Tukey test at thea significance level of $p \leq 0.05$. Pearson'sPearson's linear correlation coefficients of the

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analysedanalyzed biometric feature were calculated using the Statistica program for Windows software.

3. Results

The level of NO_3^--N and NH_4^+-N content in Alfisol and their dynamics during the growing season significantly depended on the conditions of a sustained experiment from irrigation and nitrogen fertilization (Table 4). The content of NH_4^+ dependent on the interaction of irrigation during the development phases (Table 4). At the II term (after fertilization) during ripening, the content of ammonium ions was higher at no-irrigation objects it was on average 13% less than irrigated objects. Before harvest, the higher content of these ions was observed in irrigated objects, especially with the N1 and N3 doses. In the objects fertilized with nitrogen, the lowest content of NO_3^--N $^-$ occurred in spring (germination). After applying mineral fertilization, the content of these ions increased strongly, and then slightly decreased at the end of vegetation. The content of mineral nitrogen N_{min} depended also on the applied nitrogen fertilization. Differences in content were found depending on the applied irrigation before the harvest of spring barley. The objects without irrigation contained on average 34% more mineral nitrogen than the objects with irrigated applied.

Table 4. The content of nitrate, ammonium and mineral nitrogen in investigated soil under barley (mean for 2015–2017).

		NH_4			NO_3			N_{min}		
Term	**N dose**	**IRR**	**NIRR**	**mean**	**IRR**	**NIRR**	mean	IRR	NIRR	mean
Germination	N_0	6.107	6.107	**6.107**	2.657	2.657	**2.657**	39.437	39.437	**39.437**
Ripening	N_0	4.310	3.957	**4.133**	10.023	6.497	**8.260**	64.502	47.040	**55.771**
	N_1	4.513	4.383	**4.448**	7.703	13.147	**10.425**	54.977	78.885	**66.931**
	N_2	5.217	8.040	**6.628**	21.930	29.850	**25.890**	122.16	125.51	**123.83**
	N_3	6.060	6.357	**6.208**	23.777	19.637	**21.707**	134.27	116.97	**125.62**
Average		**5.025**	**5.684**	**5.355**	**15.858**	**17.283**	**16.570**	**93.976**	**92.100**	**93.038**
Maturity	N_0	4.413	3.777	**4.095**	6.553	6.937	**6.745**	32.683	31.545	**32.114**
	N_1	4.667	2.960	**3.813**	3.150	20.260	**11.705**	35.175	104.49	**69.833**
	N_2	3.030	4.397	**3.713**	10.763	20.953	**15.525**	67.235	106.10	**86.670**
	N_3	5.603	3.573	**4.588**	25.330	19.753	**22.542**	101.87	118.31	**110.09**
Average		**4.428**	**3.677**	**4.053**	**11.449**	**16.976**	**14.213**	**59.240**	**90.111**	**74.676**
LSD for Development phases Irrigation N fertilization			n.s. n.s. n.s.			n.s. n.s. 10.754			n.s. n.s. 39.517	
Interaction: Development phases x Irrigation			1.506			n.s.			n.s.	

IRR – irrigation, NIRR – non irrigation.

The content of NH_4^+ -N and NO_3 -N^- in the years of research depending on nitrogen fertilization and irrigation is shown in Figure 1. The content of NH_4^+ -N ranged from 1.187 to 6.867 mg·kg^{-1} of soil and did not depend on the irrigation used, it increased only slightly. with increasing doses of nitrogen fertilizer. However, the content of NO_3 -N^- was within a wider range from 1.50 to 33.23 mg·kg^{-1} of soil (Figure 2). In all years of the study, a higher content of this nitrogen fraction was found in samples

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taken from non-irrigated objects compared to the irrigated ones, and the difference between these objects in subsequent years was as follows: 50%, 30% and 12%.

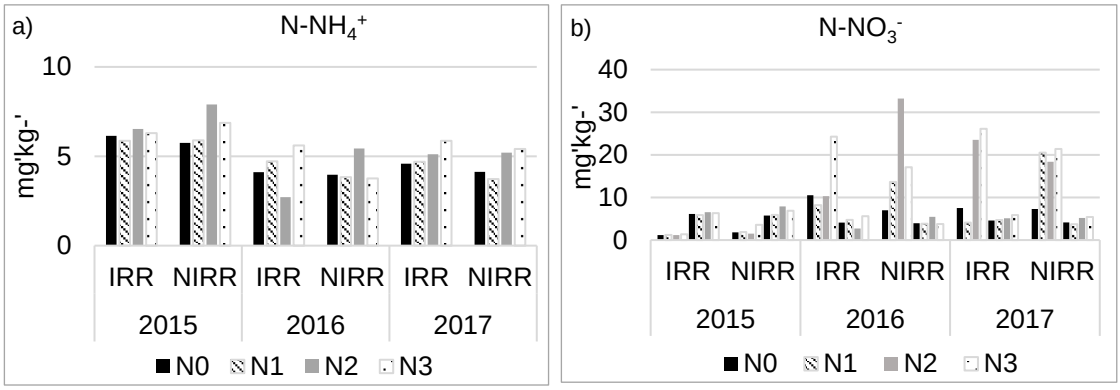

Figure 2. The content of a) ammonium and b) nitrate nitrogen in investigated soil under barley depend on fertilization in the years 2015–2017.

However, in the maturity phase, only NR activity was at 18% higher level in irrigated soils. The activity of other enzymes was higher in no-irrigated treatments by 25% in DH case, 22% in PER, 33% in CAT and 17% in UR compared to irrigated soils. Statistical analysis showed the effect of irrigation on PER, CAT and NR activity. In the case of NR, activity was influenced, apart from irrigation, by the development stage of barley. Enzymatic activity has undergone significant changes in the research years examined. Its greatest activity was found in soil samples taken in 2016, where it was on average about 4 times higher compared to the average activity determined for samples taken in 2016 and 3 times higher for the average soil activity collected in 2017. However, the activity of other oxidoreductases developed differently over the years of the study. The highest catalase activity was found in samples taken in 2015 where it was 29% higher compared to the average determined in soils from 2017.

Table 5. The enzymes' activity during the germination phase of barley vegetation in 2015, 2016 and 2017.

Year	Germination				
	DH‡	PER	CAT	NR	UR
2015	6.930	4.340	5.120	0.311	4.780
2016	25.30	4.490	2.420	3.452	6.890
2017	18.40	8.970	2.021	7.890	6.590
Mean	16.88	5.930	3.187	3.884	6.087

Table 6. The enzymes' activities during the ripening and maturity phases of barley vegetation in 2015, 2016 and 2017.

Treatment		Ripening					Maturity				
		DH‡	PER	CAT	NR	UR	DH	PER	CAT	NR	UR
Irrigation	N_0	13.55	7.717	3.192	4.800	5.032	18.21	4.819	2.887	6.260	8.144
	N_1	33.38	9.394	3.641	4.884	4.030	18.03	4.606	2.825	5.248	6.370
	N_2	29.51	8.205	3.783	3.174	3.646	24.35	5.643	3.103	4.077	4.551
	N_3	23.60	8.266	4.799	5.471	4.980	57.86	3.843	4.261	6.067	7.758
	Mean	**25.01**	**8.395**	**3.853**	**4.582**	**4.422**	**29.61**	**4.728**	**3.269**	**5.413**	**6.706**
No irrigation	N_0	27.25	7.198	3.574	7.134	4.364	16.92	6.710	2.284	2.486	6.623
	N_1	27.58	9.242	3.368	2.970	5.959	37.26	4.209	1.897	5.211	7.808
	N_2	27.33	8.601	4.310	7.901	3.195	55.62	8.235	3.069	3.888	8.040
	N_3	53.08	7.473	3.843	7.927	3.242	45.51	5.185	2.595	4.568	9.758
	Mean	**33.79**	**8.128**	**3.774**	**6.483**	**4.190**	**38.83**	**6.085**	**2.462**	**4.038**	**8.057**

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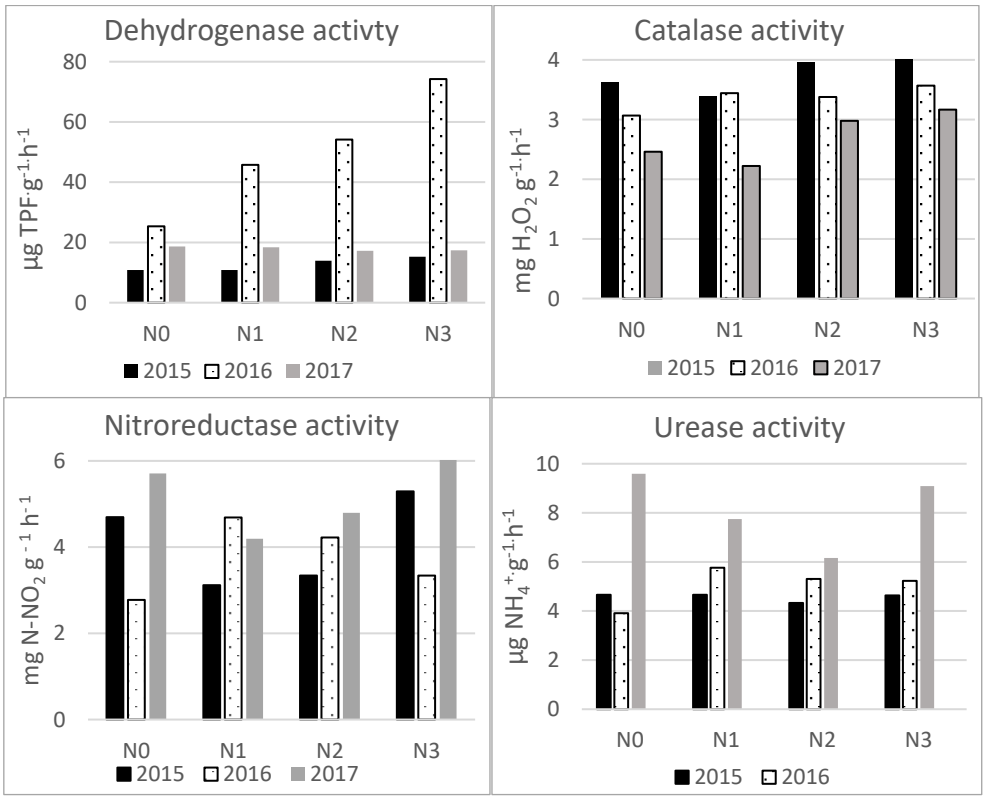
LSD for										
Development phases	ns	ns	ns	ns	ns	ns	ns	ns	1.013	ns
Irrigation	ns	ns	ns	ns	ns	ns	1.813	0.970	0.132	ns
N fertilization	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Development phases x Irrigation	ns	ns	ns	1.559	ns	ns	ns	ns	1.724	ns

‡DH -dehydrogenase activity mg TPFg $^{-1}$ h $^{-1}$. PER – peroxidase activity mmol of purpurogalin g^{-1}·h $^{-1}$. CAT catalase activity µmol H_2O_2·g $^{-1}$·min^{-1}. NR - nitroeductase activity mg N-NO_2^- kg^{-1}·24 h^{-1}. UR-urease activity mg N-$NH4^+$ kg^{-1}·h^{-1}.

Peroxidase, on the other hand, showed 70% higher activity in samples taken in 2017 compared to 2015. The influence of fertilization on enzyme activity was found; DH and CAT activity increased with increasing fertilizer doses. In the case of PER, the highest dose of fertilizer resulted in a 14% reduction in its activity compared to N_2. The activity of enzymes involved in nitrogen metabolism in soil was different compared to oxidoreductases. The activity of both these enzymes was the highest on the third date of soil sample collection. In the case of UR, the activity in this period was on average 43% higher than at the beginning of the season (germination), and nitrogenase showed 27% higher activity compared to the lowest activity in the second sampling date. The influence of nitrogen fertilization on the activity of these enzymes was also found, and on average the activity of UR was reduced by 13% when fertilized with a dose of N_2 and NR by 7% when fertilized with a dose of N_1 compared to the control objects.



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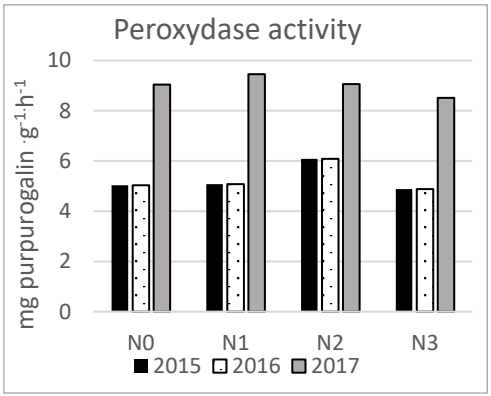

Figure 3. The enzymes' activities phases in the barley vegetation depended on nitrogen doses in 2015, 2016 and 2017.

Sprinkler irrigation applied in the barley field experiment did not significant impact on soil enzymes' activity. It is proved by non-significant coefficients of correlation obtained between the content of RAW and enzymes' activity both on irrigated and no irrigated schedules. Also, the study demonstrates the lack of response of all five soil enzymes to varying levels of nitrogen fertilization in barley cultivation (Table 7). The most sensitive enzyme to soil water content was peroxidase (r=-0.1652), while the other ones showed a similar level of response (r between -0.0712 to 0.0735). In the case of the second factor, there wasn't any response of urease to the nitrogen fertilizer level, while catalase and dehydrogenase replied in a positive, however not significant way (r=0.2001 and r=0.2576 respectively). It is worth noting that, the values of coefficients were bidirectional, depending on the type of enzyme, which confirms that the reactions of the enzymes to irrigation treatment and N-fertilizer level were ambiguous. Table 7. Coefficients of correlation (r) between soil enzymes' activity and content of ready available water (RAW) and differentiated N-fertilization level

Table 7. Coefficients of correlation (r) between soil enzymes' activity and content of ready available water (RAW) and differentiated N-fertilization level.

Types of soil enzymes	RAW	N-fertilization
Catalase	-0.0712	0.2001
Dehydrogenase	0.0735	0.2576
Peroxidase	-0.1652	-0.0087
Urease activity	0.0532	0.0000
Nitroreductase	0.0676	0.0711

RAW – ready available water in the soil, N-fertilization – nitrogen fertilization.

Table 8. Relationship between selected soil properties.

Variables dependent (y)	Variables independent (x)	Equation	Correlation coefficient (r)
Catalase activity	NH_4^+	y=2.8422+0.64010x	0.299
Dehydrogenase activity	NO_3^-	y=3.8906+0.28549x	0.523
Peroxidase activity	NH_4^+	y=3.6836+0.54160x	0.331
Urease activity	NH_4^+	y=7.6386-0,3937x	-0.297
Nitroreductase activity	Urease activity	y=6.2506-0.2875x	-0.340
Peroxidase activity	NO_3^-	y=3.6337+1.2621x	0.336
Urease activity	Peroxidase	y=6.9388-0.1986x	-0.245

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Contains and enzymes' activities were determined in this study (Table 9). Catalase, peroxidase, and urease activity were significantly correlated with NH_4^+-N contains (r = 0.299), (r = 0.331), (r =- 0.297) respectively. However, the dehydrogenase and peroxidase positively correlated with NO_3 -N content in soil. The urease activity was significantly negatively correlated with the soil enzyme activities of nitroreductase (r = -0.340) and peroxidase (r = -0.245).

Table 9. The resistance index (RS) for enzymes' activities depended on nitrogen doses during the vegetation of spring barley.

N doses	The resistance index RS				
	NR	UR	CT	PX	DH
N_0	-0.206	0.627	0.934	0.560	0.859
N_1	0.986	-0.597	0.991	0.828	0.319
N_2	0.907	0.395	0.580	0.521	0.425
N_3	0.506	0.660	0.444	0.589	0.573

The index of resistance (RS) is presented in Table 9. Differences in resistance of irrigation between enzymes were observed for doses of nitrogen. The oxidoreductases (PER, CAT, DH) enzymes with the highest RS value were observed for N_0 and N_1 doses of nitrogen. The highest RS indices (0.991 and 0.934) were calculated for CAT activity and were observed for N_0 and N_1. For these doses of N high-values RS for DH activity (0.859 for N_0) and PX (0,828 for N_1) were found. For UR the highest value of RS indices were found for N1(0.986) and N3 (0.907) doses of nitrogen. The RS indices of activities of UR and NR were negative in order at N_1 (-0.597) and at N_0 (-0.206) doses of N.

4. Discussion

Water and nitrogen are the crucial components to reduce rural production in the greatest part of the world [27]. The transforming of nitrogen in soil has a major character in the nitrogen metabolism of crop tolerance to drought stress and is engaged in nearly all physiological transformations in plants and microorganisms [28]. According to Wang et al. [29] NH_4^+ - N uptake is universally enhanced in majority plants during drought stress, and superior nitrogen uptake may increase plant drought hardiness. The outcome of the present experiment appeared the impact of irrigation on the development phases of spring barley. During the barley vegetation it was found that with the development of plants, the NH_4^+ - N content in lessive soil showed a trend of decreasing, especially in no-irrigated soil the ammonium content had decreased significantly. It may be because during maturity time, spring barley has more NH_4^+ - N uptake in the consequence way of drought stress. The result is consistent with the work of Lawlor et al. [30], who obtained increasing effective NH_4^+ nitrogen uptake and rises in the activity of NR in plants during drought stress. Compared to no irrigated soil the content of NO_3 and N_{min} in soil under irrigation treatment, were decreased during spring barley vegetation. The lowest content of NO_3^--N and N_{min} at the third term of sampling can suggest leakage NO_3^--N. Similar results were obtained by Wu et al. [31] who reported that the mineral nutrient content in the soil changed depending on irrigation and nitrogen fertilization and high irrigation water content can increase nutrient leaching and reduce soil nutrient content. Muhammad, et al. [32] show that the mechanisms of NO_3^--N leaching depend on the physical feature of soil, especially water holding capacityThe higher amount of N (300 kg N ha^{-1}) caused the higher soil SOC, total and mineral N under low (60%) irrigation. Nitrogen in the form of nitrate is highly mobile in soil and its contents is depended on soil water conditions [33]. Irrigation treatment probably contributes to increased leaching of nitrate in soil. The results of the current experiment showed that doses of nitrogen fertilizers have an impact on the contents of NO_3^--N and N_{min}. These findings are consistent with JIa [34], who presented that leakage of NO3−-N increases even using the same N fertilizer application rate due to a vast sum of irrigation.The temperature, moisture effects on enzyme diffusion and substrate availability are all critical factors influencing soil enzymes activities [35]. Drought greatly influences almost all physiological and biochemical transformations of plants: growth, development and productivity. The nitrogen content and its transformation in soil are

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decisive during drought stress for the plant and microorganisms' metabolism. The present study showed that the investigated enzymes are sensitive soil components, which are strictly connectedwith the physicochemical and biological properties of the soil. The reactions of enzymes depend on their origin and features [36]. In this study, we demonstrated the lack of responses of all five types of soil enzymes to different levels of nitrogen fertilization in barley cultivation (Table 7). Cui et al., [37] suggest that monoculture and fertilization can increase enzyme activity by improving soil nutrients and microbial richness. Zhang et al. [38] determined that water and nitrogen addition influenced to soil enzyme activity mainly by caused by soil microbial biomass carbon. Many field studies have examined the effect of nitrogen addition on the activity of enzymes in the soil. The results of these studies were inconclusive. Some results suggested that the addition of nitrogen fertilizer caused soil acidification and inhibited soil enzymatic activity [38]. Other studies indicated a stimulating effect of nitrogen on enzyme activity or no effect on it at all [40–44].

Urease hydrolysis of small organic substrates containing nitrogen into inorganic compounds (ammonia) to supply nitrogen for the normal growth and development of plants [45]. In our study, the development phases, irrigation and N mineral fertilization showed no statistical impact on urease activity. Similar results were obtained by Zhao et al. [46] in their research that single-nitrogen nor mixed-nitrogen applications did not affect urease activity significantly. However, the present study presented that the activity of this hydrolase in soil had a trend of increasing step and later decreasing, and hit the maximum at the maturity phase and during this time increased with the increasing doses of N fertilizer, especially in no irrigated treatment. Weng et al. [47] and Gong et al. [44] obtained that mineral nitrogen fertilizer often increase urease activity. Fortification of urease activity due to natural or organic nitrogen addition was observed by Nayak et al. [15] and Iovieno et al. [48]. The higher hydrolase activities may be caused by the increase in carbon and nitrogen in soil and the improvement of soil physicochemical features as well as a more appropriate soil environment for microbial growth and proliferation which stimulates microbial and enzymatic activity. Negative effects resulting from lower pH have also been observed with long-term use of nitrogen fertilizers [49].

The activity of enzymes depends on several factors but especially on the presence of substrate, in NR case is nitrate in the soil. Nitrate reductase is the controlling and reduce enzyme of nitrate assimilation in plants, which are not only responsive to outer nitrogen but also indirectly create a difference in the uptake and utilization of nitrogen by plants [50]. Waraich et al., [51]; Sardans and Peñuelas, [52] reported that drought stress reduces plant N uptake and assimilation by reducing both nutrient diffusion and N supply via mineralization [53]. In our studies, lower NR activity during the maturity phases on no irrigated treatments may result from the reaction of plants and microorganisms to long drought stress. The NR activity increased at the ripening phase and then decreased at maturity time at the no-irrigated treatment. Similar to NR activity, CAT was dependent on the irrigation treatment during the maturity time of the spring barley, and the activity of this oxidoreductase increased in irrigated soil. However, PER presented a different reaction and reached the highest activity in no irrigated soil and statistically depended on doses of water. Peroxidase is an enzyme that is expressed for a variety of reasons, including the obtained of carbon and nitrogen and protection. The enzyme moves into the environmental soil by excretion or lysis, where it mediates way ecosystem functions of lignin degradation, humification, carbon mineralization and dissolved organic carbon export [7]. Higher PER activity in no-irrigation soil indicates high oxygen availability, optimal pH conditions and mineral activity, which indicates high oxidative activity and limits the accumulation of organic matter in the soil [7].

An interesting observation regards dehydrogenases, which are one of the most important oxidoreductases and are used as an indicator of overall soil microbial activity because they are tightly linked with microbial oxidoreduction processes as occur in all living microbial cells therefore are used as indicators of microorganisms activity in soil [54]. Our research has shown that soil moisture influences dehydrogenase activity. The high DHA activity in soil during spring barley vegetation in 2016 which was the most rainy year of our investigation is coincident with the results of Gu et al. [55]. They had observed an increase in DH in high-moisture soil. The high dehydrogenase activity cay be

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due to two factors: as a result of flooding, releasing and spread of soluble organic compounds in soil can be caused, which contributes to the development of a larger number of bacteria that secrete dehydrogenases and/or the change of oxygen conditions to anaerobic conditions and the proliferation of anaerobic microorganisms [56]. Also, Dora [57], indicates that dehydrogenase and catalase activities were higher in irrigated soil. Tan et al. [58] found that long-term mulched drip irrigation (8, 12, 16, and 22 years) tends to accumulate soil nutrients and rebuild enzyme conditions. Soil enzymes, such as catalase and urease were more active in the subsoil than in the topsoil. Also, Liang et al. [59] confirmed that long-term irrigation strongly increased the activity of dehydrogenase as well as urease in the soil. Núñez et al. [60] indicated that the reduce in enzyme activity after irrigation termination in corn may point to changes in biogeochemical cycling and even a potential reduction in the decomposition of leftovers [11,61]. However, enzyme activity can also be affected by changes in soil environmental circumstances [61,62] such as reduced water availability can increase enzyme immobilization and decrease diffusion rates, decreasing enzyme efficiency and affecting residue decomposition independently of changes in potential enzyme activity [63]. A negligible effect of irrigation on the activity of soil enzymes was also reported in grassland ecosystems [64]. Moreover, it has been shown that additional water application can mitigate the effects of nitrogen enrichment on microorganisms by leaching or reducing the accumulation of inorganic nitrogen [65,66] and have a significant effect on soil enzyme activity.

The present study showed that catalase, peroxidase and urease were correlated significantly with NH_4^+- N content (appropriately $r = 0.299$, $r = 0.331$ and $r = 0.297$, $p = 0.05$), and dehydrogenase and peroxidase activity with NO_3^- N content ($r = 0.523$ and $r = 0.336$, $p = 0.05$ in order). Nitroreductase was negatively correlated significantly with urease activity($r = -0.340$; $p = 0.05$) and with peroxidase ($r = -0.254$; $p = 0.05$), indicating that some enzyme activity may affect and present other enzyme activities in soil considerably.

The resistance to the drought of investigated enzymes was different depending on the doses of nitrogen fertilization. Catalase showed the highest resistance against drought stress, followed by NR and PER. Urease and dehydrogenases showed lower resistance to soil drought. The resistance to the drought of investigated enzymes was different depending on the doses of nitrogen fertilization. Catalase showed the highest resistance against drought stress, followed by NR and PER. Urease and dehydrogenases showed lower resistance to soil drought. The results by Lemanowicz [67] show that the catalase activity has a strong resistance to the salinity stress, too.

5. Conclusions

In summary, our results suggest that no irrigation influences the NH_4^+ - N content in Alfisol soil during the maturity stage of spring barley, due to its low uptake being the consequence of drought stress. Irrigation treatment may contribute to raising nitrate leaching in the soil profile. The results of our experiment show that different dos-es of nitrogen fertilizer influenced the contents of NO_3^-–-N and Nmin. The level of nitrogen fertilization of 60 t ha-1 was optimal for the content of NO_3^-–-N and NH_4^+ - N available to plants. The present study indicates that the investigated enzymes are sensitive to soil components, which are closely related to the content of NH_4^+ - N and NO_3^-–-N in the soil. Enzymatic activity has changed in the research years examined, depending on the weather conditions. Soil enzymes' activities could be alternative natural bio-sensors for the effect of irrigation on soil biochemical reactions and could help optimize irrigation management of crop production. The resistance index could be used to sensor enzymatic water stress solution. It showed, that the highest index of resilience was presented by catalase. The obtained results indicate that there is a need to conduct further research on selected physicochemical and biochemical parameters, as well as on other types of soil and under other crops, especially in the area of the moderate transitional climatic zone, characterized by the occurrence of meteorological conditions that vary over time.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: title; Table S1: title; Video S1: title.

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Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, A. S-Z and R. K-T methodology, A. S-Z and R. K-T.; software, A. S-Z and R. K-T.; validation, A. S-Z and R. K-T.; formal analysis, A. S-Z and R. K-T.; investigation, A. S-Z and R. K-T; resources, A. S-Z and R. K-T data curation, A. S-Z and R. K-T.; writing A. S-Z and R. K-T.; supervision, A. S-Z and R. K-T.

All authors have read and agreed to the published version of the manuscript." Please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

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Data Availability Statement: We encourage all authors of articles published in MDPI journals to share their research data. In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Where no new data were created, or where data is unavailable due to privacy or ethical restrictions, a statement is still required. Suggested Data Availability Statements are available in section "MDPI Research Data Policies" at https://www.mdpi.com/ethics. 398 Conflicts of Interest: The authors declare no conflicts of interest.

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