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The Application of Biochar from Waste Biomass to Improve Soil Fertility and Soil Enzyme Activity and Increase Carbon Sequestration

Piotr Wojewódzki [1,*], Joanna Lemanowicz [1], Bozena Debska [1], Samir A. Haddad [2] and Erika Tobiasova [3]

[1] Department of Biogeochemistry and Soil Science, Bydgoszcz University of Science and Technology, Bernardynska 6/8 Street, 85-029 Bydgoszcz, Poland
[2] Department of Agricultural Microbiology, Minia University, El-Minia 61517, Egypt
[3] Department of Soil Science, Slovak University of Agriculture in Nitra, 949 76 Nitra, Slovakia
* Correspondence: piotr.wojewodzki@pbs.edu.pl

Abstract: Biochar (BC) is a material that has many applications in agricultural and environmental activities. The aim of the study was to define the influence of BC produced in low-temperature pyrolysis from various organic waste materials, including one-month-old compost (OMOC), pine bark (PB), pine needle mulch (NM), pine cones (PC) and maple leaves (ML), on soil enzyme activity as well as its relation with organic matter properties. A 60-day incubation pot experiment was set up to investigate the influence of BC amendment on soil (S) characteristics. After incubation, we investigated the activity of soil enzymes, the content of available phosphorus (AP), potassium (AK) and magnesium (AMg), total organic carbon (TOC), total nitrogen (TN), dissolved organic matter (DOM) and its fractional composition (content and share of carbon and nitrogen of humic (CHAs, NHAs) and fulvic (CFAs, NFAs) acids and humin fractions). The effect of the amended biochars differed depending on the feedstock material. In general, the use of biochar enriched the soil with AP, AK and AMg increased the soil carbon stock, increased the intensity of nitrogen transformation and influenced the soil enzyme activity. OMOC and ML biochars significantly increased soil fertility, which was expressed by the high value of the CHA/CFA ratio.

Keywords: biochar; soil; soil enzymes; carbon; soil organic matter; macroelements

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Citation: Wojewódzki, P.; Lemanowicz, J.; Debska, B.; Haddad, S.A.; Tobiasova, E. The Application of Biochar from Waste Biomass to Improve Soil Fertility and Soil Enzyme Activity and Increase Carbon Sequestration. *Energies* **2023**, *16*, 380. https://doi.org/10.3390/en16010380

Academic Editor: Gabriele Di Giacomo

Received: 6 December 2022
Revised: 22 December 2022
Accepted: 23 December 2022
Published: 29 December 2022

1. Introduction

Among the different areas of biochar (BC) utilization, there is growing interest in BC application in agriculture as an exogenous source of carbon as well as for potentially increasing soil structure and fertility. Yang and Ali [1] note that biochar is widely used in agricultural and environmental activities. The direction of BC utilization depends on its properties, which are influenced by pyrolysis parameters as well as by the biomass feedstock [2,3]. Biomass might be classified in various ways, for example, primary, secondary and tertiary biomass [4,5], natural and anthropogenic, and woody and non-woody [6]. Woody biomass mainly consists of tree and shrub remnants. It is characterized by low humidity, low ash content and high calorific value. Non-woody biomass is characterized by high humidity, high ash content and low calorific value. One of the factors influencing the properties of biochar is the content of lignin and cellulose in the biomass [7]. The content of these ingredients depends on the feedstock material for biochar production. The research of Gul et al. [8] indicated that biochar produced from crop residues had a higher content of nutrients and pH and less stable C (less resistant to decay) compared to biochar produced from raw wood material with a greater amount of lignin and cellulose. The cellulose present in the biomass helps in the formation of the so-called tar (a mixture of ketones, aldehydes and other liquid organic compounds), and the high content of lignin favors the production of solid carbon [9].

Previous works indicated that BC mixed with soil could produce a positive effect on aggregate stability [10], water use efficiency [11], soil pH and bulk density, water holding capacity, cation exchange capacity, plant growth and carbon uptake [3,12]. It was reported that biochar was a potential means to modify nutrient cycling, reduce soil N_2O emissions, and enhance soil carbon sequestration [13,14]. The research of Bi et al. [15] confirmed that fertilization with the participation of biochar increased the content of dissolved organic carbon and nitrogen, soil organic matter and total nitrogen in soil; they also revealed that biochar amendment significantly increased crop production and nitrogen uptake. In a field experiment in a sandy loam calcareous soil, Yan et al. [16] revealed positive influence of biochar-based fertilizers on soil microbial community structure as well as the effect of rising content of total and organic carbon, nitrogen, phosphorus, potassium and available forms of nitrogen, phosphorus and potassium NPK in soil. Some field experiments revealed BC's beneficial influence on many soil parameters. For example, the addition of BC produced from corn cobs to Fluvic Cambisol increased significantly the organic carbon content, soil porosity, content of available potassium and soil macroaggregates as well as soil aggregate stability [17]. The research of Baiamonte et al. [18] reported that BC produced from poplar wood chips mixed with sandy clay vertisol increased organic carbon content, soil porosity and water capacity. In a 3-year field study involving application of BC derived from a mixture of rice husks (70%) and cotton seed hulls (30%) in calcareous fluvisol, Dong et al. [19] indicated no significant influence on soil aggregate structure. However, the study demonstrated increased stability of soil aggregates as well as a significant increase of total organic carbon (TOC) and total nitrogen (TN) in the soil and in the macro-aggregates (>250 μm). The TOC represents the content of carbon in organic compounds, while inorganic carbon refers to the carbon content of inorganic compounds like carbonates or elemental carbon. The field experiment of Zhang et al. [20], which tested the application of wheat straw-derived biochar to Earth-cumuli-Orthic Anthrosols with a silty clay–loam texture, also reported no significant influence on soil aggregate structure. However this research revealed that the addition of BC significantly increased soil organic carbon content, microbial biomass carbon and nitrogen. Greater activity of urease was also found, which indicates improved transformation of nitrogen in the soil after BC addition. The activity of invertase and alkaline phosphatase were not influenced by BC.

Peiris et al. [21] reported that biochar, as an inexpensive alternative to artificial fertilizers, affects plant production in a direct and indirect way. The direct contribution is related to providing labile nutrients, while an indirect effect is the improvement of soil properties, which increases the efficiency of fertilization. The influence of biochar amendment could be assessed on the grounds of soil enzyme activity, as this is an effective means of appraising soil quality due to the high sensitivity and the rapid responses elicited to changes in the soil environment [22]. One meta-analysis [23] gathered data on the activity of soil hydrolytic enzymes under the influence of biochars produced from different biomass: wood BC, herb BC, manure BC, lignocellulose BC and sludge BC. The results of this analysis indicated that BC amendments significantly decreased the activities of C-cycling enzymes but significantly increased the N- and P-cycling enzyme activities in soils. Biochars fabricated at low temperatures from high-nutrient-content feedstock materials and their application in acidic, neutral and farmland soils significantly promoted the N and P cycling enzyme activities.

Soil enzymes fulfill an important role in soil organic matter transformation and in the circulation of nutrients (C, N, P, S). The analysis of soil enzyme activity provides information on soil processes. Therefore, to assess the condition of the soil environment, in addition to physical and chemical parameters, the activity of selected enzymes is measured [24,25].

Balancing soil organic matter is one of the strategic goals of the European Green Deal. The deficit of manure as the primary source of organic matter demands the search for alternative sources of exogenous organic matter (EOM). EOM-based biofertilizers reduce agriculture's dependence on non-renewable resources such as mineral fertilizers. Potential sources of EOM include compost, digestate, sewage sludge, bottom sediments and biochar.

Zhang et al. [20] concluded that the application of biochar is potentially a more efficient practice to improve soil aggregation and carbon sequestration than straw return into the soil. The 6-year study of Du et al. [17] also demonstrated the positive effect of biochar on soil. The authors concluded that biochar addition to soil improved soil aggregation and structural stability and resulted in increased soil organic carbon accumulation. However, the tested influence of biochar was similar to the effect of crop residues. Due to different results, the study of the impact of biochars on soil quality requires further research.

The aim of the study was to define the influence of BC produced from various organic materials—pre-composted material (OMOC), pine bark (PB), pine needle mulch (NM), pine cones (PC) and maple leaves (ML)—on soil enzyme activity and their relations with soil organic matter properties and role in the process of carbon sequestration. It was hypothesized that biochar addition into the soil would have a positive effect on stimulating soil enzyme activity and soil fertility and significantly increase the soil carbon pool.

2. Materials and Methods

The research analyzed the effect of five types of biochar produced from biomass: one-month-old compost (OMOC), pine bark (PB), pine needle mulch (NM), pine cones (PC) and maple leaves (ML). OMOC was obtained from a municipal composting plant. NM, PC and ML were obtained from the maintenance of green areas and parks. PB was a biomass obtained from a sawmill. The production of biochar was carried out in the low temperature pyrolysis (400 °C) process of air-dried feedstock biomass, under atmospheric pressure, in a muffle furnace Czylok FCF 22 M. Process execution time was 60 min. The obtained BC, prior to application into soil, was ground and homogenized in a ball mill (Retsch PM100). Parameters of milling process were as follows: time 4 min, 400 rpm, nine zirconium balls in milling chamber, and 30% power of the apparatus. The mean size distribution of BC particles was as follows: 2.0–0.05 mm: 17.0%; 0.05–0.002 mm: 76.9%; <0.002 mm: 6.1%.

The examination of BC's effect on soil was realized in a small-scale pot experiment. One experimental factor (type of BC) was assumed for six levels:

- S: soil without biochar;
- S + OMOC BC: soil mixed with one-month-old compost BC;
- S + PB BC: soil mixed with pine bark BC;
- S + NM BC: soil mixed with needle mulch BC;
- S + PC BC: soil mixed with pine cone BC;
- S + ML BC: soil mixed with maple leaf BC.

Incubation pots were filled with a mixture (1:10) of BC:soil on a dry matter basis, then incubated for 60 days under 20 ± 2 °C. Due to the planned short-term incubation experiment, the dose of biochar was selected as excessive in relation to the doses used in field studies (up to 90 Mg of BC per hectare) [10,17–20].

In order to ensure thermal conditions, the pots were kept in a thermostatic incubator (Q-Cell, Poll Lab). The soil moisture content was controlled every day to keep its value around 13.5% (the level of soil moisture in the field during soil harvesting). The soil for the incubation experiment was taken from top layer (0–25 cm) of arable field. According to the WRB classification [26], the soil was classified as Luvisol. The content of soil particle size fractions was clay (<0.002 mm) 6.18%, silt (0.05–0.002 mm) 43.77% and sand (2.0–0.05 mm) 50.06%. According to USDA classifications [27], the soil material was described as sandy loam. The bulk density of the sampled soil was 1.67 g cm^{-3}, the average humidity was 13.02%, pH_{H2O} = 6.40, and the content of available phosphorus (AP) was 152 mg kg^{-1}.

2.1. Carbon, Nitrogen and Fractional Composition of Humus

The analyses of total organic carbon (TOC) and total nitrogen (TN) content in biochars, soil and soil mixed with BC were performed with a Vario Max CN analyzer provided by Elementar (Germany). The content of TOC and TN was expressed in g kg^{-1} of dry matter (d.m.) of soil.

After incubation, the soil mixed with BC was assayed for the content of dissolved organic carbon (DOC) and dissolved nitrogen (DTN). The soil extraction solutions were prepared with 0.004 mol dm^{-3} $CaCl_2$ at the ratio of soil sample to extractant of 1:50. After 60 min of extraction, the samples were centrifuged. The content of DOC and DTN was assayed with a Muli N/C 3100 analyzer (Analytik, Jena, Germany) and expressed in mg kg^{-1} d.m. of the soil sample and as the percentage share in the pool: TOC and TN (DOC (%) and DTN (%)).

The fractional composition of humus was assayed based on the carbon and nitrogen fractions determined in the extracts using a Multi N/C 3100 Analityk Jena, according to the following procedure:

- decalcification with 0.05 M HCl (1:50 *w*/*v*), 24 h, followed by determination of carbon (Cd) and nitrogen (Nd) in solutions after decalcification;
- extraction of the remaining solid with 0.5 M NaOH (1:50 *w*/*v*) with occasional mixing, 24 h, followed by centrifugation, C(N)HAs + FAs—sum of the carbon (nitrogen) of humic and fulvic acids;
- precipitation of humic acids from the resulting alkaline extract with 2 M HCl to pH = 2 and centrifugation for 24 h; C(N)FAs—carbon (nitrogen) of fulvic acids in solutions.

The C and N content of humic acids (C(N)HAs) and carbon and nitrogen of humins (C(N)h) were calculated from the difference:

$$\text{C(N)HAs} = \text{C(N)HAs} + \text{FAs} - \text{C(N)FAs} \tag{1}$$

$$\%\text{C(N)h} = 100\% - \%\text{C(N)HAs} - \%\text{C(N)FAs} - \%\text{C(N)d} \tag{2}$$

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

Soil pH in the 1 M KCl was assayed according to the standard PN-ISO 10390 [28]. The content of available forms of phosphorus (AP) and potassium (AK) was analyzed according to protocol established by Egner et al. [29]. The content of available forms of magnesium (AMg) was assayed according to the method of Schachtschabel [30].

2.2. Enzyme Analysis

Enzyme activity analyses were performed on fresh soils, sieved on 2 mm screens. If needed, the soil samples were stored at 4 °C. The activity of soil enzymes was tested at the beginning of the experiment and then after 60 days. The activity of oxidoreductive and hydrolytic enzymes was determined as follows:

- the activity of dehydrogenases (DEH) [EC 1.1.1] was measured on the grounds of the Thalmann protocol [31];
- catalase (CAT) [EC 1.11.1.6] activity was determined with the Johnson and Temple method [32];
- the activity of alkaline phosphatase (AlP) [EC 3.1.3.1] and acid phosphatase (AcP) [EC 3.1.3.2] was assayed according to the Tabatabai and Bremner method [33];
- proteases activity (PRO) [3.4.4] was examined in reference to the Ladd and Butler protocol [34].

Actual enzyme activity values were converted according to the formula given by Chaer et al. [35] and are given as percentage changes (Relative Change—R*Ch*) in relation to the control soil:

$$\text{R}Ch = \left(\frac{\text{T}}{\text{C}} - 1\right) \cdot 100\% \tag{3}$$

where T is the average enzyme activity in soil with the addition of biochar, and C is the average enzyme activity in the control sample (without the addition of biochar, S).

The geometric mean of enzyme activities (G*Mea*) [36] is calculated as follows:

$$GMea = \sqrt[5]{(CAT \cdot DEH \cdot AlP \cdot AcP \cdot PRO)} \tag{4}$$

phosphatase, acid phosphatase, proteases, respectively.

The calculation of total enzyme activity index (TEI) was realized according to Tan et al. [37]:

$$TEI = \sum \frac{X_i}{\overline{X_l}} \quad (5)$$

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

The value of metabolic activity index (MAI) was calculated in reference to the formula of Picariello et al. [38]:

$$MAI = \sum \frac{Pij}{Pcij} \quad (6)$$

where $Pij = \frac{Aij}{\text{Ref}_\text{j}}$; $Pcij = \frac{Acij}{\text{Refc}_\text{j}}$; Aij is the value of the activity of each enzyme; Ref_j is the reference parameter, TOC; $Acij$ is the value of the activity of each enzyme in the control soil; Refc_j is the reference parameter in the control soil.

2.3. Statistical Analyses

The obtained results were assessed by one-way analysis of variance (ANOVA) according to the experimental design of randomized blocks. The normality of the data was assessed with the Shapiro–Wilk test. The multidimensional exploration technique of principal components analysis (PCA) was used to explain the multi-feature differentiation of soil with the addition of biochar obtained from different feedstock material based on two main components. The calculations were made using STATISTICA 13 software (Stat Soft Polska).

3. Results

3.1. Properties of Soil and Organic Matter

The analysis of total organic carbon in biochar indicates that biochar produced from pine cones was characterized by the highest content: 741.6 g kg^{-1} (Table 1). It was significantly higher in comparison to other tested biochars. The lowest TOC content was determined in OMOC biochar: 289.9 g kg^{-1}. This value was significantly lower compared to other samples of BC. The content of total nitrogen in biochar varied from 2.30 g kg^{-1} (PB BC) to 26.38 g kg^{-1} (OMOC BC). The nitrogen content decreased in the following order: OMOC > ML > NM > PC > PB. On the grounds of TOC and TN content, the value of TOC/TN ratio was determined. It ranged in value from 10.99 (OMOC BC) to 304.04 (PB BC). Among the biochars derived from wood biomass, the ML BC was characterized by the lowest ratio of TOC/TN (44.41). The C:N ratio value is one of the factors determining the intensity of decomposition of organic matter. The value of this ratio in the range of 20–30:1 indicates the balance of mineralization and humification processes. Lower C:N values indicate intensification of mineralization processes. Higher C:N values point to intensification of humification processes, which favors carbon sequestration [7,19,39].

Table 1. Content of total organic carbon (TOC), total nitrogen (TN) and TOC/TN ratio in biochars and soil.

Sample	TOC ($g \cdot kg^{-1}$)	TN ($g \cdot kg^{-1}$)	TOC/TN	pH KCl
OMOC BC	$289.9^{e} \pm 6.6$	$26.38^{a} \pm 2.8$	10.99^{e}	7.22
PB BC	$699.3^{b} \pm 7.4$	$2.30^{d} \pm 0.30$	304.04^{a}	3.87
NM BC	$665.1^{c} \pm 7.6$	$11.22^{b} \pm 0.65$	59.28^{c}	5.58
PC BC	$741.6^{a} \pm 8.2$	$7.64^{c} \pm 0.25$	97.07^{b}	5.48
ML BC	$641.8^{d} \pm 4.0$	$14.45^{b} \pm 0.55$	44.41^{d}	8.44
S	11.5 ± 0.93	1.42 ± 0.05	8.10	5.55

OMOC BC: one-month-old compost biochar; PB BC: pine bark biochar; NM BC: needle mulch biochar; PC BC: pine cone biochar; ML BC: maple leaf biochar; S: soil; TOC: total organic carbon; TN: total nitrogen. Values followed by the same small letter within each column are not significantly different at $p = 0.05$.

After mixing the biochars with the soil, the TOC/TN ratio values decreased and ranged from 10.04 (S + OMOC) to 53.17 (S + PB) (Table 2). After 60 days incubation of soil mixed with biochars, there was a noted decrease of TOC content in the range of 4.48% (variant S + PB) to 10.77% (variant S + OMOC) (Table 2, Figure 1). The losses of nitrogen were higher and ranged from 14.8% (variant S + ML) to 25.07% (variant S + PC). Higher intensity of nitrogen transformations as compared to carbon transformations resulted in broadening of the TOC/TN ratio. The increase of the TOC/TN ratio was highest in the variant S + PC and lowest for variant S + OMOC. The obtained results indicate that the introduction of biochar to the soil may contribute to carbon sequestration, mainly through the reduction of greenhouse gas emissions [19,39].

Table 2. Content of total organic carbon (TOC), total nitrogen (TN) and TOC/TN ratio in soil mixed with biochar before and after 60 days of incubation.

Sample	TOC_0 (g kg^{-1})	TOC_1 (g kg^{-1})	TN_0 (g kg^{-1})	TN_1 (g kg^{-1})	TOC_0/TN_0	TOC_1/TN_1
S	11.50	11.48 ^{e} ± 0.92	1.42	1.40 ^{de} ± 0.53	8.10 ^{f}	8.20 ^{f}
S + OMOC	39.34	35.10 ^{d} ± 0.70	3.92	3.17 ^{a} ± 0.16	10.04 ^{e}	11.07 ^{e}
S + PB	80.28	76.68 ^{b} ± 0.71	1.51	1.25 ^{e} ± 0.15	53.17 ^{a}	61.34 ^{a}
S + NM	76.86	70.13 ^{c} ± 1.01	2.40	1.89 ^{c} ± 0.08	32.03 ^{c}	37.11 ^{c}
S + PC	84.51	79.65 ^{a} ± 0.51	2.04	1.53 ^{d} ± 003	41.43 ^{b}	52.06 ^{b}
S + ML	74.53	70.50 ^{c} ± 1.18	2.72	2.32 ^{b} ± 0.07	27.40 ^{d}	30.39 ^{d}

TOC_0: content of total organic carbon before incubation; TOC_1: content of total organic carbon after incubation; TN_0: content of total nitrogen before incubation; TN_1: content of total nitrogen after incubation. Values followed by the same small letter within each column are not significantly different at p = 0.05.

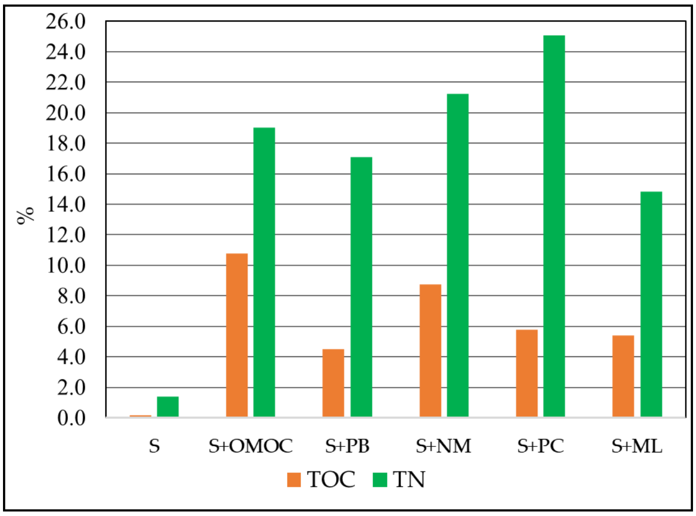

Figure 1. Percentage change in the TOC and TN content between the initial and final incubation stages.

The pH value of soil without added biochar was acidic (pH in KCl 5.10) (Table 2). The amendment of BC produced from coniferous plants changed the soil pH into slightly acidic (Table 3). The soil pH in variant S + OMOC classified it as neutral and in variant S + ML as basic. Generally, BC addition into soil raises the pH value. However, PB, NM and PC biochars, characterized with low pH, are not factors that substantially change soil pH. Earlier studies [40,41] revealed that biochar contains alkalic substances that increase soil pH. However, the formation of such substances is influenced, among other factors, by the type of feedstock material.

Table 3. Values of pH in KCl, content and share of dissolved organic carbon and nitrogen, and available phosphorus, potassium and magnesium (after 60 days incubation).

Variant	pH KCl	DOC (mg kg^{-1})	DOC (%)	DTN (mg kg^{-1})	DTN (%)	AP (mg kg^{-1})	AK (mg kg^{-1})	AMg (mg kg^{-1})
S	5.10	219.5 ^{c} ± 9.5	1.91 ^{a} ± 0.12	46.0 ^{b} ± 4.1	3.25 ^{a} ± 0.36	130 ^{b} ± 0.7	214.5 ^{d} ± 5.2	62.50 ^{c} ± 0.7
S + OMOC	6.97	491.5 ^{a} ± 9.8	1.40 ^{b} ± 0.02	116.0 ^{a} ± 0.8	3.66 ^{a} ± 0.17	153.1 ^{a} ± 0.1	240.3 ^{d} ± 1.2	92.33 ^{a} ± 1.2
S + PB	5.43	264.5 ^{b} ± 6.1	0.34 ^{d} ± 0.01	26.7 ^{cd} ± 0.6	2.14 ^{b} ± 0.16	134.2 ^{b} ± 4.1	371.3 ^{ab} ± 2.1	78.03 ^{b} ± 2.4
S + NM	5.48	264.5 ^{b} ± 4.9	0.38 ^{d} ± 0.01	22.0 ^{d} ± 0.9	1.16 ^{c} ± 001	139.0 ^{b} ± 2.7	339.5 ^{b} ± 16.1	23.09 ^{e} ± 0.3
S + PC	5.79	249.5 ^{b} ± 8.1	0.31 ^{d} ± 0.01	29.6 ^{c} ± 1.7	1.93 ^{b} ± 0.11	122.3 ^{c} ± 0.5	392.7 ^{a} ± 8.2	29.90 ^{d} ± 1.3
S + ML	7.41	488.5 ^{a} ± 6.2	0.69 ^{c} ± 0.02	24.1 ^{d} ± 0.5	1.04 ^{c} ± 0.01	151.6 ^{a} ± 0.22	283.1 ^{c} ± 9.2	26.03 ^{de} ± 0.1

DOC: dissolved organic carbon; DTN: dissolved total nitrogen; AP: available phosphorus; AK: available potassium; AMg: available magnesium. pH values: median of series (n = 3). Values followed by the same small letter within each column are not significantly different at $p = 0.05$.

According to literature reports [42], biochar introduction into the soil increases the absolute content of dissolved organic carbon, but does not alway increase the share of this carbon fraction. DOC is the most frequently used parameter to determine the dissolved organic matter (DOM) abundance in soils. DOM is the most mobile and rapidly decomposing fraction of organic matter and plays an important role in the biogeochemical cycle of carbon, nitrogen and phosphorus [43,44]. The DOM is a product of the transformation and decomposition of carbon compounds embedded in the organic material of the soil. It is a heterogeneous mixture of organic compounds of an acid, base or neutral nature (fatty acids, organic acids, amino acids, sugars) [45,46]. The addition PB, PC and NM biochars to the soil resulted in a slight increase in the DOC content, by an average of approx. 18%, compared to the biochars obtained from compost and maple leaves (Table 3). For the variants S + OMOC and S + ML, the DOC content increased on average by approximately 123%. Despite such a high increase in DOC content, the share of this carbon fraction in the TOC pool was lower than in soil samples without additives. The average share of DOC fraction in soil mixed with PB, PC and NM BC was 0.34%. The nitrogen content in the soluble organic matter pool was the highest in soil samples mixed with biochar obtained from compost (116 mg kg^{-1}) (Table 3). For the other variants, there was noted a significantly lower content and share of DTN (22.0–29.6 mg kg^{-1}, 1.04–2.14%) compared to the soil without additives (46.0 mg kg^{-1}, 3.25%).

The AP content ranged in value from 122.3 to 153.1 mg kg^{-1}, which, according to standard PN-R-04023 [47], classifies it as soil with a very high content of this macronutrient (class I). AK content ranged from 214.5 to 392 mg kg^{-1}. According to standard PN-R-04022 [48], the tested soil belongs to class I, with a very high content of this macro-element. AMg content ranged from 23.09 to 92.33 mg kg^{-1}, depending on the type of amended biochar. According to standard PN-R-04020 [49], the content of AMg was low: class IV (S + NM, S + PC, S + ML). Based on the content of AMg, soil S was classified as class II, with a high content of AMg, while variants S + OMOC and S + PB were class I, with very high content (Table 3).

Statistical analysis indicated a significant effect of various types of biochar on the content of available P, K and Mg in the soil (Table 3). The soil with pre-composted material (S + OMOC) and with maple leaves (S + ML) contained significantly more AP than soils amended with other biochars obtained from pine bark, cones and needle mulch. No significant differences were found between the AP content in the control (S), S + PB and S + NM soil. Among the tested variants of soils with the addition of biochars, the variant S + OMOC contained significantly the lowest AK (240.2 mg kg^{-1}). There was no significant difference in AK content between S and S + OMOC. However, the other variants, especially soil mixed with BC produced from pine elements, contained significantly more AK than pure soil (S). The significantly highest content of AMg was found in S + OMOC (92.33 mg kg^{-1}). The use of NM BC, PC BC and ML BC resulted in a significant reduction of AMg content in the soil. The decrease was 63%, 52% and 58%, respectively. Similarly research by Sadowska et al. [50] revealed a significant effect of biochar on the content of macro-elements (N, Ca, K, P, S), except for Mg.

Biochar has relatively poor fertilizing properties, but as an additive, it may show a supportive effect. This is due to the use of nutrient-poor materials for its production or their presence in forms that are difficult to access for plants [51]. Increasing phosphorus bioavailability by adding biochar to the soil is related to its ability to modify the soil pH and thus influence the solubility of phosphorus in the soil [40–42]. According to Børno et al. [52], biochar influenced the P pools in soil, and these effects were dependent on the BC feedstock. The results of Manolikaki et al. [53] suggest that biochar produced from agricultural residues may act as a phosphorus source in agronomic applications and improve plant growth. Qian et al. [54] report that the impending phosphorite depletion crisis initiates the development of various solid waste phosphorus fertilizers. This was confirmed in our research [42], in which the highest content of AP was found in the soil after adding BC produced from stabilized sewage sludge.

Potassium present in BC is the element most accessible to plants. In the initial stage, the addition of biochar to the soil causes a rapid growth of soluble K forms in the soil solution. Research by Widowati and Asnah [55] indicates an increase in K availability by 14–184%. This is confirmed by our results (Table 3), which demonstrate that BC addition increased the content of this macronutrient by 12% (S + OMOC), 73% (S + PB), 58% (S + NM), 83% (S + PC) and 32 % (S + ML) in comparison to control soil (S).

Another important component of organic matter is humic and fulvic acids. Their properties depend mainly on the course of the soil formation process and are a characteristic features of various types of soil. Humic and fulvic acids, as the main component of soil humus, determine its properties and the role in the natural environment. However, the composition of humus and its properties can be modified to some extent under the influence of anthropogenic factors (e.g., the cultivation method, fertilization and introduction of exogenous organic matter) [56,57].

The content of carbon in solutions after decalcitation (Cd) and carbon in solutions of humic and fulvic acids obtained from soil samples mixed with BC was generally higher than in solutions of soil samples without additives (Table 4).

Table 4. Mean content of carbon (mg kg^{-1}) in the humus fraction.

Variant	Cd	CHAs	CFAs	CHAs/CFAs
S	331.0 ^{e} ± 10.3	2176.6 ^{d} ± 25.2	3017.4 ^{d} ± 72.1	0.72 ^{c}
S + OMOC	814.6 ^{a} ± 12.8	10,970.4 ^{a} ± 108.6	4082.6 ^{a} ± 50.7	2.69 ^{a}
S + PB	484.5 ^{d} ± 8.3	2209.4 ^{d} ± 52.6	3172.6 ^{c} ± 11.25	0.70 ^{c}
S + NM	519.9 ^{c} ± 8.4	3058.5 ^{c} ± 48.6	4108.5 ^{a} ± 21.0	0.74 ^{c}
S + PC	511.6 ^{cd} ± 15.2	2218.8 ^{d} ± 38.2	3070.3 ^{cd} ± 25.7	0.72 ^{c}
S + ML	675.9 ^{b} ± 12.4	7506.3 ^{b} ± 44.5	3704.8 ^{b} ± 46.7	2.03 ^{b}

Cd: carbon content after decalcification; CHAs: carbon content of humic acids; CFAs: carbon content of fulvic acids. Values followed by the same small letter within each column are not significantly different at $p = 0.05$.

The content increase of individual carbon fractions is related to the increase of TOC content. The content of Cd, CHA, and CFA fractions did not correlate with the content of TOC. However, a significant negative correlation was determined between the share of carbon in the fractions of humic and fulvic acids and a positive correlation was found between the share of humin carbon (Ch) and the content of TOC in the tested samples (r = 0.97). The share of fulvic acid fraction decreased compared to the soil without additives (Figure 2), from 14.6 (S + OMOC variant) to 22.4 percentage points (S + PC variant), and the humic acid fraction from 8.3 (variant S + ML) to 16.1 percentage points (S + PC). The decrease in the humic acid carbon fraction included variants with BC obtained from pine materials (PB, PC, NM).

The addition of biochar obtained from compost to the soil resulted in an increase in the CHA fraction by 12.3 percentage points, while it did not contribute to an increase in the humic fraction. For the remaining variants, an increase in the share of the humin fraction was recorded from 31.2 (variant S + ML) to 40.8 percentage points (variant S + PC). In this experiment, taking into account the slow decomposition of biochar obtained from pine materials, it is more appropriate to use the term carbon in the extraction residue than in the humin fraction.

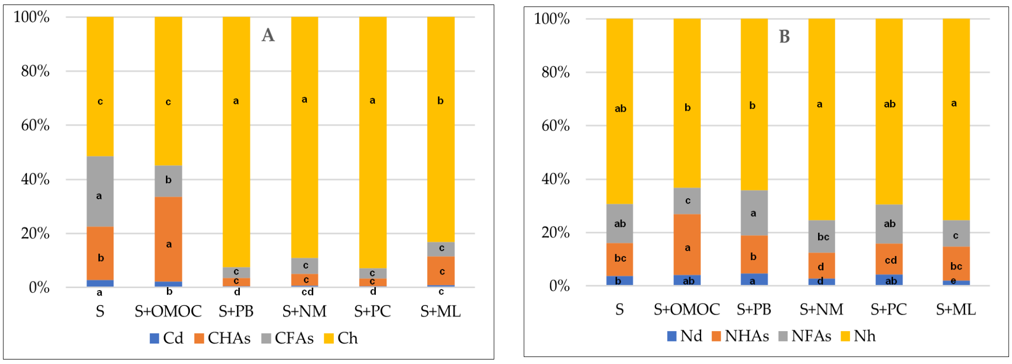

Figure 2. Share of carbon (**A**) and nitrogen (**B**) in organic matter fractions: Cd: carbon content after decalcification; CHAs: carbon content of humic acids; CFAs: carbon content of fulvic acids; Nd: nitrogen content after decalcification; NHAs: nitrogen content of humic acids; NFAs: nitrogen content of fulvic acids. Values represented by bars marked by the same small letter are not significantly different at p = 0.05.

The consequences of changes in the content of fulvic and humic acids are changes of the CHA/CFA ratio values. The highest value of this parameter was obtained for the variant S + OMOC, at 2.69, and slightly lower for the variant S + ML, at 2.03. The introduction of biochar obtained from pine cones, pine bark and needle mulch into the soil did not change the mutual proportions of humic to fulvic acid fractions. The CHA/CFA ratio values for the above-mentioned variants and soils without additives ranged from 0.70 (S + PB) to 0.74 (S + NM) (Table 4). Among the different indicators of the organic matter qualitative assessment is the value of the ratio of carbon of humic acids to carbon of fulvic acids, the so-called humification index. CHA/CFA values exceeding one are considered characteristic for fertile soils with a predominance of stable fractions of organic matter, including humins and humic acids [58].

The addition of OMOC BC to the soil significantly increased the nitrogen content in all analyzed humus fractions (Table 5). A significant increase of nitrogen content in humic and fulvic acid fractions was also noted after adding ML BC. In both variants, the increase of nitrogen content of the humic acid fraction was greater than that of the fulvic acid fraction (the values of the NHA/NFA ratio were 2.35 (S + OMOC) and 1.27 (S + ML)). For the remaining variants, there were no significant changes in the nitrogen fraction content as well as in the NHA/NFA ratio compared to the control (S).

Table 5. Mean content of nitrogen (mg kg^{-1}) in the humus fraction.

Variant	Nd	NHAs	NFAs	NHAs/NFAs
S	53.1 ^{c} ± 2.6	175.5 ^{c} ± 4.0	208.9 ^{c} ± 3.0	0.84 ^{c}
S + OMOC	131.3 ^{a} ± 9.9	725.6 ^{a} ± 5.8	308.5 ^{a} ± 14.5	2.35 ^{a}
S + PB	58.8 ^{bc} ± 2.5	177.7 ^{c} ± 2.6	212.4 ^{bc} ± 5.9	0.84 ^{c}
S + NM	53.8 ^{c} ± 1.6	183.5 ^{c} ± 4.2	230.6 ^{b} ± 4.3	0.80 ^{c}
S + PC	67.0 ^{b} ± 1.7	178.0 ^{c} ± 3.3	222.5 ^{bc} ± 4.0	0.80 ^{c}
S + ML	48.0 ^{c} ± 1.9	294.0 ^{b} ± 6.1	231.4 ^{b} ± 5.8	1.27 ^{b}

Nd: nitrogen content after decalcification; NFAs: nitrogen content of humic acids; NFAs: nitrogen content of fulvic acids. Values followed by the same small letter within each column are not significantly different at p = 0.05.

Nitrogen content in the fractions of humic and fulvic acids positively correlated with the total nitrogen content (r = 0.92 and r = 0.94, respectively). The addition of biochar did not cause significant changes in the share of Nh (Figure 2). The significant increase of nitrogen content was noted in the humic acid fraction, at 10.53 percentage points for the

S + OMOC variant (Figure 2), which is a consequence of a significantly higher TN content in the BC compared to the other biochars (Table 1).

3.2. Soil Enzymes

Our research revealed the significant influence of biochar amendment on the activity of the soil enzymes (Table 6). The significantly highest DEH, CAT and AlP activity was noted in soil mixed with ML BC. There were no significant differences in DEH activity between the variants S + PB, S + NM and S + PC (BC from pine material) where the DEH activity was the lowest. The significantly highest activity of PRO was noted in the S + NM variant (26.32 mg TYR kg^{-1} h^{-1}). However, no significant differences in PRO activity were found between S + OMOC, S + NM, S + PC and S + ML.

Table 6. The activity of enzymes in soil.

Variant	DEH	CAT	AlP	AcP	PRO
S	$0.929^{bc} \pm 0.017$	$0.823^{b} \pm 0.014$	$0.269^{b} \pm 0.061$	$1.262^{a} \pm 0.046$	$15.68^{b} \pm 0.233$
S + OMOC	$1.138^{b} \pm 0.042$	$0.849^{b} \pm 0.009$	$0.504^{a} \pm 0.018$	$0.865^{b} \pm 0.025$	$24.75^{a} \pm 0.235$
S + PB	$0.795^{c} \pm 0.022$	$0.633^{d} \pm 0.010$	$0.246^{b} \pm 0.010$	$0.808^{b} \pm 0.033$	$15.76^{b} \pm 0.583$
S + NM	$0.864^{c} \pm 0.019$	$0.746^{c} \pm 0.008$	$0.286^{b} \pm 0.025$	$0.788^{b} \pm 0.046$	$26.32^{a} \pm 1.515$
S + PC	$0.812^{c} \pm 0.008$	$0.689^{d} \pm 0.001$	$0.234^{b} \pm 0.012$	$0.850^{b} \pm 0.049$	$22.52^{a} \pm 2.917$
S + ML	$1.382^{a} \pm 0.009$	$1.097^{a} \pm 0.014$	$0.588^{a} \pm 0.020$	$1.205^{a} \pm 0.009$	$24.50^{a} \pm 1.519$

DEH: dehydrogenases (mg TPF kg^{-1} 24 h^{-1}); CAT: catalase (mg H_2O_2 kg^{-1} h^{-1}); AlP: alkaline phosphatase (mMpNP kg^{-1} h^{-1}); AcP: acid phosphatase (mMpNP kg^{-1} h^{-1}); PRO: proteases (mg TYR kg^{-1} h^{-1}). Values followed by the same small letter within each column are not significantly different at $p = 0.05$.

Earlier studies [42,59] indicate that BC feedstock material significantly determined the changes of selected soil properties. According to studies of Lehman et al. [60] and Jing et al. [59], there are two possible mechanisms through which biochar influences the activity of soil enzymes. The first is the high surface area and porosity of biochar, which absorbs the substrates on its surface or blocks the active centers of enzymes. The second mechanism is based on the change of soil physicochemical properties under the influence of biochar, which directly or indirectly affect the enzymatic activity.

Percentage changes in enzyme activity (R*Ch*) inform the direction of influence of the experimental factor (inhibition or activation) on the activity of soil enzymes [35,61]. Proteases were the only enzymes stimulated by biochar (Figure 3). R*Ch* for proteases ranged from 0.50% (S + PB) to 57.84% (S + OMOC). Negative RCh values were recorded for the AcP activity after the application of all biochar types (from −4.46% to −37.49%). The addition ML BC (S + ML) to the soil resulted in the greatest increase in the activity of DEH (R*Ch* = 48.76%), CAT (R*Ch* = 33.29%) and AlP (R*Ch* = 118.6%). The application of OMOC BC (S + OMOC) also stimulated the activity of DEH, CAT and AlP. The R*Ch* index value for the tested enzymes was as follows: PRO > AlP > DEH > CAT > AcP. Considering the influence of BC, the values of R*Ch* index were S + ML> S + OMOC> S + NM > S + PC > S + PB.

Measurement of the activity of individual enzymes under the influence of environmental factors involved in the functioning of the soil ecosystem is important. However, measuring only one soil parameter is insufficient, as soil performs multiple functions [38]. The paper presents the values of the G*Mea* and TEI coefficients as dimensionless parameters in order to compare the total activity of the tested soil enzymes. The MEI index is based on the ratio of enzyme activity in soil with added biochar to that in control soil. The enzyme activities are normalized to the content of total organic carbon in the soil (TOC). G*Mea* values in the analyzed variants ranged from 1.095 to 1.923. The significantly highest G*Mea* value was noted in the variant S + ML (1.923). According to Paz-Ferreiro et al. [62] and Lemanowicz et al. [24], higher values indicate better soil quality. The significantly lowest GMea values were found in soil with BC from pine material (S + PB, S + NM, S + PC) (Figure 4). The integrated total enzyme activity index (TEI) allows a simple comparison between the combined enzyme activity and the quality of each soil sample [63]. TEI values ranged from 1.257 to 1.950 (Figure 4).

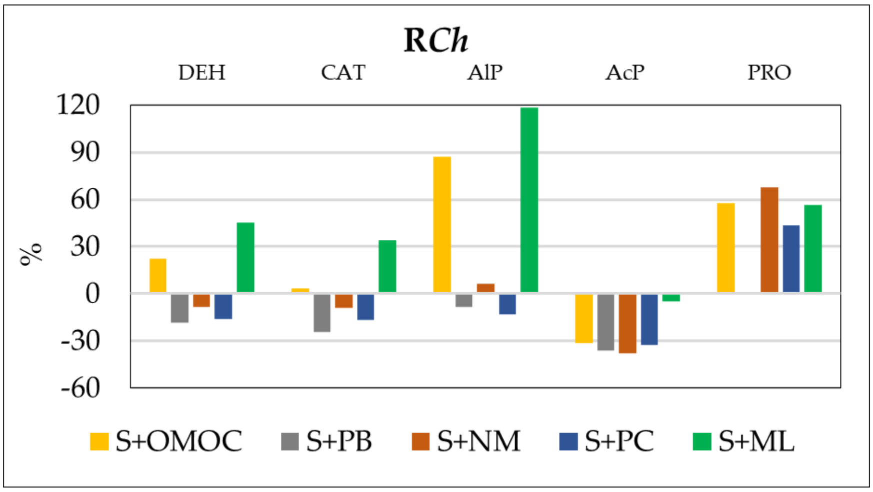

Figure 3. Percentage changes (R*Ch*) in activity of dehydrogenases (DEH), catalase (CAT), alkaline phosphatase (AlP), acid phosphatase (AcP) and protease (PRO) in soil incubated with biochars.

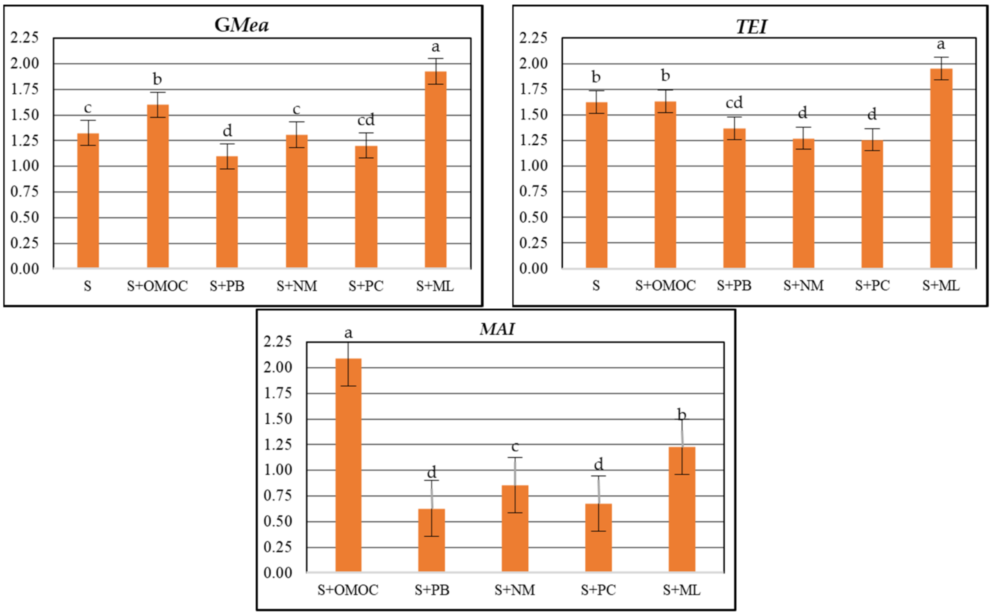

Figure 4. Index of soil enzymes: G*Mea*, TEI and MAI. Bars with the same small letter represent no significant differences at $p = 0.05$.

The trend of the influence of biochar from various feedstock was similar to that in the case of the G*Mea* coefficient. Analyzing the MAI values, the soils with the addition of biochar from pine materials (S + PB = 0.627, S + NM = 0.852, S + PC = 0.676) exposed similar trends, but these were different from the soil with the addition, OMOC BC (S + OMOC = 2.092) and ML BC (S + ML = 1.226) (Figure 4). The MAI index significantly differentiated the influence of biochar with regard to the feedstock material from which it was produced.

Enzymatic activity is a parameter very sensitive to environmental stress; therefore, it is used to assess the quality of soil [24,38,42,62]. For better understanding the processes influencing the soil environment, soil quality indicators should cover several biological parameters. To demonstrate the relationship between the G*Mea*, TEI and MAI indexes and other soil parameters, Pearson correlation analysis was performed (Table 7).

Table 7. The correlations between selected soil parameters and soil quality indexes.

	pH KCl	TOC	TN	TOC/TN	DOC	DTN	AP	Cd	CHAs	CFAs	NHAs/NFAs	CHAs/CFAs
G*Mea*	0.972	n.s.	0.720	0.732	0.908	n.s.	0.817	0.751	0.772	n.s.	0.547	0.800
TEI	0.943	n.s.	0.604	0.575	0.912	n.s.	0.812	0.705	0.737	n.s.	0.533	0.785
MAI	0.965	0.856	0.979	0.941	0.856	0.903	0.805	0.980	0.975	0.668	0.985	0.953

G*Mea*: geometric mean of enzyme activities; TEI: total enzyme activity index; MAI: metabolic activity index; TOC: total organic carbon; TN: total nitrogen; DOC: dissolved organic carbon; DTN: dissolved total nitrogen; AP: available phosphorus; Cd: carbon content after decalcification; CHAs: carbon content of humic acids; CFAs: carbon content of fulvic acids; NHAs: nitrogen content of humic acids; NFAs: nitrogen content of fulvic acids; n.s.: not significant.

The analysis indicated a significant correlation between TN and G*Mea* (r = 0.720), TEI (r = 0.604) and MAI (r = 0.979). Highly significant correlations were found between pH in KCl and G*Mea* (r = 0.972), TEI (r = 0.943) and MAI (r = 0.965), which explain 94%, 89% and 93% of changes in the calculated indicators, respectively (Table 7). The value of the TOC/TN ratio significantly positively influenced the changes of the indices. The values of G*Mea* TEI and MAI were highly correlated with the content of DOC and CHA/CFA ratio. For MAI, an additional high correlation coefficient with TOC (r = 0.856) was noted. It explained 73% of the changes of the MAI value. According to Haney et al. [64] and Wang et al. [65], the relationship between the parameters of soil enzyme activity and the content of labile fractions of organic carbon (DOC) gives the possibility of a potential assessment of changes in the biochemical cycle of elements, depending on changes in soil use. The significant correlation between the indexes of enzymatic activity and the carbon content of humic acids and the values of the CHA/CFA ratio gives the potential possibility of using these parameters as indicators of the fertility of soils amended with biochar.

To explain the differentiation of the studied soil parameters, the principal components technique (PCA) was used. PCA enables investigation of the cause-and-effect relationships between the parameters. Multivariate PCA analysis identified three components that accounted for 90.79% of the total variance, most of which were explained by PC1 (49.73%) and PC2 (24.66%) (Figure 5A). PC 1 was mainly positively related to the content of TN (0.936), DOC (0.847), DTN (0.854), Cd (0.789), CHAs (%) (0.904), N_d (0.767), NFAs (0.888), NHAs (0.940), NHAs (%) (0.830), pH KCl (0.763) and AP (0.807) and to the activity of DEH (0.719), CAT (0.588) and AlP (0.806). Positive values mean that the greater the intensity of these features, the more important their contribution to PC1. Research by Liu et al. [66] indicated that a factor can be considered strong when its value exceeds 0.750. The second component (PC2) was significantly positively related with PRO activity (0.736) and TOC content (0.735) and negatively with DTN (%) (−0.846), Cd (%) (−0.716) and CFAs (%).

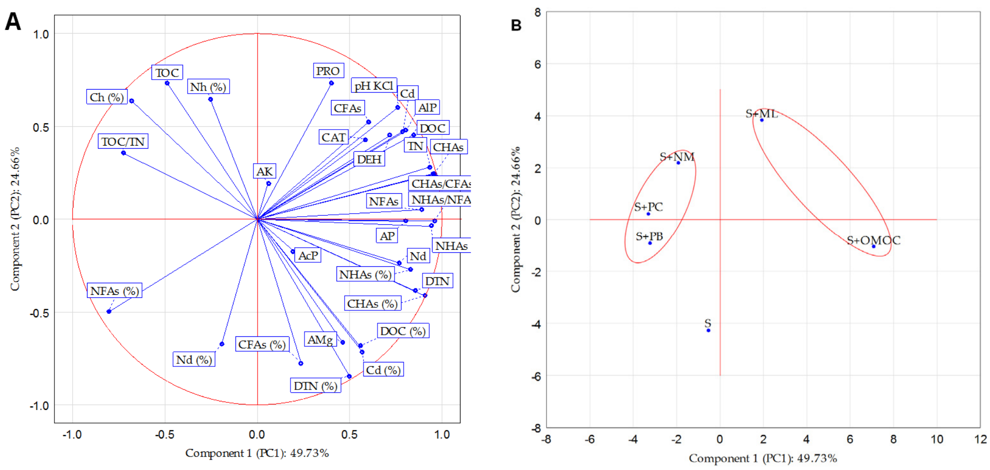

Figure 5. Principal component analysis derived from the studied soil properties; plot of the first two principal components PC1 and PC2 (**A**), cluster analysis for PC1 and PC2 (**B**).

The PCA analysis based on the examined soil parameters revealed the existence of two clusters (Figure 5B), presenting the projection of cases on the PCA factor plane. The cluster 1 (S + OMOC and S + ML) corresponded to variants with low AK content, high AP content, high DEH, CAT, AlP, AcP and PRO activity, high content and share of DOC, high content of CHAs and high value of the CHA/CFA ratio. The cluster 2 corresponds to variants S + PC, S + PB and S + NM, with the widest TOC/TN ratio, the highest AK content, low AP content, low activity of the tested enzymes, and lowest share of CHAs and the highest share of carbon in the post-extraction residue. These variants were also characterized by low values of the CHA/CFA ratio.

The use of PCA also enabled verification of the relationship significance between the studied parameters (Figure 5). Soil pH was significantly correlated with TN (r = 0.841), DOC (r = 0.977), Cd (r = 0.876), AP (r = 0.668), DEH (r = 0.917), CAT (r = 0.801), AlP (r = 0.977) and PRO (r = 0.633). To maintain catalytic activity, enzymes require an appropriate pH of the soil environment [67]. The dependence of enzyme activity on the pH of the environment is therefore one of the important factors regulating the rate of reaction. pH influences the degree of ionization of the enzyme and substrate and changes the conditions for the formation of the enzyme–substrate complex. Increased activity of alkaline phosphatase in soils amended with biochar is associated with an increase of soil pH. Soil phosphatases are the enzymes most sensitive to changes in soil pH [67]. Studies indicated that the positive effect of biochar on alkaline phosphatase activity is more likely in acidic to neutral soils (when the pH increases). There were no significant correlations between the TOC content and the activity of the tested enzymes. Similar results were obtained by Wojewódzki et al. [42] when investigating the use of biochars obtained from mellow compost, stabilized municipal sewage sludge, pine sawdust, sycamore sawdust and oak leaves.

A significant positive correlation was found between the AP content and the activity of AlP (r = 0.767) and AcP (r = 0.542). Phosphatases are enzymes involved in the circulation of phosphorus in soil [68]. With a low phosphorus content in the soil, the activity of phosphatases increases, which catalyze the hydrolysis reaction of organic phosphorus bonds to a mineral form. A significant positive correlation was found between the TN content in the soil and the PRO activity (r = 0.684). Furthermore, Chaer et al. [35] found a positive significant relationship between PRO activity and TN content (r = 0.751). Proteases are enzymes that are an important indicator of the potential rate of mineralization of organic nitrogen connections in soil. These enzymes catalyze the hydrolysis of peptide bonds in proteins and peptides to amino acids.

The conducted research revealed the influence of biochar on different soil properties and components. However, it should be noted that the impact of biochars can vary due to the material from which they are made and due to the parameters of the production process. The advances in biomass conversion mechanism [69,70] offer developed biochars [71]; however, the issue of sustainable biochar production and its environmentally safe application should be taken into consideration when using and manufacturing this material.

4. Conclusions

The biochar amendment to the soil significantly modifies the content and composition of organic matter in soil and thus affects its biochemical parameters. It significantly increases the total carbon content and the carbon share in the extraction residue (Ch), thus favoring the sequestration of this element.

The addition to the soil biochar obtained from one-month-old compost and maple leaves increased the content of available phosphorus and potassium and increased dehydrogenase, catalase and alkaline phosphatase (AlP) activity. The higher AlP activity was related to the increase of soil pH after adding the above-mentioned biochars.

Among the anlyzed enzyme indexes (G*Mea*, TEI, MAI), TEI and MAI effectively differentiated soil properties depending on the biochars used. The index that was most strongly correlated with the organic matter quality parameters is MAI.

The values of biochemical indexes (similarly to the quality indexes of organic matter) indicated that the best soil quality was obtained after the addition of biochar produced from one-month-old compost and maple leaves.

More beneficial values of soil fertility indicators were found for variants where biochars with lower values of the TOC/TN ratio and a high content of available phosphorus were amended into the soil (OMOC BC, ML BC). Taking into account the aspect of soil fertility in combination with carbon sequestration, the most favorable parameters were obtained for the variant in which the soil was mixed with biochar produced from maple leaves, characterized by the TOC/TN ratio in the range of 25–30.

Author Contributions: Conceptualization, P.W., J.L. and B.D.; methodology, P.W., J.L. and B.D.; investigation, P.W., J.L. and B.D.; data curation—compiled and analyzed the results, J.L., B.D., E.T. and P.W.; writing—original draft preparation, P.W., J.L. and B.D.; review and editing, B.D., J.L., P.W. and S.A.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data available on request.

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

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