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Mineral Components, Organic Matter Quality and Soil Enzymatic Activity under the Influence of Differentiated Farmyard Manure and Nitrogen Fertilisation

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Abstract: Research was carried out on the impact of long-term use of cattle manure (30 t ha⁻¹ FYM) and various doses of N (0, 40, 60 and 120 t ha⁻¹) in the form of ammonium nitrate on the following soil parameters: salinity, hydrolytic acidity, total exchangeable base cations, cation exchange capacity, degree of base saturation of the sorption complex, total organic carbon and total nitrogen content, dissolved organic matter, fractional composition of organic matter and content of bioavailable macroelements: phosphorus, potassium and magnesium in the soil of a multi-year static field experiment. The activities of dehydrogenases, catalase, alkaline and acid phosphatase and proteases were also tested. A significant effect of FYM and N fertilisation on the content of bioavailable macroelements was found. The application of manure at a dose of 30 t ha⁻¹ mitigated the negative effects of the application of N at a dose of 120 kg ha⁻¹. A higher content of total organic carbon (8.42 g kg⁻¹) and humic acid fraction (1761 mg kg⁻¹) and higher values of the CHA/CFA ratio (0.79; parameters that are indicators of soil quality) were found in the soil fertilised with manure compared to the soil without manure added (TOC—7.00 g kg⁻¹; CHAs—1285 mg kg⁻¹; CHAs/CFAs 0.66). The activity of the tested enzymes was also significantly determined by the applied fertilisation. Enzyme activity was highest in the soil to which manure had been applied. Nitrogen fertilisation varied in its impact on the activity of enzymes according to the specifics of each enzyme. The content of humic acids and CHA/CFA values correlated positively with the content of soil minerals and the activity of dehydrogenases, catalase, alkaline and acid phosphatase and GMea and TEI indices. Dehydrogenases and acid phosphatase can be considered enzymes that take part in transforming organic matter towards the formation of FAs.

Keywords: macroelements; oxidoreductive and hydrolytic enzymes; organic matter; sorption properties



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1. Introduction

Fertility is the natural ability of the soil to provide plants with the optimal amount of essential nutrients. In this way, the soil's physical, chemical and biological properties [1] provide plants with appropriate conditions for growth and development [2]. However, excessive application of mineral fertilisers negatively affects the soil environment. Nitrogen is, alongside carbon and phosphorus, the most important element for crop nutrition. It is a component of chlorophyll, enzymes, proteins, nucleic acids, etc. It stimulates root growth and the absorption of other nutrients [3]. Nitrogen is taken up by plants and soil microorganisms in the form of ammonia (N-NH₄⁺). When large amounts of N-NH₄⁺ are available, the use of alternative sources of N, e.g., nitrate (N-NO₃⁻), is generally inhibited. In turn, when the availability of N-NH₄⁺ is low, enzyme systems are activated to use alternative sources of N [4]. Therefore, measuring the activity of enzymes involved in the biogeochemistry of C, N and P is a way to assess the impact that anthropogenic treatments have on, for example, a soil ecosystem [5,6]. According to Steinweg et al. [7], manure

activates the release of exoenzymes, which increases the SOM content and the circulation of soil substrates through biochemical decomposition. Manure increases the activity of soil enzymes by increasing the rate of decomposition of proteins, peptides and amino acids, as well as the decomposition of cellulose and glucose. This is related to an increase in biomass, that is, a change in the composition and number of microorganisms [8]. Manure is a good source of organic carbon, which activates the biotic life of soil flora and fauna, which are the main source of enzymes in the soil. The rational use of nitrogen fertilisers improves plant yields. However, incorrect use of agrotechnical treatments and excessive doses of N disrupt the functioning of agrosystems [9]. According to Reay et al. [10], intensive use of N fertilisers increases nitrogen greenhouse gas emissions. Reactive N from agricultural crops can pollute groundwater and lead to the eutrophication of surface waters [11]. Above all, increased nitrogen leaching is a consequence of the drop in pH caused by nitrogen fertilisation.

Therefore, manure application can alleviate these problems [12]. Manure has a positive effect on crop yields by increasing SOC storage in the soil, the content of macro- and microelements and pH [13,14]. According to a long-term (94-year) fertilisation experiment, the use of mineral fertilisation increases the OM content but degrades its quality [15]. Wang et al. [13] showed, after a 23-year experiment, that only fertilisation with manure can increase the TOC content. Tang et al. [16], after a 36-year fertilisation experiment, obtained an increase in TOC content in plots where mineral fertilisation had been used in combination with organic fertilisation (using post-harvest residues, manure). Based on a 25-year field experiment, Cai et al. [14] showed that fertilisation with mineral fertilisers (NPK) alone does not cause changes in the TOC content. However, in plots where NPK was applied in combination with manure, they noted an increase in the TOC content. Importantly, the TOC content increased in the first 10 years and levelled off over the next 15 years. Based on a 62-year experiment, Mensik et al. [17] showed that fertilisation with only NPK reduces the content and quality of OM (lower content of TOC, humic substances, predominance of FAs over HAs), whereas the use of manure resulted in an increase in the content and quality of OM (increase in TOC, humic substances and humic acids). In addition to the undoubted benefits of such fertilisation, there is a danger of enriching soils with heavy metals contained in these fertilisers [18,19]. Consumption of food produced on soils with organic fertilisers containing heavy metals in their composition can cause serious health problems for both animals and humans [20]. Improper management of animal excrements (e.g., improper storage and disposal) may cause excessive greenhouse gas emissions and eutrophication. At the same time, unprocessed manure may pose a sanitary threat due to the high content of zoonotic pathogens that cause diseases in humans [21,22].

According to literature reports [2,23–26], an increase in the TOC content causes an increase in the dissolved organic matter content, but generally does not change the share of DOC in the total carbon pool. Mineral and organic fertilisation undoubtedly comprise one of the factors (alongside mineralisation processes) that directly and/or indirectly shape humification processes. Humification is a process of biological, microbiological and chemical transformations that occur in the remains of dead plants and animals and lead to the formation of humic substances, i.e., humic acids (HAs) and fulvic acids (FAs). The ratio of the carbon content of humic acids to the carbon content of fulvic acids (CHAs/CFAs) is an important parameter for determining the quality of organic matter—soil fertility. CHAs/CFAs has been used as an index to describe the humification degree of OM, with a larger value indicating a higher degree of humification and thus higher resistance to decomposition [27–31].

Long-term static fertiliser experiments are important for the development of agricultural sciences. The synthesis of the results of long-term field studies, when enriched with statistical calculations, allows for appropriate conclusions to be drawn and inspires innovations of benefit to theoretical and practical progress.

The aim of this research was to quantitatively determine the impact that long-term use of mineral fertilisers and manure has on basic physical and chemical parameters of soil,

including the content of minerals, the quality of organic matter and the enzymatic activity. In addition, relationships were determined between the content of minerals and physical, biological and chemical parameters and the quality of organic matter. The research, based on a 40-year field experiment, also allowed us to determine the role that the applied crop treatments play in carbon sequestration.

2. Materials and Methods

2.1. Experimental Design

The soils were sampled from a long-term field experiment established in 1979 at the WRiB PBŚ experimental field in Wierzchucinek, Kujawsko-Pomorskie province, Poland (53°14'58" N, 17°46'36" E). The climate in Wierzchucinek has been classified as Dfb compliant with the Köppen–Geiger system. The yearly average temperature is 8.1 °C, and the yearly average precipitation is 430 mm. A field experiment was established on Luvisols, that is, on sandy loam soil, classified in terms of agrotechnical heaviness as medium soils, with a carbon (TOC) content of 6.90 g kg^{−1} and nitrogen 0.78 g kg^{−1}. The samples were taken after 40 years of experimentation from a depth of 0–30 cm.

The experiment was conducted in a three-course crop rotation of potato, rye, and rye in a randomised split-plot design.

The experimental factors were as follows:

Factor I—dose of manure: 0 or 30 t ha^{−1};

Factor II—dose of nitrogen: 0, 40, 60, or 120 kg ha^{−1};

The samples were dried at room temperature and sieved (2 mm).

2.2. Methods

2.2.1. Physicochemical Properties of Soil

In air-dried disturbed soil samples sieved through a ø 2 mm mesh, selected physicochemical properties were determined:

- Granulometric composition by laser diffraction, using a Mastersizer MS 2000 analyser;
- pH, potentiometrically in 1 M KCl extract [32];
- Hydrolytic acidity (Hh) and total exchangeable base cations (TEB), by using the 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;
- Electrical conductivity of 1:5 soil–water extract (EC1:5), by using the conductometric method [33].

2.2.2. Properties of Organic Matter

The total organic carbon (TOC) and total nitrogen (TN) were determined with a Vario Max CN analyser (Elementar, Germany).

The content of carbon in the humus fractions was as follows:

Cd—carbon (nitrogen) in solutions after decalcification; C(HAs + FAs)—sum of carbon of HAs and FAs in extracts obtained with 0.5 M NaOH; and CFAs—carbon of fulvic acids, which, following humic acid precipitation, were assayed with Multi N/C 3100 Analytik Jena (Jena, Germany). The content of carbon of humic acids (CHAs) and humins (Ch) was calculated [33]:

$$CHAs = C(HAs + FAs) - CFAs \quad (1)$$

$$Ch = TOC - C(HAs + FAs) - Cd \quad (2)$$

The fractional composition was expressed in g kg^{−1} of dry matter of soil sample and as the % share of respective fractions in the TOC (TN) pool.

Based on changes in TOC content, the soil organic carbon sequestration rate (CSR) was calculated for one year and 40 years (experimental period) using the equation proposed by Zhang et al. [34] and Hayatu [35]:

$$CSR = (TOC_1 - TOC_0) / TOC_0 \quad (3)$$

where TOC_0 —content of total organic carbon in the initial year of study (6.90 g kg^{-1}); TOC_1 —content of the total organic carbon in the soil after 40 years of the experiment.

2.2.3. Content of Available Macroelements

The content of available macroelements in the soil sifted through a sieve (<2 mm) was determined according to the following methods:

- Available phosphorus (P) [36] and potassium (K) [37] were determined by using the Egner–Riehm (DL) method [38]. The method involves the extraction of phosphorus and potassium from the soil with a calcium lactate solution buffered to a pH of approximately 3.6. Available magnesium (Mg) was determined according to PN-R-04020 [39] by using the Schachtschabel [40] method, which involves extracting magnesium from the soil with a solution of 0.0125 M CaCl_2 and a soil-to-solution ratio of 1:10.

2.2.4. Activity of Enzymes

The activities of selected enzymes were determined on fresh-sieved (<2 mm) soils. The activity of dehydrogenases (DEH) was determined by using the Thalmann [41] method after incubating the sample with 2,3,5-triphenyltetrazolium chloride and measuring the absorbance of triphenylformazan (TPF) at 546 nm, as expressed in $\text{mg TPF kg}^{-1} 24 \text{ h}^{-1}$. Catalase (CAT) was determined via the method of Johnson and Temple [42] using 0.3% hydrogen peroxide solution as the substrate. The remaining H_2O_2 was determined by titration with 0.02 M KMnO_4 under acidic conditions. The activities of alkaline phosphatase (ALP) and acid phosphatase (AcP) were determined based on the detection of p-nitrophenol (pNP) released after incubation (37°C , 1 h) at pH ~6.5 for acid phosphatase and pH ~11.0 for alkaline [43]. The activity of proteases was determined by following the approach of Ladd and Butler [44], where the concentration of the amino acid tyrosine (Tyr) was determined in soil samples after incubation with sodium caseinate. The absorbance was measured with a spectrophotometer at $\lambda = 680 \text{ nm}$.

To assess the relationship between enzyme activity and other soil parameters, multi-parametric indices were calculated:

- The total enzyme activity index (TEI) [45]:

$$TEI = \sum \frac{X_i}{\bar{X}_i} \quad (4)$$

where X_i is the activity of soil enzyme $\bar{X}_i$ and is the mean activity of enzyme i in all samples;

- The geometric mean of enzyme activities (GMea) [46]:

$$GMea = \sqrt[5]{DEH \times CAT \times ALP \times AcP \times PRO} \quad (5)$$

2.3. Statistical Analyses

The determined physicochemical parameters (granulometric composition, pH, sorption properties, salinity, TOC and TN contents, fractional composition, macroelements and enzymatic activity) were subjected to two-way ANOVA. The results were expressed as the arithmetic mean $\pm$ standard deviation (SD). The normality of distribution of the parameters was subjected to the Shapiro–Wilk normality test. The first factor was FYM fertilisation (0 and 30 FYM), while the second was mineral N fertilisation (0, 40, 60 and 120 N). In order to assess the importance of experimental interaction factors, Tukey's post hoc test with a

95% confidence interval was performed to enable a comparison of the mean values of the analysed parameters ($p < 0.05$). The magnitude of impact that the experimental factors had on the studied parameters was determined by η^2 analysis, which describes the ratio of the variance of the dependent variable explained by the independent variable [47]. We also identified significant Pearson correlation coefficients between the examined parameters, which were calculated using the PAST 4.13 program [48]. The correlation results are presented in the form of a correlogram. Only statistically significant correlation results are shown.

3. Results and Discussion

3.1. Selected Physical and Chemical Properties of Soils

Grain size composition analysis determined that the soil samples were similar in grain size to one another. In the soils without manure fertilisation and those with, the sand fraction was found to have the largest share, whereas the clay fraction had the lowest share (Table 1). All tested soil samples were classified into one granulometric group, namely sandy loam [49]. In terms of heaviness for agricultural use, they were classified as medium soils [50]. The pH values of the tested soils ranged from 5.17 to 4.02 for soil without manure and from 5.63 to 4.44 in soil fertilised with manure (Table 1). In both cases, the reaction was acidic. Mineral and natural fertilisers used in agriculture exacerbate acidification, act indifferently or neutralise the soil environment [51]. Changes in soil reactions depend on its buffering properties, which largely determine the soil's resistance to the acidifying effects of anthropogenic factors [52].

Table 1. Selected physicochemical properties of soils.

Nitrogen (kg ha ⁻¹) II Factor	FYM (t ha ⁻¹) I Factor							
	0 FYM	30 FYM	0 FYM	30 FYM	0 FYM	30 FYM	0 FYM	30 FYM
	Sand (%)		Silt (%)		Clay (%)		pH KCl	
0 N	55.30 ±9.21	55.91 ±8.32	39.60 ±5.44	39.31 ±3.61	5.10 ±1.99	4.78 ±1.02	5.17 ±0.28	5.63 ±0.41
40 N	55.40 ±5.32	54.49 ±4.86	39.47 ±4.62	40.64 ±4.11	5.13 ±0.83	4.87 ±0.75	4.94 ±0.34	4.88 ±0.22
60 N	56.17 ±6.51	53.81 ±3.92	38.55 ±5.14	41.07 ±4.30	5.28 ±2.02	5.12 ±1.64	4.33 ±0.35	4.61 ±0.37
120 N	56.33 ±4.68	55.41 ±5.23	38.50 ±4.80	39.61 ±3.69	5.17 ±0.99	4.98 ±1.02	4.02 ±0.28	4.44 ±0.18

Hydrolytic acidity (Hh) was significantly determined by the studied factors and by their interaction. Both FYM and nitrogen fertilisation differentiated the amount of Hh (Table 2). Its highest value was recorded in soil without FYM and fertilised with nitrogen at a dose of 120 kg (2.74 cmol kg⁻¹). However, by far the lowest value was found in soil samples fertilised with 30 FYM and 0 N, at 1.46 cmol kg⁻¹. N fertilisation affected Hh similarly to soil pH. The η^2 analysis showed that the N dose had the greatest impact on the Hh value (η^2 89.86%). Commercial nitrogen fertilisers used in crop production increase acidity by oxidising NH₄⁺ to NO⁻, which generates H⁺ and lowers soil pH [53–56]. Interactions between all doses of nitrogen fertilisation without FYM fertilisation showed that the considerably highest Hh was recorded for the 120 N dose, which was slightly higher for the 60 N dose and by far the lowest for the remaining doses. For fertilisation with 30 FYM, it was found that the Hh value was highest for the two highest nitrogen doses, amounting to 2.53 cmol kg⁻¹ for 120 N and 2.34 cmol kg⁻¹ for 60 N, whereas it was lowest at 0 N (1.46 cmol kg⁻¹). One of the main causes of soil acidity is the nitrification process [57], so many researchers have shown that long-term application of nitrogen fertilisers lowers the soil pH [55,56,58].

Table 2. Selected chemical parameters of soils.

Nitrogen (kg ha ^{−1}) II Factor	FYM (t ha ^{−1}) I Factor														
	0 FYM	30 FYM	Mean	0 FYM	30 FYM	Mean	0 FYM	30 FYM	Mean	0 FYM	30 FYM	Mean	0 FYM	30 FYM	Mean
	Hh (cmol kg ^{−1})			TEB (cmol kg ^{−1})			CEC (cmol kg ^{−1})			BS (%)			EC (μS cm ^{−1})		
0 N	1.87cA ±0.01	1.46cB ±0.22	1.67 <i>d</i>	47.70 * ±0.4	45.85 ±0.1	46.78 <i>c</i>	49.57cA ±0.40	47.35cB ±0.45	48.46 <i>c</i>	96.23aA ±0.03	96.98aA ±0.21	96.60 <i>a</i>	657.2bcA ±26.91	751.2 aA ±34.05	704.2 <i>ab</i>
40 N	1.94cA ±0.03	1.93bA ±0.11	1.93 <i>c</i>	48.10 ±1.8	47.85 ±0.1	47.98 <i>b</i>	50.51bA ±0.72	49.87bA ±0.30	50.19 <i>b</i>	96.72aB ±0.03	95.95bB ±0.59	96.33 <i>a</i>	961.3aB ±84.86	764.3 aB ±30.40	862.8 <i>a</i>
60 N	2.46bA ±0.11	2.34aA ±0.03	2.40 <i>b</i>	49.20 ±1.4	48.20 ±1.6	48.70 <i>ab</i>	52.44aA ±0.23	50.54abB ±0.57	51.49 <i>a</i>	95.17bcC ±0.04	95.69bC ±0.52	95.43 <i>b</i>	576.3cC ±63.80	565.1 aC ±47.58	570.7 <i>b</i>
120 N	2.74aA ±0.23	2.53aB ±0.04	2.63 <i>a</i>	49.75 ±0.1	48.80 ±0.4	49.28 <i>a</i>	52.49aA ±0.33	50.90aB ±0.40	51.72 <i>a</i>	94.79cD ±0.41	95.03cD ±0.04	94.91 <i>c</i>	517.5 cD ±35.10	777.9 aD ±28.24	647.7 <i>b</i>
Mean	2.25 <i>a</i> *	2.06 <i>b</i>		48.69 <i>a</i>	47.66 <i>a</i>		51.25 <i>a</i>	49.68 <i>a</i>		95.72 <i>a</i>	95.91 <i>a</i>		678.1 <i>a</i>	714.6 <i>a</i>	
η ² for FYM; N η ² for interaction; error	5.36%; 89.86%; 3.32%; 1.35%			20.32%; 69.10%; 6.38%; 3.46%			21.76%; 72.50%; 3.88%; 1.01%			1.49%; 79.34%; 14.42%; 2.02%			1.50%; 51.70%; 31.04%; 12.61%		

Hh—hydrolytic acidity (cmol kg^{−1}); TEB—total exchangeable bases (cmol(+) kg^{−1}); CEC—cation exchange capacity (cmol(+) kg^{−1}); BS—basic saturation (%); a—Different small letters indicate a comparison with mineral N fertilisation (within the same FYM fertilisation) at *p* < 0.05; A—Different capital letters indicate a comparison with FYM fertilisation (within the same mineral N fertilisation) at *p* < 0.05; *a*—Different small italic letters indicate significant differences among experience factors (I factor—FYM fertilisation: 0 and 30 FYM t ha^{−1}; II factor—mineral N fertilisation: 0, 40, 60 and 120 kgN ha^{−1}); ±Standard deviation. *—No letter markings—no significant interactions.

The soil sorption complex is an essential pool of nutrients for plants. Neither total exchangeable base cations (TEB), nor cation exchange capacity (CEC), nor the degree of base saturation of the sorption complex (BS) were differentiated by manure doses, but their differences were influenced by nitrogen fertilisation. TEB and CEC values were highest for the two highest nitrogen doses (Table 2). A lack of nitrogen fertilisation significantly reduced these parameter values. However, the BS values were different. A lack of nitrogen fertilisation and its lowest dose both considerably increased BS, which was by far the highest for 0 N (96.60%) and for 40 N (96.33%). The interactions for the degree of alkali saturation of the soil complex between all doses of nitrogen and manure showed clearly the highest value for fertilisation with 30 FYM and 0 N. The η^2 analysis showed that the values of TEB, CEC and BS were most influenced by the N dose (the TEB, CEC value and the degree of BS of the sorption complex, respectively: 69.10%, 72.50% and 79.34%). The results of our research did not agree with those of other authors [54,56]. Long-term use of manure with mineral fertilisers may contribute to reducing hydrolytic acidity and increasing the total bases and sorption capacity in soils [59]. Simultaneous long-term use of organic and mineral fertilisers prevents sudden changes in the chemical properties of the soil [10].

According to the FAO [60], an EC value in the range of 800–1600 $\mu\text{S cm}^{-1}$ indicates high salinity, at which only plants resistant to very high levels of salinity can obtain high yields. According to Li et al. [61], the judicious irrigation and fertilisation of plants can control soil salinity and increase crop yields. The salinity of the analysed soils ranged from 517.5 to 961.3 $\mu\text{S cm}^{-1}$. The variations in this parameter were influenced by nitrogen doses, while manure fertilisation did not differentiate salinity (Table 2). This was also confirmed by η^2 analysis. The literature states that in agricultural practice, soil salinisation is usually caused by the use of excessively high doses of mineral fertilisers [62], while in our own research, the highest salinity was recorded with no nitrogen fertilisation and with a 40 kg dose.

3.2. Properties of Organic Matter

The main factors determining carbon sequestration in agricultural soils are fertilisation and rotation. The TOC content (Table 3) after 40 years of the experiment was higher in plots with manure compared to mineral fertilisation only (except for the 120 N plus 30 FYM variant). The nitrogen (TN) content in plots without manure was on average 0.95 g kg⁻¹, which was similar to TN in soil samples taken from plots fertilised with manure (1.05 g kg⁻¹). The determined TOC and TN contents were used to calculate the value of the TOC/TN ratio, which was highest for the soil samples of the variant with manure but the addition of N.

Table 3. Contents (g kg⁻¹) of total organic carbon (TOC) and total nitrogen (TN).

Nitrogen (kg ha ⁻¹) II Factor	FYM (t ha ⁻¹) I Factor								
	0 FYM	30 FYM	Mean	0 FYM	30 FYM	Mean	0 FYM	30 FYM	Mean
	TOC			TN			TOC/TN		
0 N	6.59bB ± 0.10	9.13aA ± 0.16	7.86a	0.91 * ± 0.02	0.99 ± 0.03	0.95b	7.24abB	9.22aA	8.23a
40 N	6.53bB ± 0.11	8.62abA ± 0.26	7.58a	0.98 ± 0.02	1.06 ± 0.02	1.02a	6.65bB	8.19bA	7.42b
60 N	7.33aB ± 0.20	8.31abA ± 0.31	7.82a	0.94 ± 0.03	1.08 ± 0.01	1.01ab	7.78aA	7.75bA	7.76ab
120 N	7.55aA ± 0.22	7.89bA ± 0.25	7.72a	0.94 ± 0.02	1.08 ± 0.01	1.01ab	8.01aA	7.33bcB	7.67b
Mean	7.00b	8.45a		0.95a	1.05a		7.42b	8.12a	
η^2 for FYM; N	59.00%; 1.27%			31.05%; 8.22%			19.49%; 13.60%		
η^2 for interaction;	20.32%;			2.28%;			46.90%;		
η^2 for error	19.44%			58.44%			20.00%		

Symbols: a, A, a, *, ± see Table 2.

According to literature reports [2,23,24,26,63–66], the impact of nitrogen and manure fertilisation on the DOC content may differ in nature, causing it to increase, decrease or have no effect. In this study, an increase in the DOC content was achieved for the 60 N and 120 N variants, and the DOC share increased for the 120 N variant. The DOC content

was considerably higher in soil samples collected in plots with manure—by an average of 21% compared to soils without manure. The DOC content correlated significantly positively with the TOC and TN contents (Figure 1). Importantly, the DOC content did not significantly correlate with the TOC and TN contents. This indicates that the share of this fraction of organic matter is relatively stable.

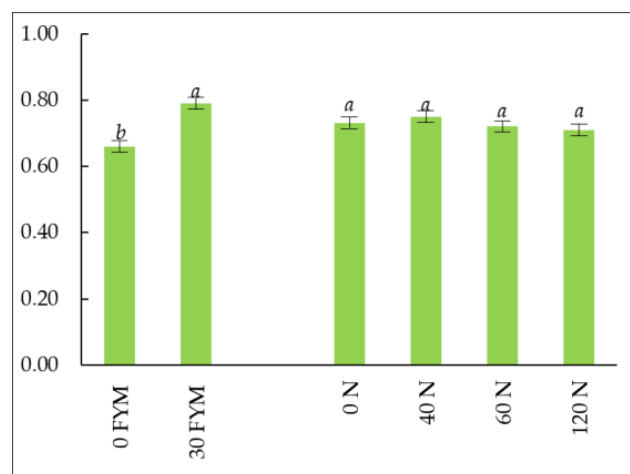


Figure 1. Values CHAs/CFAs ratio and average for I factor (FYM: 0, 30 t ha^{−1}) and II factor (N dose: 0, 40, 60, 120 kg ha^{−1}). CHAs—carbon of the fraction of humic acids, CFAs—carbon of the fraction of fulvic acids.

The quality of organic matter (OM) is determined not only by the content and share of DOC but also by the content and share of more decomposition-resistant organic matter fractions, such as humic acids, fulvic acids or humins. The contents of distinguished organic matter fractions are presented in Table 4. The process of organic matter fractionation is preceded by decalcification of soil samples (the activity of hydrochloric acid on the soil). This process also releases a small part of the organic matter, which includes simple organic compounds (the fraction marked as “Cd”, Table 5). The carbon content in the solutions after decalcification was not determined by manure fertilisation, but the highest dose of N increased the Cd content compared to the other variants. The carbon content of the humic acid fraction was strongly influenced by factor I (manure fertilisation; η^2 78.11%). In plots with manure added, the share of this carbon fraction was on average 37% higher than in plots without manure. The experimental factors (fertilisation with manure, nitrogen dose) did not significantly change the content of the fulvic acid fraction. Changes in the content of CHAs and CFAs determined changes in the CHA/CFA ratio. The CHA/CFA ratio is an indicator of soil quality and reflects the direction of organic matter transformation in the soil. According to the literature [30,67,68], humus with higher CHA/CFA ratio values has a greater degree of humification. Low CHA/CFA values are a consequence of a high CFA content and may indicate high microbiological activity in the soil [30]. As the data presented in Table 5 show, the factor determining the value of this ratio was manure fertilisation. The soil fertilised with manure was characterised by significantly higher CHA/CFA ratio values than the soil without manure added. At the same time, it should be emphasised that regardless of the type of fertilisation, the CHA/CFA ratio values were decidedly lower than one, which of course indicates that the fulvic acid fraction content was considerably greater than that of humic acids.

In determining the quality of organic matter, it is important to determine the share of individual fractions in the TOC pool. The Cd share ranged from 1.18 to 1.71; the share of the humic acid fraction ranged from 16.86 to 21.69%; and the share of the fulvic acid fraction was higher and ranged from 23.57 to 31.29%. Without a doubt, the dominant share was the post-harvest residue called the humin fraction (Figure 1). The factor influencing the share of humic and fulvic acid fractions was manure fertilisation. Manure fertilisation increased the

share of the CHA fraction and decreased CFAs. The fraction most resistant to decomposition of organic matter is that of humins, and their participation is important in the process of carbon sequestration [69]. The proportion of Ch was lower in plots fertilised with manure than those without manure. However, the use of nitrogen fertilisation increased the share of Ch. As can be seen from the presented correlation relationships (Figure 2), not only were the contents of humic acids and fulvic acids correlated significantly with the TOC content, but so too were the shares of their fractions; the share of CHAs positively and CFAs negatively impacted.

Table 4. Content of dissolved organic carbon (DOC).

Nitrogen (kg ha ^{−1}) II Factor	FYM (t ha ^{−1}) I Factor					
	0 FYM	30 FYM	Mean	0 FYM	30 FYM	Mean
	DOC (mg kg ^{−1})			DOC (%)		
0 N	75.7bB ± 5.0	90.3bA ± 5.2	83.0c	1.15abA ± 0.06	0.99bB ± 0.05	1.07c
40 N	76.3bB ± 4.9	92.4bA ± 5.8	84.3c	1.17abA ± 0.05	1.08bA ± 0.04	1.12bc
60 N	80.5bB ± 5.1	105.3aA ± 4.6	92.9b	1.10bcB ± 0.08	1.27aA ± 0.05	1.18b
120 N	93.5aB ± 3.8	105.5aA ± 5.0	99.5a	1.24aA ± 0.04	1.34aA ± 0.05	1.29a
Mean	81.5b	98.4a		1.17a	1.17a	
η^2 for FYM; N	56.25%; 35.37%			0.00%; 49.04%		
η^2 for interaction; error	4.55%; 3.84%			35.03%; 15.92%		

Symbols: a, A, a, ± see Table 2.

Table 5. Content (mg kg^{−1}) of carbon in the humus fraction.

Nitrogen (kg ha ^{−1}) II Factor	FYM (t ha ^{−1}) I Factor								
	0 FYM	30 FYM	Mean	0 FYM	30 FYM	Mean	0 FYM	30 FYM	Mean
	Cd			CHAs			CFAs		
0 N	106 ± 3	110 ± 8	108b	1175bB ± 40	1955A ± 45	1565a	1840 ± 45	2437 ± 45	2138a
40 N	112 ± 5	102 ± 5	107b	1413aB ± 45	1641A ± 40	1527a	2041 ± 55	2025 ± 60	2033a
60 N	106 ± 4	115 ± 6	110b	1277aB ± 25	1735A ± 45	1506a	1901 ± 54	2267 ± 57	2084a
120 N	122 ± 4	126 ± 7	124a	1273aB ± 28	1712A ± 47	1493a	1970 ± 32	2211 ± 58	2091a
Mean	111a	113a		1285b	1761a		1938a	2235a	
η^2 for FYM; N	0.64%; 49.71%			78.11%; 1.04%			29.46%; 1.87%		
η^2 for interaction; error	12.20%; 37.46%			13.40%; 7.45%			16.33%; 52.34%		

Cd—carbon in solutions after decalcification, CHAs—carbon of the fraction of humic acids, CFAs—carbon of the fraction of fulvic acids; symbols: a, A, a, *, ± see Table 2.

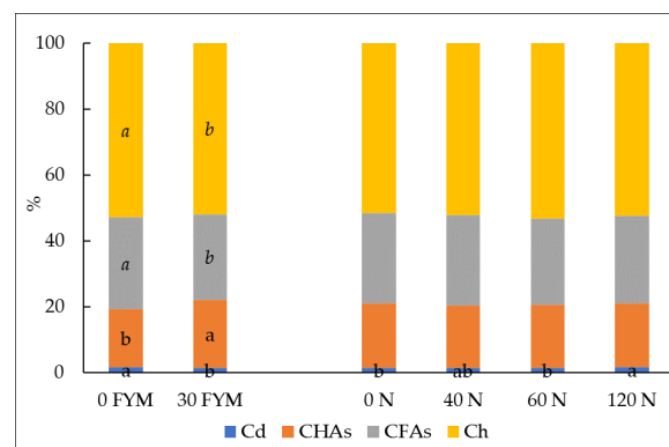


Figure 2. Average share of carbon in organic matter fractions for I factor (FYM: 0, 30 t ha^{−1}) and II factor (N dose: 0, 40, 60, 120 kg ha^{−1}). Cd—carbon in solutions after decalcification, CHAs—carbon of the fraction of humic acids, CFAs—carbon of the fraction of fulvic acids, Ch—carbon of the humin fraction.

Important information on the transformation of organic matter in the soils of the long-term field experiment was provided by the calculated carbon sequestration rate (CSR)

values. CSR values (Figure 3) were calculated for the entire experimental period and for one year. The results indicated that the applied cultivation methods did not result in the depletion of organic matter, provided that the fertiliser plan included manure. Fertilisation without manure requires the use of high doses of nitrogen. Even for the 60 and 120 N variants, lower CSR values were obtained compared to the variants on which FYM was used. The results clearly showed that manure fertilisation promotes carbon sequestration. According to Simansky [15], Tang et al. [16] and Hayatu et al. [35], only combined mineral and organic fertilisation can increase the TOC content. The results presented in this work partially agree with the research of the authors cited above, because for high nitrogen doses (variant 30 FYM 120 N), the TOC content decreased (i.e., CSR values were lower). Nitrogen fertilisation, on the one hand, simultaneously increases the yield and the mass of post-harvest residues left in the soil, and, on the other hand, increases the mineralisation processes of fresh organic matter (post-harvest residues, manure); this can lead to a decrease in the organic matter content compared to the variants fertilised with manure and lower doses of N [70].

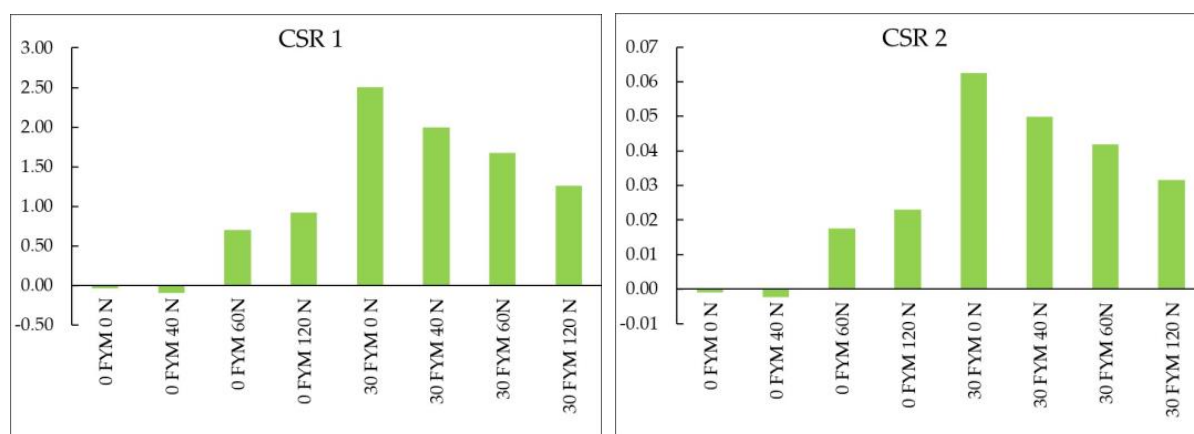


Figure 3. Soil organic carbon sequestration rates (CSR) for the one-year CSR-1 and the 40-year CSR-2.

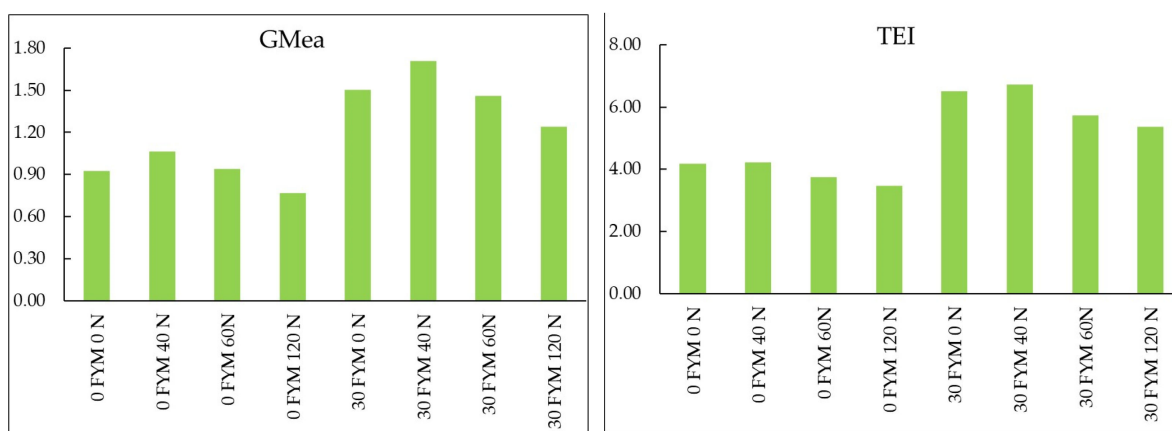
3.3. Content of Available Macroelements in Soil

Long-term fertilisation caused significant changes in the circulation of available macroelements (Table 6). Phosphorus (P) is one of the basic ingredients determining yields, alongside nitrogen and potassium. It takes part in all life processes occurring in a plant [70]. FYM and N fertilisation significantly influenced the content of available P in soil. The highest significant P content was found in the soil fertilised with 30 FYM and 20 N (58.14 mg kg^{-1}). According to PN-R-04023 [36], this soil belonged to the medium (III) class of P content. Lack of FYM fertilisation and N doses above 40 kg ha^{-1} greatly reduced the available P content in soil, which was lowest (35.66 mg kg^{-1}) in soil samples from plots fertilised with 0 FYM, 120 N. This soil can be classified as class IV—low P content. According to Wang et al. [71], long-term use of organic and mineral N fertilisation promoted a loss of P content from the topsoil, along with enrichment of P in the subsoil. Correlation analysis showed a positive and significant relationship between the TOC content and P ($r = 0.544$; $p \leq 0.05$) (Figure 4). Organic matter takes part in the chelation of cations, which limit the absorption of P. This increases the solubility of P compounds. According to Yang et al. [72], the addition of organic matter can increase phosphorus availability by reducing the P adsorption strength and maximum phosphate buffering capacity. According to Goldan et al. [22], some N and P supplied in amounts exceeding the requirements of the plant may pollute surface and groundwater.

Table 6. Contents of available macroelements in soil.

Nitrogen (kg ha ⁻¹) II Factor	FYM (t ha ⁻¹) I Factor								
	0 FYM	30 FYM	Mean	0 FYM	30 FYM	Mean	0 FYM	30 FYM	Mean
	P			K			Mg		
0 N	48.65aB ± 0.264	63.57bA ± 0.587	56.11b	58.40abB ± 0.748	65.44bcA ± 0.827	61.92b	40.29abB ± 0.582	46.01bA ± 0.448	43.15b
40 N	51.69aB ± 0.304	68.14aA ± 0.608	59.92a	60.79aB ± 0.834	72.95aA ± 0.869	66.87a	42.62aB ± 0.597	49.38aA ± 0.502	45.99a
60 N	43.82bB ± 0.256	51.59cA ± 0.782	47.71c	56.05bB ± 0.627	66.96bA ± 0.751	61.51b	38.99bA ± 0.429	50.77aA ± 0.511	44.88ab
120 N	35.66cB ± 0.185	40.52dA ± 0.439	38.09d	54.00bB ± 0.615	63.33cA ± 0.722	58.67c	37.21cB ± 0.448	41.75cA ± 0.395	39.48c
Mean	44.96b	55.96a		57.31b	67.17a		39.77b	46.97a	
η^2 for FYM; N	27.99%; 66.77%			71.08%; 25.47%			61.06%; 28.74%		
η^2 for interaction; error	4.72%; 0.478%			2.677%; 0.760%			8.97%; 1.12%		

P—available phosphorus (mg kg⁻¹); K—available potassium (mg kg⁻¹); Mg—available magnesium (mg kg⁻¹); symbols: a, A, a, *, ± see Table 2.

**Figure 4.** Index of soil enzymes: GMea and TEI. GMea—the geometric mean of enzyme activities; TEI—total enzyme activity index.

Potassium (K) is required for the activation of over 80 different enzymes. The K content in natural soils is higher than that of other nutrients, but most of the K is not absorbable by plants [73]. The highest content of available K (72.95 mg kg⁻¹) was obtained in the soil from plots fertilised with 30 FYM, 40 N. This soil belonged to the low (IV) K-content class [37]. It was 25% above the K content in the soil from the 0 FYM, 0 N treatment. There were no statistically significant differences in K content between soil samples fertilised with 30 FYM, 0 N (65.44 mg kg⁻¹) and 30 FYM, 40 N (66.96 mg kg⁻¹) (Table 6).

Magnesium helps maintain the structure of lumpy soil, prevents silting during heavy rainfall, supports water storage and deacidifies the soil better than calcium [74]. The highest content of available Mg was found in the soil from the 30 FYM, 40 N (49.38 mg kg⁻¹) and 30 FYM, 80 N (50.77 mg kg⁻¹) plots. No significant differences in Mg content were found between these plots. According to PN-R-04020 [39], these soils belongs to a high (II) Mg content class. In 0 FYM, 0 N soil samples, the Mg content was by far the lowest (40.29 mg kg⁻¹). These soils are described as medium (class III) rich in Mg. The η^2 analysis showed that the FYM dose had the greatest impact on the K and Mg content (η^2 values, respectively: 71.08% and 61.06%). In contrast, the P content was mainly influenced by N fertilisation (η^2 66.77%) (Table 6). The results of Nong et al. [75] showed that a reduction in mineral nitrogen fertiliser resulted in a decrease in the content of available P and K. In our own research, for available P, K and Mg, it was found that applying manure at a dose of 30 t ha⁻¹ mitigated the negative effects of the application of N at a dose of 120 kg ha⁻¹. Similar relationships were obtained in research by Menšík et al. [17] and Dębska et al. [31]. The authors believe that the basic strategy for proper N management in soil is to introduce organic fertilisers. This prevents acidification and maintains high soil productivity [76].

3.4. Soil Enzyme Activity

The analysis of enzymatic data showed statistically significant differences in the activity of selected redox and hydrolytic enzymes both between FYM fertilisation levels and between mineral N fertilisation levels (Tables 7 and 8). According to Sawicka et al. [9], enzymatic process dynamics in soil depend on the enzyme type. This is because each enzyme has its own specific sensitivity to environmental factors and the content of substrates. DEH activity was the highest (with a significant difference) after the application of 30 FYM (average $1.025 \text{ mg TPF kg}^{-1} 24 \text{ h}^{-1}$) and 0 N (average $1.091 \text{ mg TPF kg}^{-1} 24 \text{ h}^{-1}$). An increase in the N dose resulted in a decrease in DEH activity. Similar results were found for CAT. No significant interaction was found between the applied fertilisation factors in the case of DEH. CAT activity was much the highest in the soil after the application of 30 FYM and 0 N ($0.624 \text{ mg H}_2\text{O}_2 \text{ kg}^{-1} \text{ h}^{-1}$). The analysis of the η^2 coefficient showed that it was mainly N fertilisation that explained the variability in DEH activity (49.94%) and CAT (73.73%). However, FYM fertilisation determined 48.95% of the variability in DEH and 23.59% of the variability in CAT (Table 7). DEH and CAT are enzymes responsible for oxidative processes related to the carbon cycle [77]. These authors found that the CAT activity in soil is related to mineral fertilisation. Nutrient-poor soils do not show an absolute value of catalase activity, in contrast to fertile soils, which do. Bungau et al. [78] reported that, as the N dose increases, the biological activity of the soil increases—as does the pH value. The highest DEH activity was found in the control plot (0 N).

Table 7. The activity of dehydrogenases (DEH) and catalase (CAT)—oxidoreductive enzymes.

Nitrogen (kg ha^{-1}) II Factor	FYM (t ha^{-1}) I Factor					
	0 FYM	30 FYM	Mean	0 FYM	30 FYM	Mean
	DEH			CAT		
0 N	0.924 ± 0.030	1.258 ± 0.017	1.091a	$0.419aB \pm 0.031$	$0.624aA \pm 0.041$	0.522a
40 N	0.772 ± 0.027	1.092 ± 0.006	0.932b	$0.387bB \pm 0.028$	$0.594aA \pm 0.038$	0.491b
60 N	0.523 ± 0.041	0.881 ± 0.031	0.702c	$0.280cB \pm 0.019$	$0.428bA \pm 0.034$	0.354c
120 N	0.508 ± 0.022	0.869 ± 0.023	0.689c	$0.119dB \pm 0.014$	$0.209cA \pm 0.029$	0.164d
Mean	0.682b	1.025a		0.419b	0.624a	
η^2 for FYM; N	48.95%; 49.94%			23.59%; 73.70%		
η^2 for interaction; error	0.554%; 0.554%			2.40%; 0.31%		

DEH—dehydrogenases ($\text{mg TPF kg}^{-1} 24 \text{ h}^{-1}$); CAT—catalase ($\text{mg H}_2\text{O}_2 \text{ kg}^{-1} \text{ h}^{-1}$); symbols: a, A, a, $\pm$ see Table 2.

Table 8. The activity of alkaline (AIP) and acid (AcP) phosphatase and protease (PRO)—hydrolytic enzymes.

Nitrogen (kg ha^{-1}) II Factor	FYM (t ha^{-1}) I Factor								
	0 FYM	30 FYM	Mean	0 FYM	30 FYM	Mean	0 FYM	30 FYM	Mean
	AIP			AcP			PRO		
0 N	$0.599aB \pm 0.071$	$0.895aA \pm 0.082$	0.747a	$0.501dB \pm 0.035$	$1.132dA \pm 0.085$	0.817c	$5.8dB \pm 0.121$	$9.70dA \pm 0.259$	7.75d
40 N	$0.428bB \pm 0.065$	$0.722bA \pm 0.059$	0.575b	$0.583cB \pm 0.021$	$1.422bA \pm 0.081$	1.003b	$18.2cB \pm 0.181$	$21.70bA \pm 0.425$	19.95c
60 N	$0.307cB \pm 0.035$	$0.472cA \pm 0.051$	0.390c	$0.711bB \pm 0.025$	$1.318cA \pm 0.071$	1.015b	$23.1bB \pm 0.358$	$28.21bA \pm 0.483$	25.65b
120 N	$0.173dB \pm 0.018$	$0.286dA \pm 0.021$	0.230d	$0.853aB \pm 0.058$	$1.572aA \pm 0.069$	1.213a	$29.4aB \pm 0.745$	$35.72aA \pm 0.688$	32.55a
Mean	0.377b	0.594a		0.662b	1.361a		19.13b	23.83a	
η^2 for FYM; N	23.00%; 47.00%;			85.27%; 13.35%			5.99%; 93.59%;		
η^2 for interaction; error	2.80%; 0.20%			1.35%; 0.03%			0.39%; 0.024%		

AIP—alkaline phosphatase ($\text{mM pNP kg}^{-1} \text{ h}^{-1}$); AcP—acid phosphatase ($\text{mM pNP kg}^{-1} \text{ h}^{-1}$); PRO—protease ($\text{mg TYR kg}^{-1} \text{ h}^{-1}$); symbols: a, A, a, $\pm$ see Table 2.

The activity of AIP and AcP, which are enzymes responsible for the hydrolysis of organic phosphorus compounds in the soil, varied depending on the N dose and FYM fertilisation (Table 8). By far the highest AIP activity was obtained in the soil from the site without N fertilisation (average: $0.747 \text{ mM pNP kg}^{-1} \text{ h}^{-1}$). Increasing doses of N resulted in a decrease in AIP activity, while AcP activity increased. The highest AcP activity

was obtained in soil samples from 120 N plots (average $1.572 \text{ mM pNP kg}^{-1} \text{ h}^{-1}$). The ammonium nitrate used (ammonium form NH_4^+) has a physiological acidifying effect. Phosphatases are among the enzymes most sensitive to changes in soil pH [79]. No significant differences in AcP were found after the application of 40 N and 60 N. With the application of 30 FYM, the activity of both phosphatases increased compared to the control (0 FYM) by, respectively, 57% and 105%. In the case of ALP activity, N fertilisation determined its variability to a greater extent (η^2 47.00%) compared to AcP (η^2 13.35%). ALP activity was lower than AcP. This was related to the acidic reaction of the soil [80]. PRO is an enzyme that hydrolyses peptide bonds (CO-NH) to polypeptides and amino acids [81]. By far the highest PRO activity was found in soil after the application of 120 N (average $32.55 \text{ mg TYR kg}^{-1} \text{ h}^{-1}$). The η^2 coefficient indicated that N fertilisation explained 93.59% of the variability in PRO activity. According to Geisseler et al. [4], differences in N concentrations in the soil had no direct effect on PRO activity. The application of organic additives activated soil enzymes, but increasing doses of mineral N reduced the activity of enzymes involved in N biogeochemistry [77]. For other enzymes, these relationships were not observed. The use of cattle manure reduced the negative effect of mineral fertilisers on soil acidification [82] and thus changed the activity of soil enzymes. Multiple regression analysis of the results of Padhan et al. [83] showed that the TOC and protease activity are key regulators of nitrogen (N) mineralisation. According to Gianfreda and Ruggiero [84], the absence of trends in changes in the activity of soil enzymes in response to N fertilisation is unsurprising. The effect of inorganic fertilisers on enzymatic activity may vary depending on the method and system of cultivation, time, physical and chemical properties of soil and composition and amount of fertiliser used. Nong et al. [75] found that reducing a single N mineral fertiliser to 80% had no significant effect on CAT activity. However, it did result in a reduction in AcP activity. Supplementation with organic fertilisers (pigeon droppings) increased OM and thus CAT activity.

According to Picariello et al. [85] and Wojewódzki et al. [86], results regarding the activity of individual enzymes are important, but they are insufficient due to the many functions that the soil performs. Therefore, it is recommended to use multiparametric indices that are less sensitive to spatial and seasonal changes [87,88]. Two enzymatic indicators were used in this research: the total enzyme activity index (TEI) and the geometric mean of enzyme activities (GMea) (Figure 4). The calculated GMea shows that applying manure increased the enzymatic activity of the soil. A similar relationship was demonstrated by Hayatu et al. [35]. The GMea values calculated by these authors were statistically higher after the use of organic fertilisers than for the application of mineral fertilisers alone. This was probably related to the greater availability of SOM, N. The GMea value was highest for the application of 30 FYM, 40 N (1.71). The fact that GMea values were lower for soils without the addition of FYM and with the addition of higher doses of N (60 and 120 kg ha^{-1}) suggests that this fertilisation combination has a much smaller impact on the biological parameters of soil. The TEI values obtained from individual fertiliser combinations ranged from 3.48 (0 FYM, 120 N) to 6.74 (30 FYM, 40 N). The integrated TEI index allows for a simple comparison of total enzyme activity with soil quality [89].

The enzymatic activity of soil depends largely on its physical, chemical and biological properties. The activity of the tested enzymes was negatively correlated with clay. The correlation coefficients between the activity of DEH, CAT, ALP and AcP and clay were $r = -0.915$, $r = -0.693$, $r = -0.778$, and $r = -0.580$, respectively; $p \leq 0.05$. Clay minerals can act as effective enzyme blockers due to their ability to interact based on the surface charge, cation exchange and their provision of a precise surface [90]. The decrease in enzymatic activity observed in soil can be attributed to the presence of clay minerals, which tend to bind to organic compounds. The appearance of enzyme–clay clusters can potentially modify the entire spectrum of enzymatic activity [91]. These results are consistent with Olagoke et al. [92], who observed a decrease in potential enzyme activity with an increase in the amount of clay fraction.

Soil reactions affect a number of chemical and biochemical processes occurring in the soil [93]. Values of pH were found to correlate significantly positively between pH and DEH activity ($r = 0.853$; $p \leq 0.05$), CAT ($r = 0.857$; $p \leq 0.05$) and ALP ($r = 0.919$; $p \leq 0.05$) and significantly negatively with PRO ($r = -0.816$; $p \leq 0.05$). The pH of the soil solution affects the conformation of the enzyme, its adsorption to solid surfaces and the ionisation and solubility of substrates and cofactors [94]. Therefore, even small changes in pH can significantly change enzyme activity. The enzymes most sensitive to soil pH changes are phosphatases [93]. Soil pH affects the activity of enzymes due to the pH sensitivity of amino acid functional groups, which cause conformational and chemical changes in amino acids necessary for binding and catalysis. The pH value can also influence enzyme activity by influencing the concentration of inhibitors or activators in the soil solution and the effective concentration of the substrate [95]. However, Parham et al. [96] found that the activities of alkaline phosphatase, pyrophosphatase and phosphodiesterase were higher not only due to higher soil pH values but also due to the increased abundance and diversity of microorganisms as a result of the application of manure over the course of years.

Correlation analysis determined significant positive relationships between the TOC content and the activity of DEH ($r = 0.604$; $p \leq 0.05$), CAT ($r = 0.495$; $p \leq 0.05$), ALP ($r = 0.516$; $p \leq 0.05$) and AcP ($r = 0.776$; $p \leq 0.05$) (Figure 5). Similar relationships were obtained by Jaskulska et al. [6]. The value of the coefficient (R^2) showed that only 36.5% of the variability in DEH, 24.5% for CAT, 26.6% for ALP were related to the TOC content in soil. According to Hok et al. [97], soil organic carbon is both a source of enzymes and a substrate for the degradation of enzymes. Thus, it influences their activity. Usually, a higher TOC content increases the rate of organic matter mineralisation by soil microorganisms, which results in an increase in enzymatic activity [98]. Correlation analysis showed that only AcP and PRO were significantly positively related to TN ($r = 0.909$ and $r = 0.568$, respectively; $p \leq 0.05$). A meta-analysis by Jian et al. [99] showed that N fertilisation significantly increased the activity of hydrolytic enzymes related to the C, N and P cycle (β -d-cellobiosidase, acid phosphatase, β -1,4-xylosidase, β -1,4-glucosidase, α -1, 4-glucosidase, urease). However, the inhibition of oxidation enzymes (phenol oxidase and peroxidase) was noted. The authors suggest that adding N to soil stimulates microorganisms that produce a greater amount of enzymes that are associated with the hydrolytic acquisition of C. As reported by Li et al. [100], statistical analysis showed that NT and C were the main predictors of ALP activity.

A meta-analysis of 64 studies that was conducted by Chen et al. [98] reported that nitrogen had a negligible effect on protease activity. Our own research shows that the content of C and N from exogenously applied fertiliser is responsible for the activity of DEH, CAT, ALP, AcP and PRO. The application of an organic additive in the form of manure enriches the soil with lignin, cellulose and protein. The degradation of these compounds requires extracellular enzymes that catalyse the rate-limiting TOC- and N-mineralisation stage [99]. The enzymatic activity and chemical composition of exogenous OM, including the TOC/TN ratio, are factors determining the intensity of the mineralisation and humification process. As Figure 5 shows, DEH, CAT, ALP, AcP and the biological activity indicators GMea and TEI correlated significantly positively with the CHA content at, respectively, $r = 0.730$, $r = 0.540$, $r = 0.540$, $r = 0.775$, $r = 0.836$ and $r = 0.877$. Importantly, the content of CHAs correlated with the contents of TOC, TN, P and K. CHAs, GMea and TEI indices were also found to significantly positively correlate with values of the CHA/CFA ratio. GMea and TEI were both found to correlate particularly highly with CHAs/CFAs at, respectively, 0.940 and 0.950. The above relationships clearly indicate that GMea and TEI and CHAs/CFAs can be classified as indicators of soil fertility, and GMea and TEI, like CHAs/CFAs, can be considered indicators of the degree of humification of organic matter. GMea and TEI correlated with CFAs similarly to how they did with DEH and AcP. Therefore, DEH and AcP can be considered enzymes that take part in the transformation of OM, leading to the formation of FAs [6]. A significant correlation was found between the content of available P in the soil and the activity of DEH ($r = 0.831$; $p \leq 0.05$), CAT

($r = 0.972$; $p \leq 0.05$) and AIP ($r = 0.913$; $p \leq 0.05$). Usually, the activity of phosphatases is influenced by the P content, which depends on the macroelement bioavailability to plants [93]. This probably indicates a close relationship with the development phase of plants, the climate and soil properties [100]. Soils with low and medium mineral P contents usually have a higher production of phosphatases. This means that the rate of enzymatic release of phosphates from organic compounds is determined by the end product of several chemical reactions. Also, research by Li et al. [100] showed that the activity of AIP, unlike AcP, explained the amount and dynamics of the content of available P in the soil. This suggests a different role of AIP in regulating P availability in soil long-term fertilised with FYM and N.

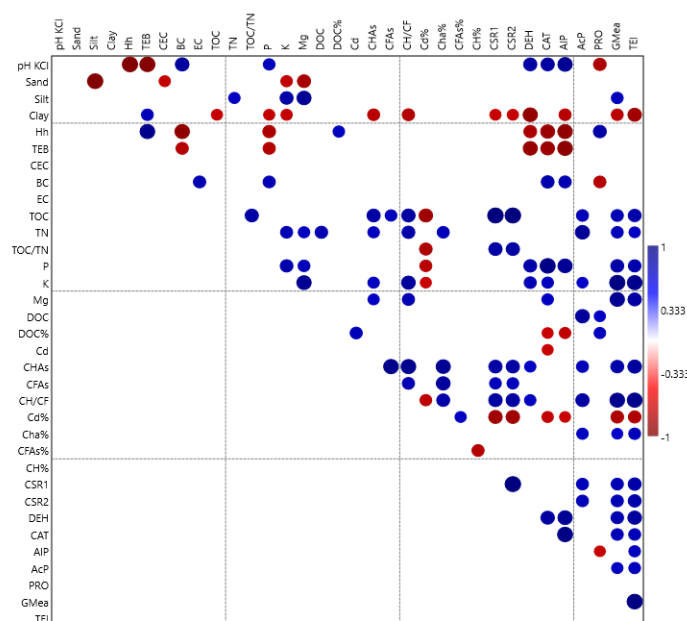


Figure 5. Correlogram of the physicochemical and organic matter variables and the activity of enzymes in soil, showing significant correlations. Abbreviations: Hh—hydrolytic acidity; TEB—total exchangeable bases; CEC—cation exchange capacity; BS—base saturation; TOC—total organic carbon; TN—total nitrogen; P—available phosphorus; K—available potassium; Mg—available magnesium; DOC—dissolved organic carbon; Cd—carbon in solutions after decalcification; CHAs—carbon of the fraction of humic acids; CFAs—carbon of the fraction of fulvic acids; Ch—carbon of the humin fraction; carbon sequestration rate (CSR); DEH—dehydrogenases; CAT—catalase; AIP—alkaline phosphatase; AcP—acid phosphatase; PRO—protease; GMea—the geometric mean of enzyme activities; TEI—total enzyme activity index.

4. Conclusions

The results obtained on the basis of a 40-year two-factor fertilisation experiment conducted on medium-heavy soil showed the following:

Increasing nitrogen doses resulted in an increase in hydrolytic acidity, total exchangeable bases and cation exchange capacity. Manure fertilisation combined with nitrogen fertilisation at doses of 40, 60 kg and 120 N ha^{−1} and nitrogen fertilisation alone at doses of 60 and 120 N ha^{−1} resulted in increased carbon contents relative to pre-experiment soil, as evidenced by positive values of the carbon sequestration rate (CSR). The use of N at a dose of 120 N ha^{−1} intensified the processes of organic matter mineralisation, leading to a significant reduction in CSR compared to the 30 FYM, 0 N variant.

The highest content of available phosphorus, potassium and magnesium was obtained in the soil fertilised with manure and nitrogen at a dose of 40 kg ha^{−1}. It was found that the application of manure mitigated the negative effects of nitrogen exposure at 120 kg ha^{−1}.

The activity of oxidoreductive enzymes was highest in the soil after the application of manure and 0 N. An increasing dose of N resulted in the inhibition of these enzymes. The

application of manure and 120 kg N ha^{−1} increased the activity of acid phosphatase and proteases and decreased the activity of alkaline phosphatase.

A higher content of the humic acid fraction and higher values of the CHA/CFA ratio (parameters that are indicators of soil quality) were found in the soil fertilised with manure compared to the soil without manure added.

The content of humic acids and CHA/CFA values correlated positively with the content of soil minerals and with the content of DEH, CAT, ALP, AcP enzymes and GMea and TEI indices. Dehydrogenases and acid phosphatase can be considered enzymes that take part in the transformation of OM leading to the formation of FAs—based on the significant positive correlations between these parameters.

Strong correlations between GMea, TEI and CHAs/CFAs clearly indicate that enzyme indices can be classified as indicators of soil fertility and, similarly to CHAs/CFAs, as indicators of the degree of humification of organic matter.

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