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Soil Extracellular Enzyme Activities and Uptake of N by Oilseed Rape Depending on Fertilization and Seaweed Biostimulant Application

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Abstract: The present study has aimed at enhancing the insufficient knowledge of functional soil enzymes properties influenced by inorganic fertilization and biostimulant application to increase the uptake of nitrogen affecting the winter oilseed rape yield. Field experiments were conducted in Poland (53° N, 18° E) in *Alfisol* (USDA). In this experiment, the NPK rates applied were as follows: high 180 N, 70 P and 160 K 132 N (kg ha^{-1}) or low 144 N, 35 P and 66 K (kg ha^{-1}); fertilization with elemental S 36 or 0 (kg ha^{-1}); and the seaweed biostimulant Kelpak was applied or there was no such treatment. Due to low NPK fertilization rates, the activity of dehydrogenases, peroxidases, and catalase increased in subsistent generative development stages from flowering to ripening. At the ripening stage, the activity of these enzymes, as well as nitroreductase activity, were inhibited by high NPK fertilizer rates. The seaweed biostimulant application and S fertilization increased N accumulation in plants of oilseed rape in generative development, by 16% and 13%, respectively, as compared with the lack of these treatments. The application of S increased the uptake of nitrogen in shoots and in whole oilseed rape plants only after application of higher rates of NPK.

Keywords: internal efficiency; nitrogen; phosphorus; potassium; reciprocal internal efficiency of nitrogen

1. Introduction

Nitrogen is one of the key sources of nutrients applied into soil to increase the efficiency of agricultural crops, however, it can trigger environmental protection problems when it is applied inadequately [1–3]. Enhancing nitrogen effectiveness is important both for sustainable agriculture and the stability of global ecosystem. More efficient N use in farming systems is possible by improving N management practices, e.g., by applying biostimulants of the crop [2].

Soil enzymes take part in the circulation of biogenic elements C, N, P, and S [4], which is of great importance in terms of ecology as the activity of microorganisms and the enzymes they secrete provide the cycle of elements to nature [4,5] and convert the substances they contain into forms available to organisms. Soil dehydrogenases are most crucial and as such, they are used as an indicator of overall soil microbial activity [4–7], as they occur intracellularly in all living microbial cells [8]. Dehydrogenases do not accumulate extracellularly in soil [9]. Similarly, catalase is an intracellular enzyme participating in the decomposition of toxic H_2O_2 produced from mitochondrial electron transport and from various hydroxylation and oxygenation reactions into water and oxygen. Catalase activity was used to characterize soil microbial activities [10]. Peroxidases mediate biogeochemical processes in soils, including microbial acquisition of carbon and nitrogen, lignin degradation and carbon

mineralization [11]. Urea and organic nitrogen compounds contained in animal urine in soil undergo hydrolysis as affected by urease produced by microorganisms [12]. The process of nitrate reduction to ammonia, and frequently to free nitrogen, which is referred to as the process of denitrification, occurs, with enzymes of nitroreductases participating. Under natural conditions, the size of the population and biological processes of soil microorganisms undergo constant fluctuations. The soil enzymatic activity shows a clear seasonal and daily variation, as well as considerable fluctuations, mostly dependent on climate conditions and substrate availability [4,10–13].

Studies of the transformations of oxidoreductases connected with nitrogen transformations due to varied mineral fertilization and the application of biopreparations at generative stages of crops are scarce. Therefore, the aim of this study was to analyze the effects of different NPK rates of fertilization and S fertilizer and biostimulant application on their interaction with soil enzymes during generative stages of rape. We also wanted to identify the level of nitrogen accumulation through the aboveground part and the roots of winter rape in successive generative development stages under varied NPK fertilization rates as well as after and without S fertilization, as well as after the application of biostimulant. Lower NPK rates were used to check the efficiency of the biostimulator applied in autumn under the stress conditions resulting from a lower availability of nitrogen.

2. Materials and Methods

2.1. Study Site and Soil Sampling

The study was based on a field experiment located in Poland, the kujawsko-pomorskie region (53°13′ N, 17°51′ E) near Bydgoszcz (Midwestern Poland). Soil samples were collected three times per year in 2011, 2012, and 2013 to determine its chemical properties. Samples were taken in the spring during flowering (between 15 and 20 May), in June during the development of fruit (between 10 and 20 June), and in July in the time of ripening (between 19 and 27 July). The experiment was conducted in *Alfisol* (USDA) [14], where the topsoil demonstrated an average content of available sulfur (13 mg S kg^{-1}), a slightly acidic reaction (pH in 1M KCl 5.7–6.1). The content of organic carbon (7.55–7.80 g kg^{-1}) and total nitrogen (0.69–0.75 g kg^{-1}) in the soil is relatively low while the content of available P (64.0mg kg^{-1}) is medium and K is high (126.0 mg^{-1}). The study involved 'Chagall' winter oilseed rape. The field rape (*Brassica napus* L. var. *napus*) was sown between 18 and 22 August after the harvesting of winter wheat. The experimental factors were fertilization NPK, elemental sulfur and doses of biostimulant Kelpak (Table 1).

Table 1. Fertilization in treatment.

Treatment	Specification
fertilization NPK	high $N_{180}P_{70}K_{132}$: 180 N, 70 P, 160 K 132 N [kg ha^{-1}] low $N_{144}P_{35}K_{66}$: 144 N, 35 P, 66 K [kg ha^{-1}]
elementary S fertilization	36 kg ha^{-1} 0 kg ha^{-1}
biostimulant Kelpak	2 dm^3 ha^{-1} in autumn + 2 dm^3 ha^{-1} in spring 0 dm^3 ha^{-1} (Control)

$N_{144}P_{35}K_{66}$ is the standard NPK fertilization dose used at the location of the study. The high dose of NPK and S were applied taking into consideration the soil's nutrient content, unit uptake, and the expected yield (3.5 Mg ha^{-1}). Biostimulant Kelpak is made from marine macroalga (*Ecklonia maxima* Osbeck) of brown algae (Phaeophyta) collected on the coast of Africa. This seaweed extract contains phytohormones: Auxins cytokinins, alginate, and amino acids, as well as small amounts of macro- and microelements [15]. Mineral, soil fertilization with K (potash salt), P (triple superphosphate), and S (Wigor S) were applied by pre-sowing in autumn. Nitrogen (ammonium nitrate) was applied at the start of growth (100 kg ha^{-1}) in spring and the other part after 3 weeks.

2.2. Plant Sampling

The field experiment was carried out in the randomized split-block design in four replications; the plot area was 13 m^2. Samples of plants (roots and shoots, i.e., stems and leaves) were collected three times every 30 days at the following developmental stages: Flowering (BBCH 65–67, development of fruit (BBCH 74–78), and ripening (BBCH 86–87). Soil samples were gathered from 0 to 20 cm of the topsoil at the same date as the sampling of the plant. The plant and soil materials were collected in four replications of all treatments at each development stage. Field-moist sampled soils were sieved (2-mm mesh) and stored in a plastic box at 4 °C for not less than two days in order to stabilize the microbial activity and then were analyzed for enzyme activity.

2.3. Biochemical Analysis

The content of nitrogen as a dry weight was determined with the Kjeldahl method [16]. Nitrogen content in a particular plant part was calculated by multiplication of its concentration and respective biomass. Dehydrogenase activity (DH, E.C. 1.1.) was measured with a buffered tetrazolium salts (TTC) reduction rate to TPF in soils after incubation at 30 °C for 24 h as described by Thalmann [17]. The activity of that enzyme is presented in mg TPF·g^{-1}·h^{-1}. For catalase (CAT, E.C. 1.11.1.6) assaying using Johnson and Temple's [18] method was applied, involving the incubation of soil with hydrogen peroxide (natural enzyme substrate) added. The results are given in units of catalase activity, equal to μmol H_2O_2·g^{-1} s.m·min^{-1}. H_2O_2 remaining in soil, undecomposed by catalase, was titrated with potassium permanganate in an acid environment. The results are given in units of catalase activity, equal to μmol H_2O_2·g^{-1} s.m·min^{-1}. Peroxidase activity (PER, EC 1.11.1.7) was measured according to Ladd [19], using pyrogallol as the substrate; the amount of purpurogaline produced is adopted as the activity unit (mmol of purpurogaline g^{-1} h^{-1}). Nitrate reductase (NR, E.C. 1.7.99.4) was measured as described by Kandeler [20] using KNO_3 as the substrate. The activity of that enzyme is provided in mg of released N-NO_2·kg^{-1}·h^{-1}. Urease activity (UR, E.C. 3.5.1.5) was assayed as described by Kandeler and Gerber [21] using urea as the substrate. After incubation at 37 °C, samples were incubated for 3 h at 37 °C. Urease activity is expressed as μg NH_4^+ released per gram of soil per hour.

2.4. Data Analysis

The parameters, internal nutrient efficiency (IE) and reciprocal internal nutrient efficiency (RIE), were calculated according to the following formulas [22]:

$$\text{IE (kg kg}^{-1}\text{)} = \text{seed yield/N uptake in plant} \quad (1)$$

$$\text{RIE (kg 1000 kg}^{-1}\text{)} = \text{nutrient uptake in plant/seed yield} \times 1000 \quad (2)$$

The results were statistically analyzed using the statistics program, analysis of variance for orthogonal experiments, by the UTP University of Science and Technology in Bydgoszcz, Poland. The differences between the values were verified with Tukey's test at significance levels marked by asterisks (* p\0.05). Besides, the paper determined the value and significance of the coefficients of Pearson's linear correlations across the parameters and evaluated the dependence between them under soil conditions with the use of Statistic 12.0 software (STASISTICA 12, StatSoft Polska 2018).

3. Results

3.1. Effect of Fertilization and Biostimulant Application on Enzyme Activity during Plant Vegetation

The activities of enzymes changed during the vegetation period of winter rape and depended on the mineral fertilization level and biostimulant application (Table 2). The highest activity of DH and NR on the highest level of $N_{180}P_{70}K_{132}$ was in soils sampled at the flowering stage. At the successive rape development stage, the activities decreased by 63% for DH and 60% for NR and then at the ripening

stage, it decreased by 47% and 70%, respectively. The highest activity of DH, PER, CAT and NR on the low level of $N_{144}P_{35}K_{66}$ fertilization was observed during the ripening stage. The activity of PER and CAT was the lowest in the flowering time and increased gradually for both levels of fertilization. The activity of UR was comparable during flowering and the development of fruit and decreased during ripening.

The effect of fertilization NPK was very pronounced on DH activity it was 3.7-fold higher in the soils fertilized with $N_{144}P_{35}K_{66}$ than the $N_{180}P_{70}K_{132}$ rate during flowering. While on the ripening time, the highest 2.7-fold activity of DH was observed with the $N_{180}P_{70}K_{132}$ rate. During the ripening time, the highest level of NPK stimulated not only DH activity but also PER by 9%, CAT by 22% and NR by 95% (Table 2.).

During fruit development time was observed the impact of the S_{36} fertilization on the CAT and UR activity. Activity of CAT was stimulated by 28% by adding sulfur to the $N_{180}P_{70}K_{132}$ fertilization. In the case of UR on both levels of NPK fertilization, sulfur application stimulated the activity of this hydrolase. S_{36} fertilization stimulated the NR activity by 51% on the $N_{180}P_{70}K_{132}$ level while on the $N_{144}P_{35}K_{66}$ it was inhibited by 30% at the ripening time. At the flowering stage, the impact of fertilization with NPK and S on the DH and UR activities has been found. The application of S_{36} inhibited the DH by 78% and UR by 31% after application of $N_{180}P_{70}K_{132}$.

A persisting, from the stage of flowering to fruit development, interaction between NPK fertilization rates and elementary sulfur fertilization was recorded. S application (S_{36}) together with $N_{144}P_{35}K_{66}$ fertilization stimulated the activity of NR by 76% on the flowering time of rape, as compared with the object without sulfur (S_0) fertilization. Whereas S application (S_{36}) together with the $N_{180}P_{70}K_{132}$ rate inhibited NR activity by 79%. Whereas the S_{36} fertilization with $N_{180}P_{70}K_{132}$ stimulated the NR activity by 25% in the fruit development. During these vegetation periods NR activity with $N_{144}P_{35}K_{66}$ fertilization was inhibited by 76%. At fruit development, the fertilization of S_{36} inhibited the activity of UR as it was 25% lower, as compared with the objects' S_0.

At the flowering stage the impact of the biostimulant on NR activity was observed. The application of Kelpak inhibited enzyme activities by 88% with $N_{180}P_{70}K_{132}$ and S_0 fertilization and by 61% with $N_{144}P_{35}K_{66}$ and S_{36} fertilization. At this stage, interactions between fertilization with NKP as well as the application of biopreparation on the activity of UR were found. However, in the objects without Kelpak, a 44% higher UR activity was recorded on the plots with $N_{144}P_{35}K_{66}$. The application of fertilizer with S_{36} and biostimulant reported a 30% lower activity than in the soil from the objects fertilized with S_0 and without seaweed extract. At the fruit development stage of DH activity, interactions between the NPK rate and the application of biostimulant were observed. The highest activity of DH was found in the objects fertilized $N_{180}P_{70}K_{132}$ and without algae extract and a 24% lower activity in the objects fertilized with $N_{144}P_{35}K_{66}$ and with biostimulant.

Table 2. Enzyme activity in soil depending on the mineral fertilization level and biostimulant application, mean from 2011–2013.

Treatment			Flowering					Fruit Development					Ripening				
			DH ‡	PER	CAT	NR	UR	DH	PER	CAT	NR	UR	DH	PER	CAT	NR	UR
$N_{180}P_{70}K_{132}$	S_0	Biostimulant †	8.316	2.260	0.028	0.188	4.074	1.134	2.333	0.029	0.088	3.843	0.588	2.425	0.030	0.067	2.594
		Control	6.090	2.217	0.032	1.612	2.601	1.512	2.449	0.030	0.085	3.357	1.092	2.535	0.031	0.018	2.202
	S_{36}	Biostimulant	1.260	2.181	0.030	0.195	2.583	1.137	2.309	0.046	0.120	2.737	0.924	2.199	0.032	0.098	2.727
		Control	1.974	2.156	0.026	0.177	2.009	2.772	2.248	0.033	0.576	2.132	0.840	2.522	0.037	0.077	2.524
Mean			4.410	2.204	0.029	0.543	2.817	1.639	2.335	0.035	0.217	3.017	0.861	2.420	0.032	0.065	2.511
$N_{144}P_{35}K_{66}$	S_0	Biostimulant	0.924	2.239	0.025	0.203	3.336	2.184	2.394	0.034	0.418	3.903	1.008	2.693	0.042	2.906	2.212
		Control	1.176	2.278	0.018	0.464	3.143	0.756	2.184	0.028	1.128	3.493	2.898	2.593	0.040	0.332	2.289
	S_{36}	Biostimulant	1.470	2.092	0.031	0.789	2.401	1.092	2.251	0.034	0.094	2.317	2.092	2.577	0.038	0.449	2.450
		Control	1.176	2.312	0.024	2.028	5.107	1.050	2.352	0.030	0.275	0.749	2.312	2.766	0.042	1.829	2.198
Mean			1.187	2.230	0.024	0.871	3.497	1.270	2.295	0.032	0.479	3.365	2.373	2.657	0.041	1.379	2.287
S_0			4.127	2.249	0.206	0.617	3.288	1.396	2.340	0.030	0.430	3.649	1.397	2.561	0.036	0.831	2.324
S_{36}			1.470	2.185	0.028	0.797	3.025	1.513	2.290	0.036	0.266	2.734	1.838	2.516	0.037	0.613	2.475
Biostimulant			2.993	2.193	0.029	0.344	3.098	1.387	2.322	0.036	0.180	3.200	1.313	2.474	0.035	0.880	2.496
Control			2.604	2.241	0.025	1.070	3.215	1.523	2.308	0.030	0.516	3.182	1.922	2.604	0.038	0.564	2.303
LSD for NPK			2.775	ns	ns	ns	ns	ns	ns	ns	ns	ns	1.029	1.013	ns	1.013	ns
S			ns	ns	ns	ns	ns	ns	ns	0.005	ns	0.647	ns	ns	0.132	0.132	ns
B			ns	ns	ns	0.703	ns	ns	ns	0.005	ns	ns	ns	ns	ns	ns	ns
NPK × S			3.924	ns	ns	0.994	0.980	ns	0.572	ns	0.572	ns	ns	ns	0.045	ns	ns
NPK × B			ns	ns	ns	ns	0.980	1.216	ns	ns	ns	ns	ns	ns	0.186	ns	ns
S × B			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.186	ns	ns

‡ DH—Dehydrogenase activity (mg TPF·g^{-1}·h^{-1}), PER—Peroxidase activity (mmol of purpurogaline g^{-1} h^{-1}), CAT—Catalase activity (μmol H_2O_2·g^{-1} min^{-1}) NR—Nitroreductase activity (mg N-NO_2^- kg^{-1} $24h^{-1}$), UR—Urease (μg NH_4^+ g^{-1} h^{-1}); † Biostimulant—Kelpak 2 dm^3 ha^{-1} in autumn and 2 dm^3 ha^{-1} in spring; Ns—differences not significant.

3.2. Effect of Fertilization on Nitrogen Uptake by Winter Rape

Nitrogen uptake by the aboveground part of winter rape changed in the vegetation period and it was the lowest during flowering (Table 3). At all the development stages, an effect of the applied factors on nitrogen uptake was found. A higher uptake always occurred in shoots with $N_{180}P_{70}K_{132}$ fertilization and the application of S_{36} fertilizer and biostimulant. It was higher than in the objects fertilized with $N_{144}P_{35}K_{66}$ 11–17%, non-fertilized with S_{36} from 12% to 15%, and without biostimulant from 10% to 19%, depending on the rape development stage.

Table 3. Uptake of nitrogen in shoots and roots of rapeseed in consecutive generative development stages depending on mineral fertilization rates and biostimulant application, mean from 2011–2013 (kg ha^{-1}).

Treatment		Flowering			Fruit Development			Ripening		
		Shoots	Roots	Total	Shoots	Roots	Total	Shoots	Roots	Total
$N_{180}P_{70}K_{132}$	S_0									
	Biostimulant †	136	13.9	150	191	13.8	205	189	18.9	208
	Control	128	16.2	144	172	12.5	185	191	18.3	209
	S_{36}									
	Biostimulant	179	20.4	199	248	17.0	265	252	23.4	276
	Control	139	13.4	152	210	13.5	224	198	18.0	216
	Mean	145	16.0	161	205	14.2	220	208	19.7	227
$N_{144}P_{35}K_{66}$	S_0									
	Biostimulant	136	12.8	148	180	10.9	191	197	17.1	214
	Control	103	10.9	114	182	11.2	193	152	16.4	169
	S_{36}									
	Biostimulant	132	13.6	146	198	12.6	210	201	19.4	220
	Control	124	12.1	136	174	10.3	185	164	16.3	180
	Mean	124	12.3	136	184	11.2	195	179	17.3	196
	S_0	143	14.9	158	208	13.4	221	204	19.3	223
	S_{36}	126	13.4	139	181	12.1	193	182	17.7	200
	Biostimulant	146	15.2	161	204	13.6	218	210	19.7	230
	Control	123	13.,1	136	185	11.9	197	176	17.3	194
	LSD for NPK	13.8	1.74	14.3	14.6	1.18	14.9	21.0	1.77	21.6
	S	13.8	ns	14.3	14.6	1.18	14.9	21.0	ns	21.6
	B	13.8	1.74	14.3	14.6	1.18	14.9	21.0	1.77	21.6
	NPK × S	ns	ns	ns	20.6	ns	21.1	ns	ns	ns
	NPK × B	ns	ns	ns	ns	ns	ns	ns	ns	ns
	S × B	ns	2.46	ns	ns	ns	ns	ns	2.50	ns

† Biostimulant—Kelpak 2 dm^3 ha^{-1} in autumn and 2 dm^3 ha^{-1} in spring. Ns—differences not significant.

At the fruit development stage, the interaction of fertilization with NPK and S in the uptake of N in the shoots and whole plants of oilseed rape was found. The application of S increased the uptake of nitrogen in shoots and in whole oilseed-rape plants only after the application of higher rates of NPK.

The uptake of nitrogen by winter rape roots changed in the vegetation period and was lowest during the fruit development stage. At all the stages, an effect of fertilization with a higher NPK rate and the application of seaweed extract on the nitrogen uptake by roots was found. During fruit development, sulfur application also increased the uptake of that nutrient. The interaction between the use of sulfur and biostimulant on nitrogen accumulation in roots was found at flowering and ripening. The fertilization with S_{36} resulted in an increase of N uptake by roots of oilseed rape only when biostimulant was applied.

3.3. Effect of Fertilization on Internal Efficiency and Reciprocal Internal Nutrient Efficiency

The internal efficiency (IE) of N (the amount of seed yield per 1 kg of the nutrient accumulated in the aboveground part) was the highest in flowering and comparable at the stages of fruit development and ripening (Table 4). This index was higher after the application of $N_{144}P_{35}K_{66}$ and without S_0 fertilization, as well as without the application of biostimulant. The nitrogen uptake per seed yield unit (RIE) increased at successive stages of generative development. This index at ripening was 1.6 times higher than the one at flowering. The application of sulfur and biostimulant resulted in at each development stage, an increase in RIE, as compared with no application of those factors at all. In contrast, the RIE in each development stage was very similar at a high and low NPK fertilization rates.

Table 4. Internal efficiency and reciprocal internal efficiency of nitrogen mean from 2011 to 2013.

Growing Stage	Treatment						Mean
	$N_{180}P_{70}K_{132}$	$N_{144}P_{35}K_{66}$	S_{36}	S_0	Biostimulant	Control	
Internal efficiency (IE) seed yield (kg)/N uptake in plants (kg)							
Flowering	23.2	24.5	23.0	24.6	23.0	24.6	23.2
Development of fruit	16.4	16.9	15.9	17.3	16.3	17.0	16.4
Ripening	17.0	18.4	17.0	18.4	16.5	18.9	17.0
Reciprocal internal efficiency (RIE) N uptake in plants (kg)/seed yield (kg) × 1000							
Flowering	46.2	44.4	47.2	43.3	47.6	43.0	46.2
Development of fruit	64.1	62.9	66.6	60.4	65.2	61.8	64.1
Ripening	75.0	77.5	78.5	74.0	80.1	72.4	75.0

† Biostimulant Kelpak 2 dm^3 ha^{-1} in autumn and 2 dm^3 ha^{-1} in spring.

3.4. Correlation between Enzymatic Activity and Researched Parameters

A significant correlation was found between the activity of PER, CAT, and UR and the accumulation and uptake of nitrogen above ground and the uptake in roots (Table 5). The uptake above ground was significantly and positively related to CAT and negatively related to UR. The activity of PER was related to the activity of CAT and both of these enzymes correlated with the uptake of nitrogen in roots. The highest correlation coefficients were observed between the uptake above ground and the accumulation of nitrogen in the aboveground part and the uptake in roots and the accumulation of nitrogen in the aboveground part.

Table 5. Correlation matrix.

Parameters	PER	CAT	UR	Uptake of N in Shoots	Uptake of N in Roots
PER—peroxidase activity	1	*	-	-	-
CAT—catalase activity	0.54	1	*	*	*
UR—urease activity	−0.18	−0.45	1	*	-
Uptake of N in shoots	−0.41	0.64	−0.44	1	*
Uptake of N in roots	−0.14	0.65	−0.29	0.72	1

The differences between the values were verified with Tukey's test at significance levels marked by asterisks (* p\0.05).

4. Discussion

In agricultural fields, fertilizer N, P, K, and S application rates lead to temporarily very high osmotic potentials and potentially toxic concentrations of the nutrient forms added [22].While plant production in agriculture is generally N limited, soil microorganisms may be carbon (C) or N limited. However, the essentially higher productivity brought on by fertilization in agricultural systems increases inputs of organic material in the form of root exudates, decaying roots, and aboveground residues, and thus

increases the pool of C sources for soil. The response of soil microbes may therefore be ambiguous and differ from the response of the plant [23].

4.1. Effect of Mineral Fertilization on Enzyme Activity

The highest activity of DH and NR at the flowering time on the highest NPK fertilization may be due to the difference in the multiplication rate of different soil microorganisms, which are usually maximum during the flowering stage [24]. Moreover, it could be attributed to the qualitative and quantitative changes in nature that were made by mineral fertilizers during different growth stages. Our research has shown that the activities of DH, NR, and UR are sensitive to mineral fertilization with NPK and its interaction with sulfur. Recent meta-analyses based on data predominantly from unmanaged ecosystems suggest that increasing N inputs suppress soil microorganisms [23]. Our results in which the highest DH, PER, CAT, and NR activities were obtained at the ripening time by the lowest NPK fertilizers are confirmed by this data. Elemental S oxidation, as well as ammonia nitrate, in soil is a microbial process and changes the soil's chemical properties, such as acidity and ionic strength [8]. Some of the acidity produced by nitrification is neutralized when plants take up more nitrate than cations [23]. Therefore, the reaction of microorganisms in soils is ambiguous. CAT activity was stimulated while UR activity was inhibited by $N_{180}P_{70}K_{132}$ fertilization and the rates of S_{36} during fruit development. The PER activity, usually correlated with environmental stress (extreme temperature, drought, mineral deficiency) and participating in detoxication [25], in our study points to a low activity, suggesting a lack of presence, for the period of three research years, of the phenomena, which would be very stressful.

4.2. Effect of Biostimulant Application on Enzyme Activity

No pronounced effect of the biostimulant on enzyme activity was found. CAT and NR were modified by the application of biostimulant whereas the activity of DH and UR was dependent on the interaction of NPK fertilization and the Kelpak rate. The biostimulant application increased catalase activity at the fruit development stage and decreased NR activity at the flowering stage. It is a different result from that reported by Bielińska et al. [26], where the fertilizing agents applied (EM-A, PRP Sol, Rosahumus, UGmax,) stimulated the activity of dehydrogenases, urease, and protease in Podzols and Cambisols. Our results suggest that the seaweed extract, Kelpak, stimulates the development of plant roots to increase the retentive area of the plant and does not clearly stimulate the activity of soil enzymes.

4.3. Effect of Fertilization and Biostimulant Application on Nitrogen Uptake by Winter Rape

The use of biostimulant increased the nitrogen accumulation both in the shoots and roots at all the winter oilseed-rape growth stages (Table 3). This effect may be compared with the effect of an increased rate of NPK fertilization. According to Jannin [27], the beneficial effect of seaweed extract application to plants seems to be the result of many components (phytohormones, betaines, polymers, nutrients), which can work synergistically. In our study, an interaction between sulfur fertilization and biostimulant application on nitrogen accumulation in roots was found.

At flowering and ripening, fertilization with S resulted in an increase in N uptake in roots only when biostimulant was applied, which could have been due to the increase in the expression of genes encoding N and S metabolism in oilseed rape, which is caused by seaweed extract application [27,28]. Wierzbowska and Bowszys [29] obtained results showing that growth regulators (gibberellin and auxin) inhibited the accumulation of nitrogen in aerial organs of wheat, especially in grain. In our study, we found that at the fruit development stage, fertilization with S increased the uptake of nitrogen in shoots and in whole oilseed-rape plants only when higher NPK rates were applied. Also, other authors have identified the relationship between the uptake of N and other macroelements. According to Gaj [30], K deficiency limits N accumulation and transport. Szczepaniak [31] demonstrated that nitrogen accumulation at the flowering stage depended on K accumulation in the aboveground part of

oilseed rape. Similar to Salviagiotti et al. [32], a synergic effect of N and S was found. Oilseed rape fertilized with sulfur accumulated more nitrogen in the aboveground part and whole plants at all generative development stages in oilseed rape (Table 3). According to Zhao et al. [8], oilseed rape and other plants of the *Brassica* species usually require larger amounts of sulfur during growth for the synthesis of both naturally occurring proteins and glucosinolates. Thus, oilseed rape is particularly sensitive to S deficiency, which was remarkably confirmed for the combined N and S application [33]. It is worth emphasizing that the highest N uptake in the total plants occurred at the ripening stage (Table 3). According to Fismes et al. [34], N can be sequestered and stored in the vegetative parts upon maturity. Therefore, the mechanisms that regulate the passage either between stems and walls or between walls and seeds remain to be elucidated as they evidently influence the seed quality and yield.

The higher level of agrotechnical practices (application of higher NPK rates, as well as S fertilization or biostimulant application) increased the reciprocal internal efficiency index value (RIE) of nitrogen (N uptake for every 1000 kg ha^{-1} of the seed yield) (Table 4). Other results were recorded by Zou et al. [22], who, with an increased fertilization rate, reported a decrease in that value.

4.4. Correlation Between Enzyme Activity on Nitrogen Uptake

Although the activities of DH and NR were not significantly influenced by the nitrogen uptake and the yield, the PER, UR, and CAT activities were significantly correlated with those properties. The enzymatic activity was significantly correlated with nitrogen uptake in aboveground parts and roots, especially at ripening (Table 5). As recorded by Zhang et al. [35], the correlation analyses revealed that the quantity of microorganisms and the activity of the urease, invertase, acid-phosphatase, and protease in soil enzymes were all positively or significantly ($p < 0.05$) correlated with the chlorophyll content, plant height, and economic and biological yields per maize plant. The findings prove that soil interactions are relevant to improving the soil environment by increasing microbial quantity and enzyme activity in soil, as well as increasing the crop yield.

5. Conclusions

The reaction of soil enzymes activity (DH, PER, CAT, NR, and UR) as well as nitrogen uptake in oilseed rape depended on NPK and S fertilization rates and biostimulant application and they differed during generative development stages from flowering to ripening. The highest activity of DH, PER, CAT, and NR at the lower NPK fertilization rates was observed during the ripening stage. NR and UR are enzymes that take part in nitrogen transformation in soils, therefore their activity depended on NPK and S fertilizer. No unequivocal effect of the biostimulant from seaweed (*Ecklonia maxima* Osbeck) on the activity of the soil enzymes was reported. The algae extract demonstrated a positive effect on the CAT activity at the fruit development stage, however, a negative effect on the NR activity at the flowering stage.

A higher rate of NPK and fertilization with S mostly increased the N accumulation in the shoots, roots, and whole plants of oilseed rape at all the development stages. At the fruit development stage, S fertilization increased the uptake of nitrogen only in the shoots and whole oilseed rape plants due to higher NPK rates.

The biostimulant had a favorable effect on N uptake in shoots and whole plants of oilseed rape at all generative development stages. It was the same effect of the biostimulant on N accumulation in roots at the fruit development stage, however, at the flowering and ripening stages, the biostimulant resulted in an increase in N uptake in roots only when fertilization with S was applied.

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