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Impact of Subsurface Application of Compound Mineral Fertilizer on Soil Enzymatic Activity under Reduced Tillage

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Abstract: Soil biochemical properties shaping soil fertility and agro-ecosystem productivity depend on the reduced tillage system and the dose and method of application of fertilizer; therefore, the research hypothesis put forward proposes that under reduced tillage system conditions, the subsurface application of a multi-component mineral fertilizer would increase soil enzymatic activity, thus favourably influencing the biodiversity of the soil environment. The objective of the three-year study was to evaluate the impact of subsurface application of varying mineral fertilizer rates on soil enzymatic activity under reduced tillage system conditions in soybean, winter wheat and maize rotations. The field experiment was set up as a split-plot design in four replicates. The first experimental factor included two methods of mineral fertilization application: fertilizer broadcast over the soil surface (S); fertilizer applied deep (subsurface placed) using a specially designed cultivator (Sub-S). The other factor was the rates of the mineral fertilizer (NPKS): 85 kg·ha^{-1} (F85) and 170 kg·ha^{-1} (F170). The method of application and rate of mineral fertilizer did not have a significant effect on the organic carbon and total nitrogen content in the soil of the plots with all rotational crops. Subsurface application of fertilizer significantly increased available phosphorus content in soil under soybean and winter wheat crops; however, it significantly decreased soil pH_{KCl} values within sites with all crops in the rotation compared to surface application. At the same time, deep application of mineral fertilizer significantly stimulated dehydrogenase activity in the soil under the winter wheat crops and acid phosphatase activity in the soil under all rotation crops. The higher level of mineral fertilization contributed to reduction of soil pH_{KCl} under winter wheat and maize, and promoted an increase in the soil P content. Additionally, significant increases of dehydrogenases and urease activity in the soil under winter wheat and maize crops, alkaline phosphatase activity in the soil under all the studied crops, and acid phosphatase activity in the soil under the soybean crops were found, compared to mineral fertilizer in the amount of 85 kg NPKS·ha^{-1}. The results of the present study have demonstrated a positive effect of subsurface application of compound mineral fertilizer on the soil biochemical parameters in reduced tillage. This may be a recommendation for the subsurface use of multicomponent mineral fertilizers in sustainable agriculture. However, a full objective characterization of the soil environment processes induced by in-depth application of mineral fertilizer in reduced tillage requires long-term monitoring.

Keywords: soil enzymatic activity; reduced tillage system; subsurface fertilization; soil biodiversity

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1. Introduction

In the approach of sustainable development, developed in response to the problems of the modern world, a balance between economic growth, care for the environment and quality of life is extremely important. The 2030 Agenda, a UN resolution promulgated in

2015, contains 17 Sustainable Development Goals (SDGs) addressing, among other things, food security, the promotion of sustainable agriculture, and the protection of biodiversity [1]. Therefore, new ways are constantly being sought to increase crop productivity while making responsible use of environmental capital, including soils. The soil's ecosystem services depend on its natural resources (i.e., soil organic matter and clay contents, soil depth and water retention capacity) and its management. Soil management in various agroecosystems to enhance food production has some trade-offs or disservices (i.e., accelerated water and wind erosion, decline in biodiversity and non-point source pollution), which must be minimized by developing sustainable agriculture. A good solution for sustainable agriculture is the use of minimum/reduced tillage systems and no-tillage systems (the absence of mechanical soil disturbance) [2–6]. Reduced cultivation is a tillage practice that does not invert the soil, combined with 30% of crop residues left on the soil surface, whereas no-tillage is defined as a system in which the soil remains undisturbed from harvest to planting and the seeds are drilled into the stubble of the previous crop [6]. Those tillage systems provide many ecosystem services: carbon sequestration, biodiversity, elemental cycling, and resistance to natural and anthropogenic disturbances, all of which can affect food security [4]. In contrast to the traditional plough tillage prevailing in Central Europe, they are less labour- and energy-intensive and have a positive effect on the physico-chemical and biochemical properties of the soil. The minimum/reduced tillage systems and no-tillage systems prevent nutrient leaching (mainly nitrogen and phosphorus) by reducing water erosion, stimulate soil microbial activity and increase soil organic matter content [6–8]. Higher soil organic carbon stocks or concentrations in the topsoil layers not only promote a more productive soil with higher biological activity but also provide resilience to extreme weather conditions. Less intensive tillage practices and no-tillage systems also have significant potential to mitigate climate change through the storage of C. These practices restore soil health and are recognized as important for offsetting greenhouse gas emissions and creating climate-resilient production systems [9,10].

According to some authors, e.g., Van den Putte et al. [11], Messiga et al. [12] and Bian et al. [13], tillage without the use of a plowcan have a negative impact on soil physical properties and lead to an increase in soil compaction, which hinders plant emergence and root system development, and consequently reduces yields, especially in the first year of applying this system. In addition, reduced tillage results in the accumulation of nutrients, particularly phosphorus and potassium, in the topsoil [8,14]. This results in a reduced capacity of plants to take up these nutrients [15]. Therefore, the way mineral fertilizer is applied has a direct impact on the availability of essential nutrients in the soil. The commonly used surface spreading of mineral fertilizers does not ensure optimal uptake of nutrients by plant roots. Agronomic techniques are now available that allow mineral fertilizers to be placed at different depths relative to the soil surface [16,17]. The techniques for fertilizer placement in soil with references were reviewed in the paper of Nkebiwe et al. [17] and include: indirect placement by pre-treatment of seeds with fertilizers before sowing; in the seed hole or furrow during seeding, on the soil surface as a band with or without incorporation; subsurface as: shallow or deep band, in a shallow or deep trench cut in the soil, as shallow or deep point placement or point injection. Fertilizer placement in soil, which refers to the precise application of specific fertilizer formulations close to seeds or plant roots to ensure high nutrient availability, may be a more effective alternative to broadcast application [16,17]. The subsurface (deep) application of mineral fertilizers prevents from it accumulating in the limited soil volume and can contribute to an increased nutrient efficiency [6,18]. Deep subsurface fertilizer placement may be an additional tool for the mitigation of negative consequences of increasingly frequent extreme weather events, such as high temperatures, droughts or heavy rainfall, which affect food production for an expanding global population [17,19].

Agricultural management related to factors such as, fertilization, tillageand cropping systems, determine the properties of the world's soils, and soil microorganisms and enzymes both mediate and respond to these changes [20]. Enzyme activity in soil is considered to be a potentially sensitive indicator of changes in soil under the influence of tillage, as well as an indicator of the activity of microorganisms involved in nutrient transformation processes [21–23]. Soil enzymes are natural catalysts and mediators of many processes in the soil environment, including: decomposition of organic matter, processes of decomposition and formation of soil humus, molecular nitrogen fixation, the release of mineral nutrients and their supply to plants, and the flow of carbon, nitrogen and other major components of the biochemical cycle [24,25]. According to Dick et al. [26], soil enzyme activity should be a fundamental parameter in assessing the quality and fertility of mineral soils because of its rapid response to environmental factors compared to other soil properties. Tillage intensity influences the biological and enzymatic activity of the soil [8,27,28], and therefore tests based on enzymatic tests should also be used to evaluate the tillage system. Research reveals that some enzymatic tests reliably reflect the impact of agronomic practices on soil ecological status [29–31].

In the present study, a research hypothesis was formulated proposingthat under reduced tillage system conditions, the subsurface application of a multi-component mineral fertilizer, compared to its surface application, would increase the enzymatic activity of the soil, thus having a beneficial effect on the biodiversity of the soil environment. The aim of the three-year field experiment was to evaluate the effect of subsurface application of varying doses of multi-nutrient mineral fertilizer on soil enzymatic activity under reduced tillage system conditions in soybean, winter wheat and maize rotations.

2. Materials and Methods

2.1. Study Area and Field Experiment

The field reaserch was established in the autumn of 2014 in Rogów, Municipality of Grabowiec, Zamość County (location 50°48′22.4″ N; 23°30′00.5″ E), Poland. The experiment was set up on brown soil (CAMBISOLS) developed from post-glacial tills [32]. The particle size distribution of arable layer (0–30 cm) of this soil was as follows: 2.0–0.5 mm fraction −23.6%; 0.5–0.25 mm fraction −70.6%; <0.002 mm fraction –5.8%. The total content of organic carbon (TOC) in 0–30 cm layer was 7.91 g·kg^{-1}. Properties of the starting soil of the experiment site were presented in the research Kraska et al. [6].

The experiment was set up as a split-plot design with four replicates in plots with an area of 175 m^2. The first experimental factor included two methods of mineral fertilization application under reduced tillage conditions (Table 1). In the first treatment, the compound mineral fertilizer was broadcast over the soil surface (S). In the second treatment, the fertilizer was placed deep (S-Sub), by a specially designed cultivator, evenly at a depth of 10–30 cm of the operation of the soil loosening and fertilizer spreading attachment. Another factor considered included was the different rates of the mineral fertilizer: 85 kg NPKS·ha^{-1} (F85) and 170 kg NPKS·ha^{-1} (F170). Between the plots with the different mineral fertilization treatments, there was a 20 m wide buffer zone necessary to properly perform specific agronomic operations [6].

In the experiment, the soybean cultivar ‘Annushka’, was grown in crop rotation with winter wheat varieties ‘Patras’ and maize varieties DKC 3711 (FAO 250). ‘Annushka’ originate from the soybean breeding company ‘Hodowla Soi Agroyoumis Poland’and it is listed in the Common Catalogue of Varieties of Agricultural Plant Species (CCA) in 2009 [33]. It is a very early variety (earliness group 0000), and its growing season lasts about 100–130 days. ‘Annushka’ is recommended for cultivation all over Poland. The winter wheat variety ‘Patras’ by the breeder Saaten Union was entered in the National Register in 2012 and was classified in quality group A. Patras is characterized by good prolificacy, less reaction to intensive agrotechnical level and medium tolerance to soil acidification. The maize variety DKC 3711 (FAO 250) of the breeders Caussade Semences was registered in Poland in 2014 and is recommended for cultivation on poor, medium and good soils [34].

Table 1. Machinery and tools for tillage, pre-sowing fertilization and sowing of soybean, winter wheat and maize.

MFA	Machinery and Tools Used	
S	After harvesting the pre-crop:	TERRADISC 6001 T (6 m) (Pöttinger, Austria) + John Deere 8230 (USA)
	Pre-winter cultivation (does not apply winter wheat):	TERRADISC 6001 T (6 m) + John Deere 8230
	Preparing the field for sowing (does not apply to maize):	tiller SYNKRO 5030 K (5 m) (Pöttinger, Austria) + Case Magnum 280 (Case IH, USA)
	Mineral fertilization:	ZA TS 4200 (Amazone, Germany) + John Deere 8230
	Sowing (does not apply to maize):	tillage and sowing unit TERRASEM C6 (6 m) (Pöttinger, Austria) + John Deere 8230
	Pre-sowing and sowing (does not apply to soybean and winter wheat):	generator of our own design STRIPTILL + seeder Gaspardo Manta (Maschio Gaspardo, Italy) + Case Magnum 280
Sub-S	After harvesting the pre-crop:	TERRADISC 6001 T (6 m) + John Deere 8230
	Pre-winter cultivation (does not apply winter wheat):	TERRADISC 6001 T (6 m)+ John Deere 8230
	Pre-sowing and sowing (does not apply to soybean and winter wheat):	tiller Pöttinger SYNKRO 5030 K (5 m)+ Case Magnum 280
	Sowing (does not apply to maize):	tillage and sowing unit TERRASEM C6 (6 m) + John Deere 8230
	Pre-sowing and sowing (does not apply to soybean and winter wheat):	generator of our own design STRIPTILL + seeder Gaspardo Manta + Case Magnum 280

Before the starting the experiment, winter oilseed rape was grown in the condition of conventional tillage. After harvesting forecrop liming was applied by spreading chalk (with CaO: 39.2% and CaCO3: 70%) at a rate of 5 t·ha^{-1}) [6].

Before sowing the winter wheat, soybean and maize seeds, mineral fertilizer was applied in the form of Polifoska®6 NPK(S) 6-20-30(7), at a rate of 200 kg·ha^{-1} (F85) or 400 kg·ha^{-1} (F170). The percentage content of all nutrients in the applied fertilizer was as follows: N is 6%; P_2O_5 is 20%; K_2O is 30%; SO_3 is 7%. In total, the mineral fertilization was the following (per hectare):

85 kg NPKS·ha^{-1} (F85) = 12 kg N, 17.5 kg P, 50 kg K, 5.5 kg S;

170 kg NPKS·ha^{-1} (F170) = 24 kg N, 35 kg P, 100 kg K, 11 kg S [6].

In winter wheat, as soon as spring vegetation started, post-emergence nitrogen doses were applied in the form of RSM 26 fertilizer at a density of 1.28 kg·dm^{-3} (with N-26% and SO3-7.5%) in the amount of 300 dm^3·ha^{-1} (99.84 kgN·ha^{-1} and 11.5 kgS·ha^{-1}) and in the last week of flowering—RSM 32 at a density of 1.32 kg·dm^{-3} (with N-32%) in the amount of 250 dm^3·ha^{-1} (105.60 kgN·ha^{-1}). In a maize field a pre-sowing application was made of RSM 32 in the amount of 300 dm^3·ha^{-1}, and next in the phase of 3-6 leaves RSM 32 in the amount of 200 dm^3·ha^{-1} (in total 211.2 kg·ha^{-1} N). As soybean is a plant that fixes atmospheric nitrogen, no nitrogen top dressing was applied in the soybean crop. Furthermore, the soybean plants were not irrigated during the growing season.

Machines and tools for soil tillage, pre-sowing fertilization and sowing are shown in Table 1. Cultivation was performed before sowing all crops of the crop rotation, while in the plots with surface application of fertilizer this treatment was performed immediately after sowing, while in the variant with deep application of fertilizer—during the same pass. The surface placement of the fertilizer was carried out using a ZA TS 4200 spreader (Amazone, Germany), whereas the subsurface application was performed using an own design rigid tine cultivator with its sweeps adapted to subsurface fertilizer placement. The sweeps were connected with a fertilizer hopper via a compressed air turbine, used to feed the fertilizer to the sweeps through the distribution mechanism. Furthermore, this device places the fertilizer evenly at a depth of 10–30 cm of the operation of the soil loosening and fertilizer spreading attachment during one travel [6].

Seeds of soybean cultivar 'Annushka' were sown in the amount of 120 kg·ha^{-1}, and winter wheat grain of cultivar 'Patras' in the amount of 170 kg·ha^{-1}. On the other hand, maize grain of cultivar DKC 3711 was sown at a density of 9 units per 1 m^2. For sowing soybean and wheat, the TERRASEM C6 tillage set was used, while for sowing maize the STRIPTILL + Gaspardo Manta seeder was used. The chemical plant protection was as shown in Table S1.

2.2. Weather Conditions

Weather conditions were described on the background of the multiyear period 1974–2010 based on the average monthly air temperature (°C) and total precipitation (mm). Additionally, hydrothermal Sielianinov coefficient (k) was calculated according to the Formula (1):

$$k = (P \times 10) / \sum t \quad (1)$$

where P is the total monthly rainfall in mm, $\sum t$—sum of mean daily temperatures >0 °C [35].

The average air temperature during the field experiment was above the long-term average for most of the months. In the 2014–2015, 2015–2016 and 2016–2017 seasons the air temperature exceeded the long-term average by 2.0 °C, 2.2 °C, and 1.0 °C, respectively (Figure 1). The total rainfall in the 2014–2015 season was only 77% of the long-term average. The highest rainfall deficits occurred in the period from September to November 2014, in February 2015 and also from July to August 2015 (Figure 1). In 2015, the Sielianinov hydrothermal coefficients confirmed that these months were very dry and extremely dry (Table 2). The 2015–2016 season was very humid, since during this season the total rainfall was higher than the long-term average by 31%. The months of September and July were most abundant in rainfall, and the total rainfall in these months was about 60% higher than to the long-term average. The Sielianinov hydrothermal coefficients confirmed that early spring months were extremely humid (March) and humid (April), whereas May was a rather dry month, similar to June. In the third seasons of the experiment (2016–2017), the total rainfall was similar to the long-term average, but the distribution of precipitation in individual months varied. The highest amount of rainfall was recorded in October, in which the sum of precipitation was three times higher than the long-term average. At the same time, the highest rainfall deficit was found in the months of September, June, and August. During these months, the total rainfall was only 19%, 35%, and 56% of the long-term average, respectively. The humidity characteristics of the analysed months of the growing season tended toward humid periods. Only September was extremely dry and June and August were very dry months (Table 2). During the spring and summer period, the highest rainfall was recorded in May and July, which is confirmed by Selyaninov's coefficient, according to which these months were rather humid [6,36].

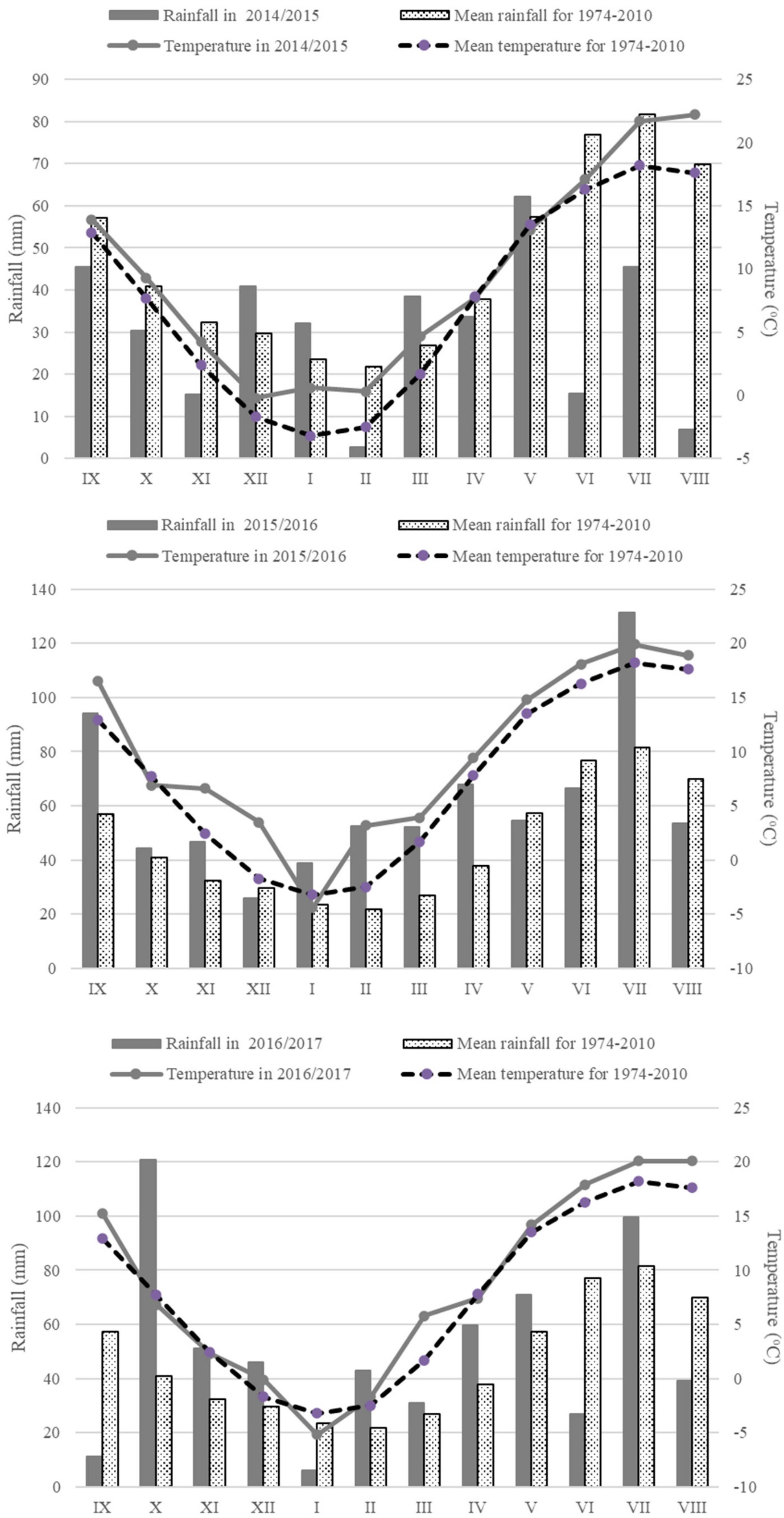

Figure 1. Rainfall and air temperature as compared to the long-term mean figures (1974–2010) according to the Meteorological Station at Bezek.

Table 2. Sielianinov hydrothermal coefficients (k) during the growing seasons in the years of the experiment (2015–2017) according to the Meteorological Station at Bezek [6]. The Roman numerals from III to X represent the following months of the growing season: from March to October.

	III	IV	V	VI	VII	VIII	IX	X
2015	k = 2.73 very humid	k = 1.47 optimal	k= 4.75 extremely humid	k = 0.30 extremely dry	k = 0.70 very dry	k = 0.10 extremely dry	k = 1.90 rather humid	k = 2.14 Humid
2016	k = 4.49 extremely humid	k = 2.40 humid	k = 1.23 rather dry	k = 1.23 rather dry	k = 2.20 humid	k = 0.94 dry	k = 0.24 extremely dry	k = 5.89 extremely humid
2017	k = 1.79 rather humid	k = 2.66 very humid	k = 1.67 rather humid	k = 0.50 very dry	k = 1.66 rather humid	k = 0.65 very dry	k = 2.50 very humid	k = 3.97 extremely humid

The Roman numerals from III to X represent the following months of the growing season: from March to October.

2.3. Sampling and Analyses

In July each year of the study, soil samples were collected for analysis from soybean, winter wheat and maize crops, using a modified soil auger. Soil samples were taken at 10 randomly selected sites from each experimental plot, at a soil depth of 0–30 cm. Then, the collected soil samples were combined into one aggregate sample from each plot. The total number of samples was 36 per year. Each sample was assayed in three replications. Soil samples intended for biochemical analyses were collected and stored in accordance with the principles specified by International Organization for Standardization 18400 [37].

The chemical analyses consisted in determining the following parameters: pH_{KCl} with the potentiometric method for measurement of the pH of soil suspensions in a 1 $mol \cdot dm^{-3}$ KCl (1:2.5) solution [38], the content of total organic carbon (TOC) by the Tiurin method [39], total nitrogen (TN) [40] and available phosphorus (P) by the Egner-Riehm method [41].

The methodology for determination of soil enzymatic activity was based on a detailed study conducted by Schinner et al. [42] and Dick [43]. The activity of four soil enzymes, i.e., dehydrogenases, urease, acid and alkaline phosphatase, was determined. These enzymes are directly involved in the biogeochemical cycle of carbon (dehydrogenase), nitrogen (urease) and phosphorus (acid and alkaline phosphatase), in the environment. They react clearly to the action of stressors, and the magnitude of changes in their activity is related to the intensity of stress factors [36]. The classification of the soil enzymes tested, their abbreviations, units used to present the analytical data, as well as substrates and products used in the assays are presented in Table 3. Activity of dehydrogenases (ADh) was determined by Thalmann's method [42] using a 1% solution of 2,3,5-triphenyl tetrazolium chloride (TTC) as a substrate. Urease activity (AU) was determined following Zantua and Bremner [42] using a 2.5% urea solution as a substrate. Determination of alkaline phosphatase activity (APh_{al}) and acid phosphatase activity (APh_{ac}) was performed according to Tabatabai and Bremner [42] using a 0.8% disodium p-nitrophenyl phosphate solution as a substrate in buffer pH 8.5 and pH 5.4, respectively. The activities of the enzymes were determined using a CECIL CE 2011 (Cecil Instrumentation Ltd, UK) spectrophotometer at the following wavelengths: λ = 485 nm for dehydrogenases, λ = 410 nm for urease and λ = 410 nm for acid and alkaline phosphatase.

Table 3. Determination of the activity of soil enzymes.

Enzymes	EC	Acronym	Substrate Name	Product Name	Unit Name
Dehydrogenses	EC 1.1	ADh	2,3,5-triphenyltetrazolium chloride (TTC)	triphenyl formazane (TPF)	mg TPF kg^{-1} DM 24 h^{-1}
Urease	EC 3.5.1.5	AU	urea	$N\text{-}NH_4^+$	mg $N\text{-}NH_4^+$ kg^{-1} DM h^{-1}
Alkaline Phosphatase	EC 3.1.3.1	APh_{al}	*p*-nitrophenyl phosphate disodium	*p*-nitrophenol (PNP)	mmol PNP kg^{-1} DM h^{-1}
Acid Phosphatase	EC 3.1.3.2	APh_{ac}			

2.4. Statistical Analysis

Analysis of variance (ANOVA) was used to statistically analyse the results. The differences between the means for the main factors (methods of fertilizer application; fertilizer dose; years; plant) were checked with Tukey's multiple comparison test. A significance level of $p < 0.05$ was assumed, which indicated the presence of statistically significant differences. The statistical analysis of the study results was performed using Microsoft Office Excel 2003 and Statistica PL 13.3 (TIBCO Software Inc., Tulsa, OK, USA).

3. Results

The study showed that the chemical properties and enzymatic activity of the soil in the plots studied depended on the type of parameter analysed, the method of application of compound mineral fertilizer and its dose, the plant grown and the years of the study (Tables 4–9).

Table 4. Effect of the interaction of the experimental factors on the chemical properties of soil in soybean plots (average values from 2015 to 2017).

Treatmet	Fertilizer Dose (FD)	pH_{KCl}	TOC	TN	P
S	F85	5.34 [a]	14.42 [a]	1.30 [a]	17.88 [a]
	F170	5.40 [b]	14.36 [a]	1.28 [a]	19.47 [b]
Sub-S	F85	5.27 [c]	14.57 [a]	1.39 [a]	19.26 [c]
	F170	5.37 [d]	15.73 [a]	1.43 [a]	20.83 [d]
	$LSD_{0.05}$	0.005	n.s	n.s	0.399
Mean for MFA	S	5.37 [a]	14.39 [a]	1.29 [a]	18.68 [a]
	Sub-S	5.32 [b]	15.15 [a]	1.41 [a]	20.05 [b]
	$LSD_{0.05}$	0.004	n.s	n.s	0.728
Mean for FD	F85	5.31 [a]	14.50 [a]	1.35 [a]	18.57 [a]
	F170	5.39 [b]	15.05 [a]	1.36 [a]	20.15 [b]
	$LSD_{0.05}$	0.004	n.s	n.s	0.728
Years (Y) *	2015	5.27 [a]	11.83 [a]	1.21 [a]	17.76 [a]
	2016	5.42 [b]	13.65 [b]	1.25 [b]	19.31 [b]
	2017	5.31 [c]	18.71 [c]	1.58 [c]	21.03 [c]
	$LSD_{0.05}$	0.011	2.978	0.049	0.407

* Regardless of the method of application and dose of fertilizer; S—Surface fertilizer application, Sub-S—Subsurface fertilizer application; F85—fertilizer in a dose 85 kg NPKS·ha^{-1}; F170—fertilizer in a dose 170 kg NPKS·ha^{-1}; TOC—total organic carbon in g·kg^{-1}; TN—total nitrogen in g·kg^{-1}; P—available phosphorus in mg·kg^{-1}; $LSD_{0.05}$—the lowest significant difference at $p = 0.05$; n.s—not significant; different letters indicate significant difference at $p \leq 0.05$.

Table 5. Effect of the interaction of the experimental factors on the soil enzymatic activity of soybean plots (average values from 2015 to 2017).

Treatmet	Fertilizer Dose (FD)	ADh	AU	APh_{al}	APh_{ac}
S	F85 F170	11.45 [a] 7.51 [b]	14.11 [a] 13.83 [b]	50.96 [a] 69.89 [b]	70.67 [a] 98.50 [b]
Sub-S	F85 F170	11.60 [c] 9.19 [d]	14.17 [c] 12.54 [d]	55.27 [c] 65.57 [d]	128.74 [c] 138.89 [d]
	$LSD_{0.05}$	2.016	0.239	2.202	4.237
Mean for MFA	S Sub-S	9.48 [a] 10.40 [a]	13.97 [a] 13.36 [b]	60.43 [a] 60.42 [a]	86.84 [a] 133.82 [b]
	$LSD_{0.05}$	n.s	0.261	n.s	6.734
Mean for FD	F85 F170	11.53 [a] 8.35 [b]	14.14 [a] 13.19 [b]	53.12 [a] 67.73 [b]	99.71 [a] 118.70 [b]
	$LSD_{0.05}$	1.482	0.261	4.252	6.734
Years (Y) *	2015 2016 2017	12.15 [a] 6.55 [b] 11.11 [c]	13.85 [a] 15.06 [b] 12.08 [c]	82.58 [a] 32.38 [b] 66.31 [c]	94.80 [a] 98.23 [b] 134.57 [c]
	$LSD_{0.05}$	1.580	0.188	1.726	3.321

* Regardless of the method of application and dose of fertilizer; S—Surface fertilizer application. Sub-S—Subsurface fertilizer application; F85—fertilizer in a dose 85 kg NPKS·ha^{-1}; F170—fertilizer in a dose 170 kg NPKS·ha^{-1}; ADh—dehydrogenases in mg TPF kg^{-1}·24 h^{-1}; AU—urease in mg N-NH_4^+ kg^{-1}·h^{-1}; APh_{al} and APh_{ac}—alkaline phosphatase and acid phosphatase in mmol PNP kg^{-1}·h^{-1}; $LSD_{0.05}$—the lowest significant difference at $p = 0.05$; n.s—not significant; different letters indicate significant difference at $p \leq 0.05$.

Table 6. Effect of the interaction of the experimental factors on the chemical properties of soil in winter wheat plots (average values from 2015 to 2017).

Treatmet	Fertilizer Dose (FD)	pH_{KCl}	TOC	TN	P
S	F85 F170	5.47 [a] 5.32 [b]	12.22 [a] 12.71 [a]	1.02 [a] 1.04 [a]	22.04 [a] 26.12 [b]
Sub-S	F85 F170	5.40 [c] 5.29 [d]	13.08 [a] 12.60 [a]	1.14 [a] 1.09 [a]	30.23 [c] 33.95 [d]
	$LSD_{0.05}$	0.009	n.s	n.s	1.047
Mean for MFA	S Sub-S	5.40 [a] 5.35 [b]	12.47 [a] 12.84 [a]	1.03 [a] 1.12 [a]	24.08 [a] 32.09 [b]
	$LSD_{0.05}$	0.018	n.s	n.s	2.536
Mean for FD	F85 F170	5.44 [a] 5.31 [b]	12.65 [a] 12.66 [a]	1.08 [a] 1.07 [a]	26.14 [a] 30.04 [b]
	$LSD_{0.05}$	0.018	n.s	n.s	2.536
Years (Y) *	2015 2016 2017	5.28 [a] 5.35 [b] 5.47 [c]	11.22 [a] 12.48 [b] 14.36 [c]	0.98 [a] 1.05 [b] 1.19 [c]	21.54 [a] 27.82 [b] 34.91 [c]
	$LSD_{0.05}$	0.006	1.66	0.009	0.723

* Regardless of the method of application and dose of fertilizer; S—Surface fertilizer application, Sub-S—Subsurface fertilizer application; F85—fertilizer in a dose 85 kg NPKS·ha^{-1}; F170—fertilizer in a dose 170 kg NPKS·ha^{-1}; TOC—total organic carbon in g·kg^{-1}; TN—total nitrogen in g·kg^{-1}; P—available phosphorus in mg·kg^{-1}; $LSD_{0.05}$—the lowest significant difference at $p = 0.05$; n.s—not significant; different letters indicate significant difference at $p \leq 0.05$.

Table 7. Effect of the interaction of the experimental factors on the soil enzymatic activity of winter wheat plots (average values from 2015 to 2017).

Treatmet	Fertilizer Dose (FD)	ADh	AU	APh_{al}	APh_{ac}
S	F85	3.08 ^{a}	15.62 ^{a}	57.14 ^{a}	105.74 ^{a}
	F170	10.91 ^{b}	18.47 ^{b}	101.83 ^{b}	86.48 ^{b}
Sub-S	F85	6.98 ^{c}	16.19 ^{c}	67.60 ^{c}	103.23 ^{c}
	F170	10.16 ^{d}	16.49 ^{d}	69.59 ^{d}	103.04 ^{d}
	$LSD_{0.05}$	1.027	0.313	1.730	5.249
Mean for MFA	S	7.00 ^{a}	17.05 ^{a}	79.49 ^{a}	96.11 ^{a}
	Sub-S	8.95 ^{b}	16.34 ^{b}	68.60 ^{b}	103.14 ^{b}
	$LSD_{0.05}$	1.004	1.529	4.378	3.244
Mean for FD	F85	5.03 ^{a}	15.91 ^{a}	62.37 ^{a}	104.49 ^{a}
	F170	10.54 ^{b}	17.48 ^{b}	85.71 ^{b}	94.76 ^{b}
	$LSD_{0.05}$	1.004	1.529	4.378	3.244
Years (Y) *	2015	6.97 ^{a}	13.69 ^{a}	65.71 ^{a}	59.09 ^{a}
	2016	5.66 ^{b}	15.47 ^{b}	45.69 ^{b}	101.29 ^{b}
	2017	10.72 ^{c}	20.92 ^{c}	110.73 ^{c}	138.49 ^{c}
	$LSD_{0.05}$	0.805	0.246	1.356	4.114

* Regardless of the method of application and dose of fertilizer; S—Surface fertilizer application. Sub-S—Subsurface fertilizer application; F85—fertilizer in a dose 85 kg NPKS·ha^{-1}; F170—fertilizer in a dose 170 kg NPKS·ha^{-1}; ADh—dehydrogenases in mg TPF kg^{-1}·24 h^{-1}; AU—urease in mg N-NH_4^+ kg^{-1}·h^{-1}; APh_{al} and APh_{ac}—alkaline phosphatase and acid phosphatase in mmol PNP kg^{-1}·h^{-1}; $LSD_{0.05}$—the lowest significant difference at p = 0.05; different letters indicate significant difference at $p \leq 0.05$.

Table 8. Effect of the interaction of the experimental factors on the chemical properties of soil in maize plots (average values from 2015 to 2017).

Treatmet	Fertilizer Dose (FD)	pH_{KCl}	TOC	TN	P
S	F85	5.51 ^{a}	11.80 ^{a}	1.09 ^{a}	17.91 ^{a}
	F170	5.45 ^{b}	11.71 ^{a}	1.10 ^{a}	18.83 ^{b}
Sub-S	F85	5.40 ^{c}	12.02 ^{a}	1.18 ^{a}	17.32 ^{c}
	F170	5.32 ^{d}	11.91 ^{a}	1.18 ^{a}	18.08 ^{c}
	$LSD_{0.05}$	0.006	n.s	n.s	0.146
Mean for MFA	S	5.48 ^{a}	11.76 ^{a}	1.10 ^{a}	18.37 ^{a}
	Sub-S	5.43 ^{b}	11.97 ^{a}	1.18 ^{a}	18.08 ^{b}
	$LSD_{0.05}$	0.008	n.s	n.s	0.063
Mean for FD	F85	5.46 ^{a}	11.91 ^{a}	1.14 ^{a}	17.62 ^{a}
	F170	5.39 ^{b}	11.81 ^{a}	1.14 ^{a}	18.46 ^{b}
	$LSD_{0.05}$	0.008	ns	ns	0.063
Years (Y) *	2015	5.27 ^{a}	10.14 ^{a}	0.97 ^{a}	17.60 ^{a}
	2016	5.38 ^{b}	12.12 ^{b}	1.18 ^{b}	17.48 ^{b}
	2017	5.62 ^{c}	13.47 ^{c}	1.26 ^{c}	19.03 ^{c}
	$LSD_{0.05}$	0.004	1.086	0.072	0.215

* Regardless of the method of application and dose of fertilizer; S—Surface fertilizer application, Sub-S—Subsurface fertilizer application; F85—fertilizer in a dose 85 kg NPKS·ha^{-1}; F170—fertilizer in a dose 170 kg NPKS·ha^{-1}; TOC—total organic carbon in g·kg^{-1}; TN—total nitrogen in g·kg^{-1}; P—available phosphorus in mg·kg^{-1}; $LSD_{0.05}$—the lowest significant difference at p = 0.05; n.s—not significant; different letters indicate significant difference at $p \leq 0.05$.

Table 9. Effect of the interaction of the experimental factors on the soil enzymatic activity of maize plots (average values from 2015 to 2017).

Treatmet	Fertilizer Dose (FD)	ADh	AU	APh_{al}	APh_{ac}
S	F85	9.36 [a]	15.82 [a]	70.89 [a]	102.30 [a]
	F170	13.42 [b]	19.64 [b]	100.01 [b]	77.79 [b]
Sub-S	F85	10.01 [c]	17.05 [c]	67.68 [c]	145.96 [c]
	F170	10.59 [d]	16.56 [d]	70.17 [d]	134.11 [d]
	$LSD_{0.05}$	0.621	0.342	1.899	4.769
Mean for MFA	S	11.39 [a]	17.73 [a]	85.45 [a]	90.05 [a]
	Sub-S	10.30 [b]	16.81 [b]	68.93 [b]	140.04 [b]
	$LSD_{0.05}$	0.073	0.482	0.972	3.670
Mean for FD	F85	9.69 [a]	16.44 [a]	69.29 [a]	124.13 [a]
	F170	12.01 [b]	18.10 [b]	85.09 [b]	105.95 [b]
	$LSD_{0.05}$	0.073	0.482	0.972	3.670
Years (Y) *	2015	8.68 [a]	14.70 [a]	57.58 [a]	106.98 [a]
	2016	4.18 [b]	17.48 [b]	36.45 [b]	101.95 [b]
	2017	19.68 [c]	19.63 [c]	137.57 [c]	136.19 [c]
	$LSD_{0.05}$	0.487	0.268	1.488	3.738

* Regardless of the method of application and dose of fertilizer; S—Surface fertilizer application. Sub-S—Subsurface fertilizer application; F85—fertilizer in a dose 85 kg NPKS·ha^{-1}; F170—fertilizer in a dose 170 kg NPKS·ha^{-1}; ADh—dehydrogenases in mg TPF kg^{-1}·24 h^{-1}; AU—urease in mg N-NH_4^+ kg^{-1}·h^{-1}; APh_{al} and APh_{ac}—alkaline phosphatase and acid phosphatase in mmol PNP kg^{-1}·h^{-1}; $LSD_{0.05}$—the lowest significant difference at p = 0.05; different letters indicate significant difference at $p \leq 0.05$.

3.1. Chemical Properties and Soil Enzymatic Activity of Soybean Growing Plots

The soil within the soybean plots was characterized by an acid reaction. A significant increase in pH_{KCl} was observed in the soil of plots with surface application of mineral fertilizer (S) in comparison with deep application (Sub-S). A higher rate of mineral fertilization (F170) also significantly increased soil pH_{KCl}. Regardless of the tillage treatments applied, the lowest soil pH_{KCl} values were found in 2015 and the highest in 2016 (Table 4). During the three-year study period, the soil of the plot with the deep application of mineral fertilizer was characterized by a significantly higher content of TOC, TN and P in comparison with the variant with surface application of the fertilizer preparation. An application of 170 kg NPKS·ha^{-1} (F170) of fertilizer resulted in a significant increase in soil TOC and P content. The content of TOC, TN and P differed between the years. The highest value of TOC and TN were found in the last year of the experiment, whereas the values for 2015 and 2016 were not statistically different. On the other hand, the P content of the soil increased steadily in successive years of observation.

Within the soybean plots, there was no significant influence of the method of multi-nutrient fertilizer application on the activity of dehydrogenases (ADh) and alkaline phosphatase (APh_{al}) in the soil. Surface application of compound mineral fertilizer (S) significantly increased urease activity (AU) in the soil. An opposite trend was observed for acid phosphatase activity (APh_{ac}). Irrespective of the application method, fertilizer in the amount of 170 kg NPKS·ha^{-1} (F170) caused a significant inhibition of dehydrogenase) and urease activity in the soil. An opposite relationship was noted for APh_{al} and APh_{ac} activities (Table 5). Regardless of the method (MFA) and mineral fertilization rate (FD), the first year of the study (2015) recorded the highest activity of ADh and APh_{al} in the soil, and significantly the lowest in the second year of the experiment (2016). In turn, the activity of APh_{ac} in soil increased systematically in subsequent years of observation. The highest AU activity was determined in the second year of the study, and the lowest in the last year of the study (Table 5).

3.2. Chemical Properties and Enzymatic Activity of the Soil on Winter Wheat Plots

As in the case of soybean crops, the soil within winter wheat plots was characterized by an acid reaction (Table 6). The subsurface application of fertilizer (Sub-S) resulted in a statistically significant reduction in the value of pH_{KCl}, as with the use of F170. The pH_{KCl} value of the soil increased systematically in subsequent years of observation, irrespective of the tillage treatments carried out. The method of application of the compound fertilizer (MFA) and its dose (FD) did not significantly affect the content of TOC and TN in the soil. The subsurface application of the fertilizer preparation caused a significant increase in the P content of the soil. A higher dose of fertilizer (F170) resulted in a significantly higher accumulation of available P in the soil environment. The content of TOC, TN and P in soil increased, generally statistically significantly, in subsequent years of observation (Table 6).

Within winter wheat plots, under the conditions of deep application of mineral fertilizer (Sub-S), the activity of ADh and APh_{ac} in soil was significantly higher than in the variant with surface application of fertilizer (S), (Table 7). However, in the case of AU and APh_{al}, their higher activity was found under surface application of mineral fertilizer compared to its deep application. Introduction of 170 kg of NPKS·ha^{-1} into the soil stimulated, in most cases significantly, the activity of dehydrogenases, urease and alkaline phosphatase (Table 7). In the case of APh_{ac}, higher activity was observed in the soil of the plots fertilized with the amount of 85 kg of NPKS·ha^{-1}. During the period of the present study (2015–2017), AU and APh_{ac} activities significantly increased in successive years of the experiment regardless of the application method and mineral fertilizer dose. In contrast, the activities of ADh and APh_{al} in the second year (2016) of the study were significantly lower than in the other years. The highest, statistically significant, activity of all monitored soil enzymes was found in the last year of the experiment (Table 7).

3.3. Chemical Properties and Enzymatic Activity of the Soil on Maize Plots

A significant increase in soil pH_{KCl} values was observed in the soil of plots with surface application of mineral fertilizer (S) in comparison with the deep method (Sub-S). Fertilization application at a rate of F170 also significantly increased soil pH_{KCl} values. Regardless of the tillage methods applied, a significant increase in the pH_{KCl} value was observed in subsequent years of the experiment. In the last year of the study (2017), the soil reaction changed from acidic to slightly acidic. The method of application of the fertilizer preparation as well as its dose did not significantly affect the changes in soil TOC and TN content. The surface application of the fertilizer (S) resulted in a significantly higher accumulation of available phosphorus in the soil compared to the subsurface application. The F170 dose of fertilizers significantly influenced higher P content in the soil in comparison with the F85 dose. In subsequent years of the study, a significant increase in the content of TOC and TN in soil was observed. The lowest content of P was determined in the second year of the study and the highest in the last year of the experiment (Table 8).

Within the maize plots, the surface application of compound fertilizer significantly stimulated the activity of most of the enzymes studied, with the exception of acid phosphatase activity. Introduction of a higher dose of mineral fertilizer (F170 objects) into the soil, regardless of the method of its application, significantly increased the activity of soil ADh, AU and APh_{al} (Table 9). An inverse relationship was observed for the activity of APh_{ac}, as a higher activity of this enzyme was determined in the soil of plots fertilized with a lower dose of mineral fertilizer (F85). Between 2015 and 2017, AU activity increased significantly in the following study periods. In contrast, the activities of ADh, APh_{al} and APh_{ac} in the second year of the study were significantly lower than in the other years. The highest, statistically significant, activity of all monitored soil enzymes was found in the last year of the experiment (Table 9).

3.4. Effect of Method of Fertilizer Application and the Fertilizer Dose on Soil Biochemical Properties of Plant Plots

Analyzing the results obtained over the three-year study period, it was found that surface application of fertilizer caused a significant increase in soil pH_{KCl} values under all crops in rotation compared to Sub-S application (Table 4, Table 6, Table 8). The application of fertilizer at F170 rate caused a decrease in soil pH_{KCl} under winter wheat and maize crops. An opposite relation was observed in the soil of the plot with soybean. Irrespective of the method of application (MFA) and the dose (FD) of fertilizer, the highest pH_{KCl} values were found in the soil under maize crop (Table 4, Table 6, Table 8). It was shown that the method of application and rate of mineral fertilizer did not have a significant effect on the TOC and TN content in the top soil. Soybean plots were characterized by the highest content of these components in comparison with other analysed crops, irrespective of the treatments applied (Table 4, Table 6, Table 8). Under conditions of deep application of mineral fertilizer (Sub-S), a significantly higher content of available form of phosphorus (P) was observed in soil under soybean and winter wheat crops. An opposite relationship was shown in the case of maize crop. A higher dose of compound fertilizer (F170) significantly increased soil P content in all plant plots in the crop rotation compared with F85. Independently of MFA and FD, the highest P content in soil was found in plots with winter wheat crop (Table 4, Table 6, Table 8).

Under conditions of deep application of mineral fertilizer (Sub-S) dehydrogenase activity in the soil of plots with winter wheat crops and acid phosphatase activity in the soil under all plants of crop rotation were significantly higher than in the variant with surface fertilization (S), (Table 5, Table 7, Table 9). An opposite trend was observed for ADh activity in soil under maize, APh_{al} in the variant with winter wheat and maize crops, and AU in the soil of plots with all the crops studied. There was no significant effect of the method of application of compound fertilizer on the activity of dehydrogenases and alkaline phosphatase in soil under soybean crops. The application of mineral fertilizer in the amount of 170 kg NPKS·ha^{-1} significantly increased the activity of ADh and AU in the soil under winter wheat and maize crops, APh_{al} in the soil of plots with all the plants studied and APh_{ac} in the soil of the plot with soybean crop in comparison with the variant F85, (Table 5, Table 7, Table 9). Irrespective of the tillage treatments (MFA and FD), in general, the highest activity of the analysed enzymes was found in the soil under the maize crop (Table 5, Table 7, Table 9). The enzyme tests used proved to be good discriminators of the tested soil plots.

4. Discussion

The tillage system, mineral fertilization and appropriate choice of plants in crop rotation condition the formation of favourable biochemical properties of soil, which consequently affect its quality and fertility as well as agro-ecosystem productivity [44,45]. Such a system of relations was confirmed in the presented studies. The results showed that the physicochemical and biochemical parameters of the soils of the selected study plots varied according to MFA, FD and years of study. Surface application of fertilizers in reduced tillage system, where fertilizers remain on the field surface unmixed with soil, causes stratification of nutrients in the soil, which may affect proper plant nutrition and biomass production [46]. In addition, fertilizers spread in this way may be subject to surface run-off and pollute water bodies [47]. Deep fertilizer application in soil, which refers to precise application of fertilizer close to seeds or plant roots to ensure high nutrient availability, may be a more effective alternative to broadcast placement [17,48]. In the present study it was shown that subsurface application of NPKS fertilization increased the content of organic carbon and total nitrogen in the soil of all objects, but the increase was not statistically significant. There was also no effect of fertilization rate on the soil parameters in question. Similar results were obtained by Kraska et al. [6] analysing soil properties from soybean crops. The subsurface application of fertilizer significantly increased available phosphorus (P) content in the soil under soybean and winter wheat crops. Such a relationship was not observed in the soil of the maize plot. The higher level of mineral fertilization promoted an increase in the soil P content. According to Rychel et al. [49], deeply placed fertilizer is not exposed to greater downward mobility, probably due to smaller changes in soil moisture after rainfall at this depth. According to Hasan et al. [50], urea deep placement is a proven technology that reduces N losses by up to 50% when compared with the conventional broadcast application of urea. This fertilization method is primarily recommended in the no-tillage system [14,51,52]. The benefits of placing nitrogen deeper are likely to depend on the climate and soil type, but could be the next step in precision farming and environmentally sustainable farming [49].

In reduced tillage systems, compared to conventional tillage, there is a tendency towards higher acidity of the soil surface layer [6,53], which is due to the difference in the mineralization process of organic matter, concentration of nutrients, mainly nitrogen, and root secretions [54]. The use of balanced fertilizer application strategies, and rational crop rotations will relieve soil acidification under no-tillage [55]. Our study shows that surface application of fertilizer (S) significantly influenced the increase in soil pH_{KCl} value within the plots with all crops grown in the crop rotation as compared to Sub-S application. Such a relationship was also observed by Kraska et al. [6]. Biomass production is generally associated with the uptake of more cationic than anionic nutrients by the plant root system, resulting in an increase in H+ concentration in the rhizosphere. Deep application of nutrients can promote soil acidification. A dose of a fertilizer also influences the pH of the soil. The mineral fertilizer applied at the double rate (170 kg NPKS) contributed to lowering of the pH_{KCl} of the soil under winter wheat and maize. pH is a critical parameter that influences the bioavailability of many nutrients and toxic elements and the physiology of the roots and rhizosphere microorganisms [56].

There is a lack of information in the global literature on the effect of subsurface application of inorganic fertilizers on soil biochemical parameters in zero-tillage, especially enzymatic activity. In the present study, the activities of dehydrogenases, urease and acid and alkaline phosphatase were investigated. They play a critical role in organic matter decomposition and nutrient cycling of carbon, nitrogen and phosphorus [57]. In addition, studying the activity of these enzymes can provide information on the metabolic or functional responses of soil to changes in farming practices [8,21,23,29,30,58–60]. The direction and intensity of the observed activity changes depended on the individual properties of the enzyme studied, their resistance to environmental stress factors, as well as the content of specific substrates for enzymatic reactions in the soil [61,62]. Reducing the intensity of soil tillage reduces the rate of mineralization of organic matter, which in turn has a positive effect on improving soil structure. Numerous studies [8,28,59,60] have shown that conservation tillage significantly stimulated the activity of soil microbes and enzymes.

The study showed that subsurface application of mineral fertilizer significantly stimulated the activity of dehydrogenases in the soil under winter wheat crop and acid phosphatase in the soil under all rotation plants. There was no significant effect of the multi-nutrient fertilizer application method on ADh and APh_{al} in soil under soybean. The application of mineral fertilizer in the amount of 170 kg NPKS·ha^{-1} (F170) significantly increased ADh and AU activities in soil under winter wheat and maize crops, APh_{al} in soil of the plots with all the studied crops and APh_{ac} in soil with soybean crop. Csitari and Hoffmann [63] showed higher dehydrogenase activity and microbial biomass under conditions of increased mineral fertilization. The increase in dehydrogenase activity in plots with higher fertilization could be due to a higher concentration of root secretions produced by the root system [61]. According to many authors [22,30,64], root secretions are an excellent source of nutrients for microorganisms, especially those living in the rhizosphere. However, it should be remembered that the effect of higher plants on soil enzymes depends on the chemical composition of the plant, which even in the case of root secretions alone may be different in different genera, species and even cultivars [23,65–67]. Dehydrogenases are sensitive indicators of changes in soil microbial activity analysis, as they are active only in living microbial cells [67,68]. Being intracellular enzymes, dehydrogenases are more sensitive to natural and anthropogenic stress factors than enzymes associated with soil colloids. The measurement of dehydrogenase activity using TTC, as a reducing substrate, reflects the activity of the entire microbial population of the soil environment [69]. Dehydrogenases are commonly found in organic matter-rich soils and they are regarded as good indicators of the respiratory metabolism of microbes [21,26,70]. Bielińska and Mocek-Płóciniak [22] and Harasim et al. [8] point to the usefulness of dehydrogenases for assessing changes in the soil environment under the influence of the tillage system. Gajda et al. [59,71] found an increase in the activity of enzymes, including dehydrogenases in soil under no-tillage under winter wheat, while Niewiadomska et al. [72] found this increase under spring wheat. On the other hand, Woźniak [73] noticed higher activities of dehydrogenases and phosphatase in the soil tilled in the investigated soils, the activity of acid phosphatase was significantly higher than that of alkaline phosphatase. Acid phosphatase, an enzyme with low substrate specificity, has the ability to hydrolyse many phosphate bonds of different molecular structures [74]. The supply of fertilizers to the soil can increase the microbial activity of the soil, while decreasing the activity of some enzymes [61]. By analysing the relationship between acid and alkaline phosphatase activity and the content of different forms of phosphorus (total, organic and available), Nahas [75] showed positive correlations only for total phosphorus and organic matter. Phosphatases have the ability to hydrolyse organic phosphorus compounds in excess of the plants' phosphorus requirements and the limiting factor for their activity in soil is the availability of hydrolysable organic phosphorus. An excess of inorganic phosphorus in the soil may inhibit the synthesis of phosphatases [75].

Changes in soil enzymatic activity in the vegetation periods turned out to depend mainly on individual characteristics of the enzymes under study and the different reaction of enzymes to atmospheric conditions in the study years [61,76]. Enzymatic activity in soils depends on a number of factors, such as the content of organic matter, soil pH, the content of biogenic elements, and the quantity and diversity of microorganisms [22,77]. There was a significant correlation between the activity of dehydrogenases, acid phosphatase and alkaline phosphatase with the content of TOC, TN and bioavailable form of P. Moreover, the activity of APh_{ac} was positively correlated with pH_{KCl} (Table 10). It proves that nutrients significantly stimulate the activity of soil enzymes. Numerous authors [59,61] have found that soil enzymatic activity depends on the TOC and TN content of the soil. The lack of correlation between urease activity and the soil parameters studied confirms the reports that urease is resistant to external factors and an increase in its activity is observed even in extreme conditions. The only factor limiting urease activity is the availability of the urea substrate, because as an extracellular enzyme, it is synthesized only in its presence [78]. Nannipieri et al. [74] report that urease participate in ammonification, during which ammonia is released from urea, amino acids, and purine bases.

Table 10. Correlation coefficients between the activity of the examined enzymes and pH_{KCl} and the content of organic carbon (TOC), total nitrogen (TN), and available phosphorus (P) form (n = 36).

Enzymes	pH_{KCl}	TOC	TN	P
Dehydrogenases	n.s.	0.649 *	0.691 *	0.571 *
Urease	n.s.	n.s.	n.s.	n.s.
Alkaline phosphatases	n.s.	0.734 **	0.696 **	0.685 *
Acid phosphatases	0.751 *	0.718 **	0.729 **	0.597 **

** significant at α = 0.0001; * significant at α = 0.001; n.s = not significant at p > 0.05.

The soil environment is a highly diverse system in terms of microorganisms. One essential microbial function in soils is the processing and recovery of key nutrients from detrital inputs and accumulated soil organic matter. This requires the activity of extracellular enzymes to process complex organic compounds into assimilable subunits (sugars, amino acids, NH_4^+, PO_4^{+3}). Ratios of various nutrient-processing enzyme activities can provide insight into how the soil community is responding physiologically to changes in the soil environment [79]. Soil microbial communities shift with tillage treatment and soil depth. In Mathew et al. [80] study, soil under the long-term no-till treatment had higher soil carbon and nitrogen contents and phosphatase activities at the 0–5 cm depth than that under the conventional tillage treatment. Differences between tillage treatments at the 5–15 cm depth were negligible with the exception of alkaline phosphatase activities. Tillage practice and soil depth were two important factors affecting soil microbial communities [80]. Therefore, subsurface application of mineral fertilizer can have a positive effect on increasing soil microbial diversity. Tabatabai et al. (1992) found that mineral fertilization can affect soil microorganisms and enzymes through higher crop yield, which in turn affects the amount of crop residue. Vetanovetz and Peterson [81] and Sawicka et al. [61] are of the opinion that mineral nitrogen fertilization increases the population of soil microorganisms and, thus, the biological activity of the soil, but only within certain limits for the optimal fertilization rate. However, further research is needed to determine the effect of changes induced by deep application of mineral fertilizer in reduced tillage on soil enzyme activity, which reflects soil microbial composition and dynamics.

Key strategies to improve nutrient efficiency should include optimal fertilization dose, time and method of application and the use of nutrients appropriate to crop species, varieties and genotypes [61]. As summarized by several reviews [17,82,83], the effectiveness of deep placement of fertilizers may be determined by factors including climatic condition, soil texture, tillage, fertilizing history, nutrient mobility, and crop species. However, further investigations are needed before deeper placement of fertilizer can be recommended as a sustainable farming practice as indicated by our study.

5. Conclusions

The method of application and rate of mineral fertilizer did not have a significant effect on the TOC and TN content in the soil of the plots with all rotational crops. However, subsurface application of fertilizer significantly increased available phosphorus (P) content in soil under soybean and winter wheat crops. Such a relationship was not observed in the soil under maize crop. The higher level of mineral fertilization promoted an increase in the soil P content. Surface application of fertilizer (S) significantly increased soil pH_{KCl} values within sites with all crops in the rotation compared to subsurface application. Deep application of nutrients may promote soil acidification. The mineral fertilizer applied at the double rate (170 kg NPKS) contributed to a reduction of soil pH_{KCl} under winter wheat and maize. Deep application of mineral fertilizer significantly stimulated dehydrogenase activity in the soil under the winter wheat crops and acid phosphatase (APh_{ac}) in the soil under all rotation crops. There was no significant effect of the method of application of a compound fertilizer on ADh and APh_{al} in the soil under soybean crop. Application of mineral fertilizer in the amount of 170 kg NPKS·ha^{-1} significantly increased ADh and AU activity in the soil under winter wheat and maize crops, APh_{al} in the soil under all the studied crops and APh_{ac} in the soil under the soybean crops. As mentioned, there is a lack of information in the global scientific literature on the effect of subsurface application of fertilizer on the biochemical properties of soils. The assessment of soil quality is not easy due to the complexity of the soil environment and the variability of its conditions. The results of the present study have demonstrated a positive effect of subsurface application of compound mineral fertilizer on the soil biochemical parameters in reduced tillage. This may be a recommendation for the subsurface use of multicomponent mineral fertilizers in sustainable agriculture. However, a full objective characterization of the soil environment processes induced by in-depth application of mineral fertilizer in reduced tillage requires long-term monitoring.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11112213/s1. Table S1. Chemical plant protection during the growing seasons.

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