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The Effect of Enzyme Activity on Carbon Sequestration and the Cycle of Available Macro- (P, K, Mg) and Microelements (Zn, Cu) in Phaeozems

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Abstract: The study objective was to determine the relationship of selected enzyme activities with carbon sequestration and N, P, K, Mg, Zn and Cu contents in Phaeozem soils. Soil samples were taken from a 10 ha area. A selection of their physical and chemical properties and the contents of the available forms of selected macro- and microelements were determined. The activities of dehydrogenases (*DEH*), catalase (*CAT*), peroxidases (*PER*), alkaline (*AlP*) and acid (*AcP*) phosphatase, β-glucosidase (*BG*) and proteases (*PR*) were also determined. The relationship between enzymatic soil fertility indices (*AlP/AcP*, *BIF*, *GMea*, *TEI*, *BA12* and *BA13*) and selected soil parameters was also determined. The research used principal component analysis (PCA) to distinguish significantly correlated parameters of a Phaeozem used for agricultural purposes. The study area showed low TOC and K contents and average P and Mg contents. Significant positive correlations were found between the TOC content and activity of the tested enzymes, evidencing that soil enzymes are an important parameter in carbon sequestration and soil nutrient dynamics.

Keywords: dehydrogenases; catalase; peroxidases; alkaline and acid phosphatase; β-glucosidase; proteases; Phaeozem; soil texture; total organic carbon; total nitrogen; available macro- and microelements

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Citation: Lemanowicz, J.; Bartkowiak, A.; Zielińska, A.; Jaskulska, I.; Rydlewska, M.; Klunek, K.; Polkowska, M. The Effect of Enzyme Activity on Carbon Sequestration and the Cycle of Available Macro- (P, K, Mg) and Microelements (Zn, Cu) in Phaeozems. *Agriculture* **2023**, *13*, 172. https://doi.org/10.3390/agriculture13010172

Academic Editors: Riccardo Scotti and Ryusuke Hatano

Received: 11 November 2022
Revised: 16 December 2022
Accepted: 6 January 2023
Published: 10 January 2023

1. Introduction

Soil fertility consists of a set of physical, chemical and biological soil properties providing plants with suitable conditions for growth [1]. Phaeozems (black earths) are agricultural soils that cover approximately 20.2% of the world's total arable [2]. These soils are among the most fertile in Poland and constitute a small proportion of arable land, and only a little over 3% of the country's total area. They were formed mainly by excessive moisture caused by the long-term impact of a high water table that was often rich in calcium cations [3]. The fertility of black earths is influenced by their high humic content, abundance of nutrients and good buffer properties. The use value of black earths varies depending on the thickness of the humic horizon and its physical and chemical properties. The soils of Poland are not particularly rich in humus, although black earths are usually rich in colloidal material and calcium carbonate, which stabilizes processes that transform humic substances. The relationship between the quantity and quality of soil organic matter and the sorption complex's richness in alkaline cations are clear because the organic matter in soils constitutes an important proportion of the sorption surface and, at the same time, an important source of nutrients. Cation exchange capacity (CEC) is also an indicator of soil fertility, as it indicates the soil's capacity to supply important plant nutrients [4–6]. Preserving soil humus is important not only to maintain the soil's productive functions but also with regard to the soil's role in sequestering (binding) carbon from the atmosphere.

Unsustainable fertilizer use, intensive cultivation and the reduced use of organic fertilizers ultimately lead to soil degradation. The rapid increase in temperatures and atmospheric CO_2 concentrations is affecting plant productivity globally. The increase in CO_2 is associated with an increase in the allocation of carbon to soils [7]. This can upset the balance of the soil and provoke changes in nutrient cycling and enzyme activity. One major measure to mitigate climate change is carbon sequestration in the soil environment. Slight changes in soil carbon resources affect the biogeochemistry of this element [8]. Introducing nutrients and organic materials into the soil increases the abundance of microorganisms, which produce extracellular enzymes in response [9]. Enzymes are biological catalysts that affect the rates of chemical reactions. They are involved in element cycles, and they influence the efficiency with which natural, organic and mineral fertilizers are taken up and used by plants. They are also considered to be indicators of soil quality and changes and play a key role in the decomposition of organic matter, bio-availability of energy and nutrients, detoxification of xenobiotics and nitrification and denitrification processes [10–14]. The most important enzymes in soil agroecosystems are those involved in breaking down cellulose and other plant cell components and in the transformations of C, N, P and S [15]. In the case of carbon sequestration and the circulation of nutrients in the soil, some oxidoreductase and hydrolase enzymes have been considered indicators or predictors of organic carbon decomposition and nutrient mineralization [12]. The simplicity and speed with which they can be measured and how they are associated with most soil properties increase the attractiveness of soil enzyme activities as indicators of soil condition [16,17]. However, the global literature has very little data on the use of many different multiparametric enzyme indices to assess soil quality. Having soil quality indicators within appropriate ranges should indicate that the soil is optimized for maximum possible yields and reduced soil degradation [18].

The successive reduction of soil organic matter through the limited use of natural and organic fertilizers, as well as the emission and leaching of gases, has resulted in activities that focus on increasing the binding of carbon into the soil, i.e., its sequestration. In the assessment of soil properties, the results of enzyme activity can be used. Multiparametric enzymatic indices must be determined first on small agricultural fields to be able to manage the soil on a larger scale. We hypothesized that (i) in the humus, horizon changes of the studied enzymes activity would depend on the soil physicochemical properties (mainly TOC content) and (ii) individual enzyme activities will be more positively correlated with soil physicochemical properties than enzyme activity indices.

Therefore, the objectives of this study were (i) to examination of the activity of some enzymes in the soil (dehydrogenases, catalase, peroxidases, alkaline and acid phosphatase, b-glucosidase, proteases) and selected physicochemical properties in Phaeozems; (ii) to undertake the assessment of selected enzyme activity on carbon sequestration and the cycle of soil elements and (iii) to examination the relationships between soil physicochemical properties and enzyme activities, using soil enzyme activity indices (*AlP/AcP*, *GMea*, *BIF*, *TEI*, *BA12*, *BA13*, *TEI*).

2. Materials and Methods

2.1. Location of Soil Sampling

The research involved soil samples from an area of intensive agriculture on the Inowrocław Plain. The Inowrocław Plain is a physico-geographical mesoregion in north-central Poland that constitutes the north-eastern part of the Wielkopolskie Lakeland macroregion [19]. A characteristic feature of the region is its relatively low annual precipitation of up to 500 mm (the lowest in Poland). The Inowrocław Plain is a primarily agricultural region with one of the most fertile soils in Poland—Phaeozems (black earths) [20].

Due to the production potential of the soil in the analyzed area, mainly cereals (winter wheat and spring barley) and sugar beets are grown. The field study was conducted in September 2021 after the winter wheat harvest. Before sowing wheatgrass, the field under study was ploughed to a depth of 22–23 cm. Before the autumn sowing, soil mineral fertilization was performed with a 250 kg dose of NPK Ultra 8 (8–20–30), and then in the spring, ammonium nitrate 34 was applied in two doses of 200 and 240 kg.

The research material was soil samples collected from the study area along a transect set out on a 10 ha cultivated field. From the entire surface of the field, 20 representative samples with a disturbed structure were taken from the humus horizon at a depth of 0–30 cm. The overall sample consisted of five single samples. In terms of sampling density, the area that single samples represented did not exceed 0.5 ha.

2.2. Soil Analysis

Chemical analyses were performed on air-dried and sieved samples (<2 mm). Each sample was analyzed in triplicate. In the adequately prepared soil samples, the following were assayed:

- Particle-size distribution was determined using laser diffraction with a Mastersizer MS 2000 (Malvern Panalytical, UK). Based on the percentage shares of granulometric fractions determined, the granulometric group and subgroup were determined according to the classification of the Soil Science Society of Poland [21], as were the categories of agrotechnical heaviness;
- Active acidity was determined in demineralized water, whereas exchangeable acidity was determined in 1 M KCl potentiometrically using a CPC-551 pH meter [22];
- Hydrolytic acidity (Hh) and total exchangeable basic cations (TEB) were determined using Kappen method. Based on TEB and Hh, the cation exchange capacity (CEC) was calculated, and the sorption complex’s degree of saturation with bases (BS) was calculated from CEC and TEB;
- Total organic carbon (TOC) and total nitrogen (TN) contents were determined using a Vario Max CNS analyzer (Elementar, Langenselbold, Germany). Based on the results of organic carbon and total nitrogen, the TOC/TN ratio was calculated;
- The contents of available forms of phosphorus (P) [23] and potassium (K) were also defined using the Egner–Riehm method (DL) [24], as was the content of magnesium available to plants (Mg) using the Schachtschabel method [25],
- Available forms of zinc (Zn) and copper (Cu) were extracted with 1 M HCl using Rinkins’ method.

Available phosphorus content was determined using a Marcel Pro spectrophotometer. The content of forms K, Mg, Zn and Cu available to plants was determined using atomic absorption spectroscopy and atomic emission spectroscopy with a Solaar S4 spectrometer. To verify the accuracy of the results, the analysis of the certified material Loam Soil No. ERM–CC141 as well as so-called zero tests were made, which were exposed to the identical analytic procedure as the soil samples. Good compatibility between the certified and determined values was obtained.

2.3. Enzyme Analysis

The activities of selected enzymes were assayed on fresh sieved (<2 mm) soils that had been stored at 4 °C for two weeks. Each activity was assayed in triplicate. The following oxidoreductase and hydrolase enzymes were analyzed:

- The activity of dehydrogenases (*DEH*) (EC 1.1.1) in soil was determined using the Thalmann method [26] after incubation of the sample with 2,3,5-triphenyltetrazolium chloride and measurement of triphenylformazan (TPF) absorbance at 546 nm and expressed in mg TPF kg^{-1} 24 h^{-1}.
- Catalase activity (*CAT*) (EC 1.11.1.6) was determined using the method of Johnson and Temple [27] with 0.3% hydrogen peroxide solution as a substrate. The remaining H_2O_2 was determined using titration with 0.02 M $KMnO_4$ under acidic conditions.
- The activity of peroxidases (*PER*) (EC 1.11.17) was determined according to Barth and Bordeleau [28] by measuring the amount of purpurogallin (PPG) produced by oxidation of pyrogallol in the presence of H_2O_2.

- Alkaline (*AlP*) (EC 3.1.3.1) and acid (*AcP*) (EC 3.1.3.2) phosphatase activities in the studied soil were assayed using the method of Tabatabai and Bremner [29], which involves the determination of p-nitrophenol released by incubation at 37 °C for 1 h of 1 g soil with 4 mL MUB (modified universal buffer) at pH 6.5 for acid phosphatase and pH 11.0 for alkaline.
- β-glucosidase (*BG*) activity (EC 3.2.1.21) was measured according to Eivazi and Tabatabai [30] using p-nitrophenyl-β-D-glucopyranoside as a substrate. P-nitrophenol concentrations were determined using direct detection in the sample at 400 nm after alkalization with a Tris/NaOH buffer (pH 10.0) and $CaCl_2$.
- The activity of proteases (*PRO*) (EC 3.4.21) was determined using the method described by Ladd and Butler [31]. One gram (1 g) of soil was incubated with Tris buffer (0.2 M, pH 8.0) and sodium caseinate solution at 40 °C for 2 h.

Based on the enzyme activity results, the following indices were calculated:
Enzymatic pH indicator defining the right soil reaction [11]:

$$AlP/AcP \tag{1}$$

Biological index of fertility *BIF* was calculated according to Stefanica et al. [32]:

$$BIF = \frac{1.5DEH + 100kCAT}{2} \tag{2}$$

where k is the factor proportionality equal to 0.01.

The geometric mean GMea [33]:

$$\mathrm{G}Mea = \sqrt[7]{DEH \times CAT \times PER \times AlP \times AcP \times PR \times BG} \tag{3}$$

where *DEH*, *CAT*, *PER*, *AlP*, *AcP*, *PRO* and *BG* are dehydrogenases, catalase, peroxidases, alkaline phosphatase, acid phosphatase, proteases and β-glucosidase, respectively.

To assess the total enzyme activity index (*TEI*), the following was calculated [34]:

$$TEI = \sum \frac{Xi}{\overline{\overline{Xi}}} \tag{4}$$

where Xi is the activity of soil enzyme i, and $\overline{X_l}$ is the mean activity of enzyme i in all samples.

The indices of biochemical soil activity (*BA12* and *BA13*) [35] were proposed based on the activities of soil enzymes, the content of clay and the content of organic carbon:

$$BA12 = log_{10}TOC\ \sqrt{DEH + CAT + AlP + AcP} \tag{5}$$

$$BA13 = log_{10}clay\ \sqrt{DEH + CAT + AlP + AcP} \tag{6}$$

2.4. Statistical Analysis

To study the trends (mean, median) and variability (standard deviation SD, minimum and maximum) of the sample population, classical statistics were used in Statistica.PL 13.3. The coefficients of variation (CV%) of the analyzed parameters were also calculated. CV values of 0–15%, 16–35% and >36% indicate low, moderate and sufficiently high variability, respectively [36]. Granulometric composition, pH in H_2O and KCl, contents of TOC, TN, available macronutrients (P, K and Mg) and micronutrients (Zn and Cu), sorption properties (Hh, TEB, CEC and BS) and the activities of selected enzymes (*DEH*, *CAT*, *PER*, *AlP*, *AcP*, *BG*, *PR*) were also assessed using principal component analysis (PCA). PCA can reduce the number of variables describing a given object and can indicate the influence that principal variables have on principal components and the mutual correlations between principal variables. A p value of <0.05 was considered significant. This method can also be used to determine the influence of primary variables on the principal components and the mutual correlations between primary variables. Standardized PCA was performed on a correlation matrix.

3. Results and Discussion

3.1. Granulometric Composition, pH and Sorption Properties of the Soil

The analyzed soils had a highly homogeneous granulometric composition, as evidenced by the low coefficient of variation (Table 1). The tested soil samples from the arable horizons were classified into two granulometric groups: clays and silts. Most samples had a particle-size distribution typical of the sandy clay subgroup. Only 4 of the 20 samples were classed as silt loam [37]. In the humus horizon, the silt and sand fractions dominated, while the clay fraction was the lowest (Table 1). The silt fraction ranged from 40.27 to 59.57%, averaging 47.29%. The clay fraction averaged 5.66%. According to the guidelines of the Polish Soil Science Society, the granulometric compositions of the soils from across the entire studied field surface classed them agronomically as medium soils [21].

Table 1. Selected physical and chemical properties (n = 20).

Parameters *	Min	Max	Mean	Median	SD	CV
Sand	35.27	54.54	47.06	47.14	4.32	9.18
Silt	40.27	59.57	47.29	46.61	4.27	9.03
Clay	4.59	6.33	5.66	5.72	0.42	7.50
pH H_2O	5.53	7.76	6.94	7.10	0.57	8.26
pH KCl	5.22	7.55	6.92	7.09	0.61	8.86
Hh	0.075	1.575	0.416	0.26	0.39	94.22
TEB	0.6	41.3	16.82	13.75	11.90	70.75
CEC	1.425	41.45	17.27	13.83	11.67	67.72
BS	42.10	99.82	92.94	98.69	12.91	67.72
TOC	10.85	28.27	14.72	13.75	3.95	26.87
TN	0.98	2.08	1.30	1.25	0.24	18.51

* Min—minimum, Max—maximum, SD—standard deviation, CV—coefficient of variation, Sand, Silt, Clay (%), Hh—hydrolytic acidity (cmol kg^{-1}), TEB—total exchangeable bases (cmol(+) kg^{-1}), CEC—cation exchange capacity (cmol(+) kg^{-1}), BS—degree of saturation of the sorptive complex with base cations (%), TOC—total organic carbon (g kg^{-1}), TN—total nitrogen (g kg^{-1}).

In the humus horizon, the soil pH was variable, ranging from slightly acidic to alkaline, despite the coefficient of variation being low. On average, it was pH 6.94 in distilled water and pH 6.92 in 1 M KCl, indicating a neutral reaction of the tested samples. The results coincide with those obtained by other researchers analyzing Phaeozems [38,39]. In examining the black earths of the Inowrocław Plain, the authors found these soils' reaction to be slightly acidic to alkaline. The coefficient of variation (CV) for hydrolytic acidity (Hh) and the total exchangeable base cations (TEB) indicated that these parameters were highly differentiated, at 94.22% for Hh and 70.75% for TEB. The total base cations (TEB) were favorable for growing crops (0.60–41.30 cmol(+) kg^{-1}) in most of the soil samples, averaging 16.82 cmol(+) kg^{-1} (Table 1). The studied soils had an average CEC of 17.27 cmol(+) kg^{-1}, with a high coefficient of variation of 67.72%. They were also characterized by up to 99.82% saturation of the base complex with base cations (BS). The content of the base and acid exchangeable cations in the soil sorption complex affects plant nutrition and the ion balance of plants. The cations adsorbed in soil colloids constitute a pool of nutrients for plants [40–42]. The cation exchange capacity (CEC) and saturation of the sorption complex with base cations (BS) determine soil fertility and resistance to chemical degradation. The CEC value is determined by the total cations neutralizing the negative charges on the surface of soil colloids and on the soil reaction [40].

3.2. Content of Macro- and Microelements in Soil

The TOC content in the study area ranged from 10.85 to 28.27 g kg^{-1}, with an average of 14.72 g kg^{-1} and a standard deviation of 3.95. According to the European criterion for assessing organic carbon content in soils that was developed based on the European Soil

Database (ESB), these values were low [43]. The low content of organic carbon may have been caused by intensive agricultural cultivation on the land under study. The CV coefficient of 26.87 indicated that the TOC variability was average. The median calculated in the distribution analysis showed that most of the results were below the mean (Table 1). The results are confirmed by other authors' research [39,44,45]. The cited authors showed that the organic carbon content in the black soils of the Inowrocław Plain averages 17 g kg^{-1}. Intensive agricultural production may, when combined with simple crop rotation or monoculture, reduce the amount of organic residues entering the humus transformation cycle and, consequently, decrease the humic content in soils [39]. Humus may also be decomposed and biodegraded by the use of physiologically acidic fertilizers and the activation of soil microorganisms under the influence of intensive mineral fertilization. However, the variability in the TOC content depends mainly on the soil type and class [46,47]. Soil organic carbon (SOC) has been proposed as the most important single indicator of soil quality and agricultural sustainability because it affects most soil properties [48].

The TN content in the analyzed soil samples averaged 1.30 g kg^{-1}, with a SD of 0.24. Most of the samples were below average, as indicated by the median value. Studies by other authors indicate that the total nitrogen content in the black earths of the Pomerania and Kuyavia region does not exceed 1.8 g kg^{-1} on average [49].

The intensity of changes in soil organic matter is presented using the TOC/TN index (Figure 1). The narrow TOC/TN ratio that was calculated attests to a rapid transformation of organic matter in the analyzed soil [14]. Lower C/N ratios are of key importance for soil microorganisms' use of TOC and TN, which promotes greater mineralization of organic substances and the release of mineral forms of N. They affect the release of larger amounts of nutrients into the soil and, thus, soil fertility.

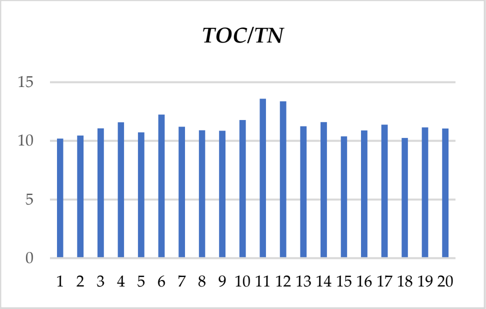

Figure 1. TOC/TN ratio in the soil.

Nutrient abundance is one of the determinants of soil fertility. The parent material, granulometric composition and the course of soil-forming processes are the greatest determinants of the amount and distribution of nutrients in the soil profile. The mean content of available P in the tested soil samples of the humus horizon was 59.08 mg P kg^{-1} of soil with an SD of 11.78 and low variability (CV 19.93) (Table 2). The content of available phosphorus at the surface of the study area averaged 59.08 mg kg^{-1}. On this basis, the studied soil was classified, according to PN-R-04023 [23], as having an average content of available P (fertility class III).

The abundance of available K in the soil varied across the entire field, ranging from 68.08 to 137.7 mg K kg^{-1}. The mean soil abundance of bioavailable K that was determined is classed, for a soil in the "average soil" agronomic category, as category IV (low) [24]. These results are in line with those obtained by Kobierski et al. [45]. They stated that the content of bioavailable potassium in the black earths of the Inowrocław Plain ranged from 19.2 to 357.3 mg K kg^{-1}.

Table 2. Content of available forms of macro- and microelements (n = 20).

Parameters *	Min	Max	Mean	Median	SD	CV
P	47.13	93.77	59.08	53.53	11.78	19.93
K	68.08	137.7	99.31	100.0	16.44	16.55
Mg	4.84	9.61	7.63	7.47	1.27	16.63
Zn	7.17	9.98	8.34	8.09	0.75	8.99
Cu	3.39	5.23	4.24	4.18	0.45	10.56

* Min—minimum, Max—maximum, SD—standard deviation, CV—coefficient of variation, P—available phosphorus, K—available potassium, Mg—available magnesium, Zn—available zinc, Cu—available copper (mg kg^{-1}).

The content of available Mg in the analyzed samples ranged from 4.84 to 9.61 mg Mg kg^{-1}. The average content of available Mg in the humus horizon was 7.63 Mg kg^{-1}. Analyzing the results for the "medium soils" agronomic category, the soil can be placed in class III—average richness in magnesium for mineral soils [25].

The soil was highly homogeneous in its content of available Zn and Cu. The coefficient of variation for both was low, amounting to 8.99% for Zn and 10.56% for Cu. The content of bioavailable Zn and Cu in the soil was low (Table 2), averaging 8.34 mg kg^{-1} for Zn and 4.24 mg kg^{-1} for Cu. The neutral and alkaline reaction of the studied soils reduces the amount of bioavailable Zn and Cu [50]. Comparing the bioavailable zinc and copper content in the studied soils against the critical values for arable soils, the soil samples were above the threshold for a deficit of these elements [51]. The content of bioavailable forms of these microelements was close to the values obtained by other authors in studies of black earths [3,52].

3.3. Activity of Selected Enzymes in Soil

The *DEH* activity ranged from 1.513 to 1.713 mg TPF kg^{-1} 24 h^{-1} (with an average activity of 1.639 mg TPF kg^{-1} 24 h^{-1}) (Table 3). The CV for *DEH* was 3.40, indicating little variability. The distribution analysis showed most of the results to be above average, as indicated by the median value being above the mean. Dehydrogenases play an important role in the biological oxidation of soil organic matter by transferring hydrogen from organic substrates to inorganic acceptors [53]. Their activity can be considered an indicator of oxidative metabolism in soil. The functioning of dehydrogenases is related to many biochemical processes in soil, which include the greenhouse gas emissions of CO_2 and N_2O.

Table 3. Activity of enzymes in soil (n = 20).

Parameters *	Min	Max	Mean	Median	SD	CV
DEH	1.513	1.713	1.639	1.650	0.056	3.40
CAT	1.028	1.491	1.216	1.220	0.108	8.90
PER	1.885	2.467	2.140	2.157	0.171	8.00
AlP	0.594	1.804	1.083	0.979	0.366	33.80
AcP	1.323	2.395	1.822	1.809	0.266	14.60
BG	0.583	1.300	0.784	0.763	0.168	21.40
PR	25.31	58.92	34.05	32.06	8.001	23.50

* Min—minimum, Max—maximum, SD—standard deviation, CV—coefficient of variation, *DEH*—dehydrogenases (mg TPF kg^{-1} 24 h^{-1}), *CAT*—catalase (mg H_2O_2 kg^{-1} h^{-1}), *PER*—peroxidases (mM PPG kg^{-1} h^{-1}), AlP—alkaline phosphatase (mM pNP kg^{-1} h^{-1}), AcP—acid phosphatase (mM pNP kg^{-1} h^{-1}), BG—β-glucosidase (mM pNP kg^{-1} h^{-1}), *PR*—proteases (mg TYR kg^{-1} h^{-1}).

The *CAT* activity ranged from 1.028 to 1.491 mg H_2O_2 kg^{-1} h^{-1}(average 1.216 mg H_2O_2 kg^{-1} h^{-1}) H_2O_2 (Table 3). The low CV (8.90%) showed *CAT* to be homogeneous in the tested transect. Catalase is an important cellular antioxidant enzyme that protects against oxidative stress, catalyzing the breakdown of hydrogen peroxide into H_2O and O_2. Catalase activity is used together with dehydrogenase activity to obtain information on microbial activity in soil [14].

Peroxidases catalyze the decomposition of hydrogen peroxide while also oxidizing organic and inorganic substances. The activity of this enzyme ranges from 1.885 to 2.467 mM

PPG kg^{-1} h^{-1} (average 2.140 mM PPG kg^{-1} h^{-1}) (Table 3). Peroxidase participates in the biogeochemical processes of lignin degradation, the oxidation of toxic substances, mineralization and carbon sequestration [54,55]. There was not much variation in *PER* (CV = 8.0%). Dehydrogenases, catalase and peroxidases are enzymes belonging to the class of oxidoreductases and are responsible for oxidative processes in soil [56]. They catalyze the cleaving of bonds in electron-rich substrates over a wide range of redox potentials. This is a key process in degrading biotic and xenobiotic aromatic compounds in soil [57]. Oxidoreductases are often produced to degrade humic complexes rather than obtaining plant nutrients directly.

Alkaline phosphatase (*AlP*) and acid phosphatase (*AcP*) are enzymes that play a key role in the P cycle. The production of these enzymes in the soil is the most important biological strategy for obtaining phosphate ions from organic molecules [58]. In the studied area of the cultivated field, *AlP* activity ranged from 0.594 to 1.804 mM pNP kg^{-1} h^{-1} (averaging 1.083 mM pNP kg^{-1} h^{-1}) (Table 3). The coefficients of variation indicate moderate and low variability of *AlP* and *AcP* in the soil (33.80 and 14.60, respectively).

The *BG* activity in the studied soil samples averaged 0.784 mM pNP kg^{-1} h^{-1} (from 0.583 to 1.300 mM pNP kg^{-1} h^{-1}) (Table 3). The research showed a moderate variability this parameter (CV = 21.40%). β-glucosidase is the enzyme responsible for the final step in the hydrolysis of cellulose breaking down disaccharides into glucose that is assimilable by microorganisms.

The average activity of *PR* in the soil of the experimental field was 34.05 mg TYR kg^{-1} h^{-1} (Table 3). The coefficient of variation calculated for *PR* was in the range of moderate variation (CV of 16–35%) according to the classification given by Wilding [59]. The activity of specific enzymes involved in the N cycle attests to the intensity of changes in nitrogen compounds in the environment and may be an indicator of the bioavailability of nitrogen. Proteases are involved in hydrolyzing the peptide bonds (CO-NH) of proteins to polypeptides and then to free amino acids [60].

Research on the spatial heterogeneity of agricultural soils showed that enzyme activity (urease, phosphatase and protease) was more variable (CVs 31–88%) than the organic C and total N contents [61].

Research by Nedyalkov et al. [62] has indicated that soil type (Vertisol = Luvisol > Cambisol) more strongly influenced the activity of enzymes than their method of use. Du et al. [63] also found soil type to be a greater determinant of soil activity than contamination with polychlorinated biphenyls.

3.4. Enzyme Activity Indexes in Soil

According to Gil-Sotres et al. [64], soil enzymatic activity can, in combination with selected physical and chemical properties, reflect the intensity of soil processes and, thus, provide information about its fertility. It is very difficult to develop a universal fertility index for soils regardless of their specificity. Based on the results of alkaline and acid phosphatase activity, an enzymatic soil pH level index (*AlP/AcP*) has been developed [11]. The mean *AlP/AcP* value for the tested samples was 0.62 (Figure 2A). An *AlP/AcP* value above 5.0 (>0.5) indicates an alkaline soil reaction. In most cases, the *AlP/AcP* values of the tested soil exceed 0.50 (0.30–1.24). An alkaline reaction was confirmed using potentiometric testing in H_2O and KCl (slightly acidic to alkaline reaction—Table 1).

The *BIF* values ranged from 1.65 to 2.029 (mean 1.84) (Figure 2B). Earlier studies have shown that the *BIF* value is higher in meadow and forest soils than in arable fields [65]. Those authors argue that forest soils exhibit a strong root system and contain a large amount of organic matter, which differentiates them significantly from agricultural soils. The G*Mea* and *TEI* indices are dimensionless parameters used to compare the total activity of tested soil enzymes. According to Paz-Ferreiro and Fu [66], G*Mea* constitutes an integrative approach that can combine multiple properties relating to different soil functions. The G*Mea* values for the soil samples ranged from 0.85 to 1.03 (Figure 2C). Higher values of this index indicate better soil quality [12,67]. The integrated total enzyme activity index (*TEI*) allows for simple comparisons between the combined enzyme activity and the quality of each soil sample [68]. The *TEI* values ranged from 5.14 to 7.77 (Figure 2D). Based on the activities determined for the tested enzymes and the clay and TOC contents, two indices of soil biochemical activity were calculated, *BA12* and *BA13* [35] (Figure 2E,F). The *BA12* index ranged from 2.32 to 3.68 (average 2.78). Meanwhile, *BA13* averaged 1.80. The authors of the *BA12* and *BA13* indices [35] found that the activity of these indicators depends mainly on dehydrogenase activity and the carbon content. Kobierski et al. [69] showed that soil from an organic farm (OF) in which reduced tilling with the application of manure or compost and biodynamic preparations stimulating enzymatic activity were used had significantly higher enzymatic indices values than soil from conventional farming.

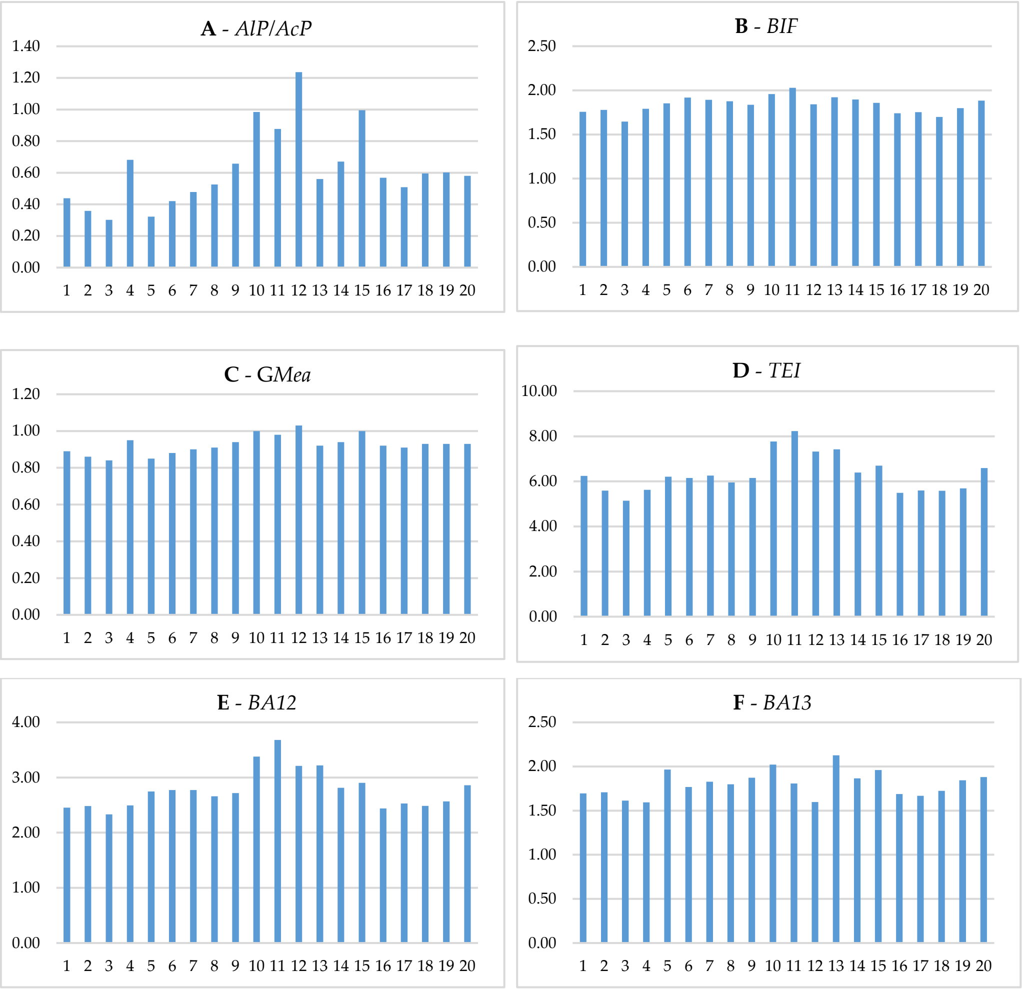

Figure 2. (**A**–**F**) Enzymatic indices of soil quality. (**A**) *AlP/AcP*—enzymatic index of soil pH level; (**B**) *BIF*—biological index of fertility; (**C**) G*Mea*—geometric mean; (**D**) *TEI*—total enzyme activity index; (**E**) *BA12* and (**F**) *BA13*—indices of biochemical soil activity.

The enzymatic activity parameter is very sensitive to environmental factors (biotic and abiotic) [14,67,70]. In order to demonstrate the relationship between the *AlP/AcP*, *BIF*, G*Mea*, *TEI*, *BA12* and *BA13* indices and other soil parameters, a Pearson correlation analysis was performed at $p < 0.05$ (Table 4). TOC was found to have significant positive relationships with *AlP/AcP* ($r = 0.647$), *BIF* (r = 0.748), G*Mea* ($r = 0.612$) and *BA12* ($r = 0.915$), which explain, respectively, 41.8, 55.9, 37.4 and 83.7% of the variation in the calculated indices. TN was found to have highly significant correlations with *AlP/AcP* ($r = 0.651$), *BIF* ($r = 0.798$), G*Mea* ($r = 0.627$) and *BA12* ($r = 0.945$). The study did not show positive correlations between TEI, GMea and the content of assimilable P and K, contrary to the research [34]. CEC and TAB also positively correlated with *AlP/AcP*, *BIF*, G*Mea* and *BA12*. According to

Mierzwa-Hersztek et al. [68], *TEI* is usually positively correlated with the content of C and N, which our study did not show. The results of studies by Nurzhan et al. [71] showed that enzymatic indices (geometric mean (*GM*), weighted mean (*WM*) and total enzyme index (*TEI*)) correlated better with selected physical and chemical soil properties than with the activity of a single enzyme.

Table 4. Relations between the activity of selected enzyme indices and soil properties.

Parameter	**Index**					
	AlP/AcP	**GMea**	***BIF***	***TEI***	***BA12***	***BA13***
TOC	0.647	0.612	0.748	n.s.	0.915	n.s.
TN	0.651	0.627	0.798	n.s.	0.945	n.s.
P	−0.526	−0.584	n.s.	n.s.	n.s.	n.s.
K	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Mg	n.s.	n.s.	0.508	n.s.	0.549	0.540
Zn	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Cu	n.s.	n.s.	0.570	n.s.	0.539	n.s.
Hh	−0.594	−0.680	−0.474	n.s.	−0.522	n.s.
CEC	0.723	0.707	0.452	n.s.	0.701	n.s.
BC	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
TEB	0.729	0.715	0.459	n.s.	0.705	n.s.
pH H_2O	0.799	0.864	0.484	n.s.	0.641	n.s.
pH KCl	0.698	0.779	n.s.	n.s.	0.513	n.s.
Clay	n.s.	n.s.	n.s.	n.s.	n.s.	0.784

n.s.—not significant.

3.5. Statistical Analyses

Principal component analysis (*PCA*) was used to clarify the differentiation in the black earth in terms of the tested physicochemical and biochemical parameters (sand, silt, clay, pH in H_2O and KCl, Hh, TEB, CEC, BC, *TOC*, TN, P, K, Mg, *DEH*, *CAT*, *PER*, *AlP*, *AcP*, *BG* and *PR*) based on the principal components. Accordingly, three components were found to account for 66.80%; of these, the first two components (PC1 and PC2) represent 61.78% of the dependence, while the next component contributes only 5.02% to the model. Therefore, only the first two components are projections of the variables on the factor plane presented graphically (Figure 3). The *PCA* analysis showed that the first component (PC1) generated 50.49% and was significantly negatively associated with the activity of *AlP* (−0.783), *CAT* (−0.843), *DEH* (−0.705), *PR* (−0.921), *BG* (−0.894) and *PER* (−0.832). Significant negative relationships were also found between PC1 and silt (−0.888), active acidity (−0.782), TEB (−0.795), CEC (−0.788), TOC (−0.926), TN (−0.936) and TOC/TN (−0.751). The relationship between these parameters may reflect the influence of the agricultural technology used on the chemical and enzymatic properties of Phaeozem. The second component (PC2) accounted for 11.29% of the total variance and was significantly positively related to *AcP* activity (0.560). According to Liu et al. [72], load values of >0.75, 0.75–0.5 and 0.5–0.3 are defined as "strong", "moderate" and "weak", respectively. According to Ghaemi et al. [73], PCA is a method for selecting effective indicators that play a key role in soil sustainability. Makó et al. [74] used PCA analysis to determine that soil types such as Luvisols and Chernozems are easily identifiable by their chemical properties, whereas Arenosols and sandy Cambisols are recognized by their physical properties.

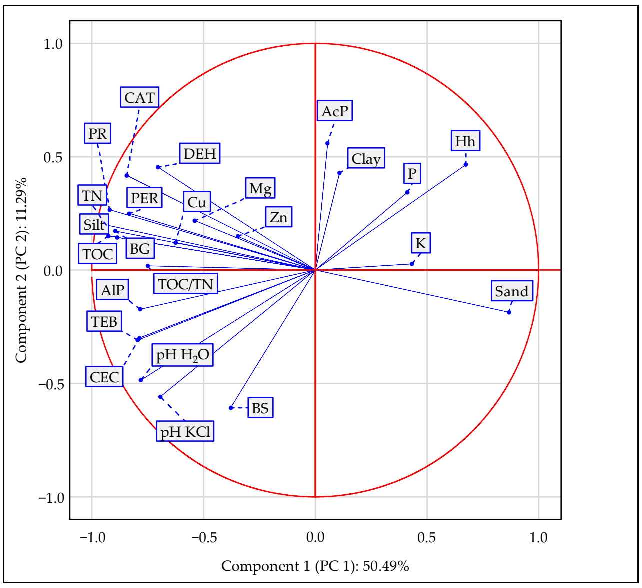

Figure 3. Principal component analysis soil properties' plot of the first two principal components (PC) for the measured soil properties. Sand, Silt, Clay, Hh—hydrolytic acidity, TEB—total exchangeable bases, CEC—cation exchange capacity, BS—degree of saturation of the sorptive complex with base cations (%), TOC—total organic carbon, TN—total nitrogen, P—available phosphorus, K—available potassium, Mg—available magnesium, Zn—available zinc, Cu—available copper, *DEH*—dehydrogenases, *CAT*—catalase, *PER*—peroxidases, AlP—alkaline phosphatase, AcP—acid phosphatase, BG—β-glucosidase, *PR*—proteases.

The activity of enzymes in the soil environment depends on abiotic and biotic factors alike, which include the content of mineral and organic colloids, temperature, water–air properties, soil pH, the content of biogenic elements and the abundance and species status of microorganisms [75,76]. These factors are greatly affected by the soil tillage system. PCA was used to verify the significance of correlation between individual soil parameters (Figure 3). *TOC* content was significantly positively correlated with the activity of *AlP* (r = 0.629), *CAT* (r = 0.815), *DEH* (r = 0.603), *PR* (r = 0.970), *BG* (r = 0.973) and *PER* (r = 0.832). There was also a significant and sufficient relationship between the TN content and the activity of the tested enzymes, with the exception of *AcP*. The results show a strong relationship between the organic carbon content and enzymatic activity in the studied soil. Soil organic matter is transformed with the participation of soil microorganisms and enzymes [77]. The literature states [78] that enzymatic activity varies depending on type of soil use and is higher in sodded soil and in soil used for crop rotation than in soils used for monocultures. Crop soils are generally lower in organic matter, which reduces the biomass of enzyme-producing microorganisms. Higher levels of organic carbon in the soil can provide a sufficient amount of substrate to affect microbial biomass and thus increase enzyme production [79]. Soil enzymes can be immobilized and thus accumulate on soil organic matter. Research by Wolińska and Stępniewska [80] confirmed that the high correlation coefficient for enzymatic activities and the *TOC* level suggests that these enzymes play a significant role in transforming the basic components of soil organic matter. Enzymes from the oxidoreductase class mediate key processes in the soil ecosystem, e.g., lignin degradation, humification and the mineralization of *TOC* to dissolved organic carbon [35]. As the activity of oxidative enzymes mediates both the degradation and formation of the most resistant components of detritic organic matter, they are closely associated with carbon sequestration in soil [81]. A significant correlation was also found between the TN content and activity of the tested enzymes, except for *AcP*. Chaer et al. [82], too, found a positive significant relationship between *PR* activity and TN content (r = 0.751). This can be explained by the increased accumulation of SOM (soil organic matter) being able to increase the variety of substrates, including N, that increase enzyme activities.

Studies by Bowles et al. [83] concluded that the activity of enzymes responsible for the C cycle increased with the availability of inorganic N, while the activities of enzymes related to N increased with the availability of C.

Research by Margalef et al. [58] into the effect of soil and climate variables on soil phosphatase activity showed that the TN content in soil, average annual rainfall, average annual temperature, thermal amplitude and total carbon in soil explain up to 50% of the spatial variability of phosphatase activity on a global scale. The content of available P was significantly negatively correlated with the value of *AlP* ($r = -0.444$). Soil phosphatase is necessary for the mineralization of organic phosphorus in soil [84]. As a large proportion of P in soil is organically bound, its mineralization is of great importance to agriculture [75]. Usually, a high inorganic P content in the soil reduces phosphatase activity [85]. In this case, phosphorus acts as an inhibitor of soil phosphatase activity. According to Olander and Vitousek [86], the activity of nutrient-mineralizing enzymes is inversely proportional to nutrient availability. Therefore, soil enzymatic activity can provide information on the circulation of nutrients in soil [10]. A negative correlation is more common between P content and phosphatase activity than between N availability and nitrogen-mineralizing enzymes [87]. This is because organic phosphorus is mainly in the form of phosphate esters and is mineralized by phosphatase catalysis. Organic nitrogen, on the other hand, comes in various forms and can also be closely associated with organic carbon. Soil enzymatic activity can provide information on the soil nutrient cycle [15]. Research by Xiao et al. [88] has shown that soil enzymatic activity is more sensitive to changes in nutrient availability than to changes in atmospheric CO_2, temperature and precipitation. *AlP* correlated positively with pH in H_2O ($r = 0.797$) and pH in KCl ($r = 0.655$). In addition, a positive correlation was obtained between *PER* activity and pH in H_2O ($r = 0.513$) and pH in KCl ($r = 0.464$). TEB and CEC correlated positively with *AlP* activity ($r = 0.722$ and $r = 0.716$, respectively). The pH of soil solution strongly controls the activity of enzymes because it affects the conformation of an enzyme, its adsorption to solid surfaces and the ionization and solubility of substrates and cofactors [89,90]. Increased soil acidity weakens soil enzymatic activity by destroying hydrophobic, ionic and hydrogen bonds in the active centre of enzyme proteins. As the catalytic performance of enzymes is closely related to the conformation of the chain, especially the active site, even slight changes in pH can significantly reduce enzyme activities [90–92]. Those most sensitive to and dependent on pH are acid and alkaline phosphatases. *AcP* is dominant in acidic soils with a slight alkaline admixture, while *AlP* is more active in alkaline soils [11]. TEB and CEC correlated positively with *AlP* activity ($r = 0.722$ and $r = 0.716$, respectively). A significant positive correlation was found between clay content and *AcP* activity ($r = 0.566$). The persistence and stability of soil enzymes is generally attributed to their association with clays and humus. The adsorption of enzymes to clay minerals significantly changes enzyme properties such as optimal pH, stability, activity and kinetics [93]. The amount of mineral and organic colloids determines sorption capacity and thus affects a soil's biochemical activity. In the case of the complexes of enzymes with minerals and organic colloids, it makes them more durable and resistant to denaturation.

The content of Cu in Phaeozems was significantly positively correlated with the activity of *CAT* ($r = 0.550$), *DEH* ($r = 0.475$), *PR* ($r = 0.534$) and *BG* ($r = 0.436$). The Zn content was positively correlated only with *DEH* activity ($r = 0.458$). Heavy metals are natural components of soil, but they can have long-term negative effects on soil and its biological processes [94]. The inhibition of soil enzymes depends on the concentration and nature of the heavy metals, and levels vary by enzyme. However, at certain concentrations, some heavy metals can increase enzyme activities [95]. Enzyme activity can be inhibited by interactions within the enzyme–substrate complex denaturing the enzyme protein. Many metal ions are enzymatic activators, e.g., Mg^{2+} ions activate phosphatases, Fe^{2+} ions activate peroxidases, Mn^{2+} ions activate phosphotransferases, and Zn^{2+} ions activate dehydrogenases. Both TOC and TN were also significantly positively correlated with the assimilable Cu content, and the correlation coefficient was $r = 0.502$ for TOC and $r = 0.511$

for TN, respectively. According to Kwiatkowska-Malina [47], organic matter in acidic conditions is the basic adsorbent of trace elements. Murray et al. [96] presented the need to consider the quantity and quality of SOM, pH and clay content when establishing threshold criteria for metal contents as part of a human risk assessment. Rafiq et al. [97] observed that soil pH, CEC and SOM were the main factors influencing the bioavailability of heavy metals in various soil types.

4. Conclusions

The research results based on the correlations obtained between selected soil physicochemical properties and enzymatic activity and enzymatic indices suggest that soil enzymes are important for carbon capture in soil and nutrient dynamics.

In the study area, according to the relevant assessment criterion adopted in Europe, the organic carbon content was found to be low. This may be due to intensive agricultural cultivation in the area, combined with simplified crop rotation or monoculture and improper organic fertilization. To improve the balance of organic matter, natural fertilization should be applied and/or catch crops sown. To restore soil organic matter and optimize the nutrient cycling of agricultural systems, management practices need to be developed that take into account the principles of soil enzyme activity.

Enzymatic activity can be used to indicate the availability of nutrients in the soil. The studied soil parameters significantly influenced the enzymatic quality indices calculated for Phaeozems. The strongest determinants were TEB, TOC and TN. The indicator least well associated with the tested physical and chemical properties of the soil was *TEI*. The Phaeozems' spatial properties are better reflected using the single enzyme activities compared with an integrated soil enzyme index.

The conducted analysis of Phaeozems emphasizes the key role of the interaction of enzymes with selected physical and chemical properties in driving soil C dynamics in agricultural soils. Used in study indicators, this can be used for soil quality assessments. This ensures a more accurate view of how soil environments work. This helps in making a decision about which preventive measures should be taken to maintain the sustainability and fertility of soil.

Author Contributions: Conceptualization, A.B., A.Z. and J.L.; methodology, J.L. and A.B.; software, J.L. and A.B.; validation, J.L., A.B., A.Z., I.J., M.R., K.K. and M.P.; formal analysis, A.B., J.L., A.Z., M.R., M.P. and K.K.; investigation, J.L., A.B., M.R., K.K., I.J. and A.Z.; resources, A.B. and J.L.; data curation, A.B. and J.L.; writing—original draft preparation, A.B., A.Z. and J.L.; writing—review and editing, J.L., A.B., A.Z., I.J., M.P., M.R. and K.K.; visualization, J.L. and A.B.; supervision, A.B., J.L., I.J., K.K., M.R. and M.P.; project administration, J.L. and A.B. All authors have read and agreed to the published version of the manuscript.

Funding: Bydgoszcz University of Science and Technology under Grant BN-WRiB-2/2022.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to thank the Faculty of Agriculture and Biotechnology, Bydgoszcz University of Science and Technology for their support in this research work.

Conflicts of Interest: The authors declare no conflict of interest.

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