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Enzyme Activity and Dissolved Organic Carbon Content in Soils Amended with Different Types of Biochar and Exogenous Organic Matter

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Abstract: Biochars are often proposed as a strategy for long-term carbon sequestration. Nevertheless, application of pyrolysed feedstock, particularly along with exogenous organic matter, may affect carbon dynamics in soil through introduction of labile carbon pools and stimulation of extracellular enzymes activity. The main aim of this research was to evaluate the influence of biochars and unprocessed organic amendments addition in two agricultural soils on the dissolved organic carbon (DOC) content and activity of three enzymes involved in carbon turnover. In the incubation experiment, activity of dehydrogenase, β-glucosidase, cellulase and DOC content were measured on day 30, 60, 90, 180 and 360. Addition of biochars stimulated the activity of dehydrogenase and β-glucosidase, while cellulase was suppressed. Fresh biomass amendment enhanced activity of the enzymes through priming effect. DOC content tended to be the highest in treatments with high enzyme activity, suggesting that DOC introduced with amendments acted as a source of energy for microbes. Our findings support the hypothesis that biochar properties and presence of exogenous organic matter affect microbial response in soil, what might be crucial for carbon sequestration potential of biochar. However, long-term studies are recommended to fully understand the mechanisms that determine response of soil biota to biochar addition.

Keywords: biochar; soil; organic amendments; incubation; enzyme activity; dissolved organic carbon

1. Introduction

In the last decades, the issue of rising greenhouse gases (GHG) emissions has gained particular interest [1,2]. The subject of main concern is carbon dioxide (CO_2) due to observed imbalances between CO_2 release to the atmosphere and carbon sequestration. It is estimated that the increase of CO_2 content in the atmosphere reaches billions of tons per year [3]. Therefore, international efforts of governments and scientists aim to mitigate GHG emissions. One of the strategies is carbon (C) capture and storage, that allow to retain its stable forms in the environment [4]. In this context, soils are particularly important carbon sinks, as their content of C is many times higher than in the atmosphere [5]. Moreover, it is possible to increase soil carbon pool by proper land management strategies [6], that include afforestation [7,8], non-tillage cultivation, organic farming or application of soil amendments, such as crop residues, compost, manure or sewage sludge [9,10]. However, the long-term effect of these treatments is often debatable in terms of the amount of carbon stored and the amendments have to be applied regularly, to ensure efficient soil carbon storage [4]. Another approach for soil C sequestration is the use of the amendments highly resistant for decomposition processes, with low decay rates and long estimated lifetime in the environment. In this context, biochar (BC) has attracted a lot of attention as a promising carbon sequestration tool [11,12]. Advantages of the biochars reported in the literature include long residence time – many times greater than unprocessed biomass and potential to be applied as a soil fertilizer, due to the proven

positive impact on soil chemistry, water retention and crop yields, consequently. Another argument for the use of biochar is its great availability, limited only by the supply of biomass [4].

The positive effect of carbonized organic matter on soils and crop yields has been known since ancient times and widely studied in the literature [13]. There is also a lot of research on the biochar effect on soil chemical properties [14,15], heavy metal availability or soil remediation potential [10,16]. However, the knowledge about interaction between biochar and soil microorganisms, and consequently dynamics of carbon pool in biochar-amended soil is still limited. Soil carbon pool is complex and consists both of labile fractions with short residence time of few years to decades, and recalcitrant compounds with estimated lifetime of hundreds years [17]. Labile carbon fractions are considered a good indicator of soil quality, as they timely reflect the processes ongoing in the environment [18,19]. Particularly interesting part of the carbon pool is dissolved organic matter or dissolved organic carbon (DOC), defined as the most mobile portion of soil organic matter with particle sizes smaller than 0.45 μm [20]. DOC does not participate in C sequestration and promotes carbon losses with water runoff [21]. According to the current state of knowledge, DOC fluxes play an important role in the global carbon cycle, therefore this indicator may be useful in research on C sequestration [22]. Another factor with rapid responses to environmental changes in amended soils is microbial activity. Microbes are involved in short-term utilization of nutrients, therefore their activity reflects organic matter turnover. Via a variety of enzymes, microorganisms are able to decompose organic substances in soil and these processes start from the most labile, easily available compounds that will not contribute to the long-term carbon sequestration [23]. Therefore, we hypothesized that microbial activity along with dissolved organic carbon content can reflect the changes in soil organic pool in soils amended with biochar. In order to ensure effective C sink, it is necessary to identify changes after biochar application. Measurements of the most mobile carbon fractions in amended soils seem to be crucial for understanding the changes in soil organic carbon quality and quantity.

The aim of presented research was to evaluate DOC pool and microbial activity in biochar-amended soils, considering biochars derived from six different feedstocks and their co-application with other organic amendments: compost, manure and fresh legume biomass, commonly used in agriculture. We measured the activity of β-glucosidase (GA), dehydrogenase (DHA) and cellulase (CA), recommended as indicators of soil organic matter (SOM) turnover [18], along with DOC content. On that basis, carbon sequestration potential of tested biochars and impact of organic amendments on carbon pool dynamics were evaluated.

2. Materials and Methods

2.1. Soils, biochars and organic amendments

An incubation experiment in laboratory conditions has been carried out to study the influence of biochar and organic amendments on DOC content and enzyme activity in tested soils. The experimental soil samples included silt loam (SiL) and loamy sand (SA), collected from the topsoil layer (0-25 cm) of arable land near Trzebnica, Poland (51°18′ 17″ N; 17° 3′ 41″ E). Before the experiment started, moist soil samples were stored in the refrigerator at 4 °C, to keep them biologically active. Biochars were derived from six different feedstocks, accepted as biomasses available for pyrolysis [24]: kitchen wastes (BC1), cut green grass (BC2), coffee grounds (BC3), wheat straw (BC4), sunflower husks (BC5) and beech wood chips (BC6). Each biomass was pyrolyzed at 550 °C for 60 minutes in nitrogen atmosphere. Additionally, three organic amendments commonly used as organic fertilizers in agronomic practices: compost (CO), cattle manure (MA) and fresh legume biomass (LE) were tested. Compost was produced from kitchen and garden organic waste at home composter. Cattle manure was obtained as a dry fertilizer from Fertigo company, Poland. Legume plant biomass of red and white clover (*Trifolium repens L., Trifolium pratense L.*) originated from meadows around Wrocław city, Poland.

Basic properties of the substrates were evaluated before the experiment. Prior to laboratory analyses, samples of all materials were air-dried, sieved with 2 mm mesh and prepared following

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standard methodologies. Particle size distribution of soils was determined by mesh and hydrometer method. Cation exchange capacity (CEC) was measured in soil and biochar samples on Microwave Plasma-Atomic Emission Spectrometer MP-AES 4200 (Agilent Technologies, Santa Clara, CA, USA), after sample extraction with 1 M ammonium acetate and pre-treatment with isopropanol [25]. Total organic carbon (TOC) and total nitrogen (TN) content in the substrates were analyzed on TOC/TN analyzer (Elementar, Langenselbold, Germany). Ash content was calculated based on the loss of mass after combustion at 550 °C in a muffle furnace [26]. Properties of the substrates are presented in Table 1.

Table 1. Characteristics of soils, biochars and organic amendments used in the experiment.

Abbr. in paper		**Substrate**	**pH** (H_2O)	**CEC** [1] [cmol (+) kg^{-1}]	**TOC** [g 100 g^{-1}]	**TN** [g 100 g^{-1}]	**Ash** [%]
Soils	SA	Loamy sand	4.62	1.62	0.72	0.04	n/a
	SiL	Silt loam	6.40	11.70	0.99	0.07	n/a
Biochars	BC1	Food wastes	9.41 ± 0.05	228	53.0 ± 1.10	2.05 ± 0.16	10.1 ± 1.00
	BC2	Cut green grass	10.43 ± 0.04	228	52.0 ± 1.00	2.37 ± 0.01	31.3 ± 3.10
	BC3	Coffee grounds	6.91 ± 0.07	35.0	68.0 ± 1.40	3.16 ± 0.37	3.70 ± 0.40
	BC4	Wheat straw	7.20 ± 0.13	7.41	76.0 ± 1.50	0.32 ± 0.26	1.30 ± 0.1
	BC5	Sunflower husk	10.29 ± 0.02	35.3	78.0 ± 1.60	0.80 ± 0.06	5.60 ± 0.60
	BC6	Wood chips	6.96 ± 0.07	22.7	70.0 ± 1.40	1.23 ± 0.07	9.80 ± 1.00
Organic matter	CO	Compost	5.66	10.8	17.6	2.01	n/a
	MA	Manure	7.00	n/a	28.0	1.90	n/a
	LE	Legume plants	n/a	n/a	51.8	n/a	12.20

[1] In table: Abbr. – abbreviation, CEC – cation exchange capacity, TOC – total organic carbon content, TN – total nitrogen content, n/a – analysis not applicable. Values are means ± standard deviation from three replicates, if available.

Soils in the experiment differed in terms of texture and basic chemical properties. Loose loamy (SA) sand was characterized by low cation exchange capacity, organic carbon content (0.72 %) and total nitrogen (0.04 %), along with acidic pH. Silt loam (SiL) was more fertile, with well-developed sorption complex and significantly higher content of organic carbon (0.99 %) and nitrogen (0.07). Biochars obtained from different biomass exhibited varied with basic properties. pH of BCs was neutral to alkaline, carbon content from 52 % for kitchen waste BC, with lowest carbonization rate, up to 78 % of C in highly carbonized biochar from sunflower husk. In general carbonization rate of BCs obtained under similar temperature and time regime conditions was correlated with the content of lignocellulose. The highest content of nitrogen (3.16 %) was in coffee grounds biochar, while even tenfold lower content of TN was determined in wheat straw and sunflower husk BC (Table 1).

2.2. Incubation experiment

Prior to the experiment, all of the substrates were manually crushed or, in case of fresh clover, cut with scissors, to pass 2 mm sieve. Before cutting, clover plants were rinsed with distilled water, to avoid introducing contaminants with soil and dust particles. Amendments were thoroughly mixed with sandy and loamy soil at rates: 2% (v/w) of biochar, corresponding to 0.565 – 0.915 t ha^{-1}, depending of biochar's bulk density, and 1% (w/w) of organic matter, what is an equivalent of 37.5 t ha^{-1} (Table 2). Then, 100 g of mixed substrates were placed in 550 mL glass vessels in three replicates, and left open to allow gas exchange. The vessels were incubated at constant temperature of 22 °C, in place protected from direct sunlight, and watered with distilled water to maintain the moisture at 20% by weight.

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Table 2. Summary of the treatments.

Description	Abbreviation
Loamy sand without amendments	SA
Loamy sand + 6 types of biochar	SA BC1 - SA BC6 [1]
Loamy sand + 6 types of biochar + 3 types of organic matter	SA BC1- BC6 + CO for compost SA BC1- BC6 + MA for manure SA BC1- BC6 + LE for legumes
Silt loam soil without amendments	SiL
Silt loam soil + 6 types of biochar	SiL BC1 - SiL BC6
Silt loam + 6 types of biochar + 3 types of organic matter	SiL BC1- BC6 + CO for compost SiL BC1- BC6 + MA for manure SiL BC1- BC6 + LE for legumes

[1] - respectively for 6 biochar types.

2.3. Activity of enzymes

Activity of all tested enzymes and dissolved organic carbon content in incubated samples was determined at the day 30th, 60th, 90th, 180th and at the end of incubation (day 360). Concentration measurements based on colorimetry were performed using the Cary 60 UV-Vis spectrophotometer (Agilent, Santa Clara, CA, USA).

β-glucosidase

β-glucosidase (GA) in soils participates in microbial degradation of sugars: maltose and cellobiose, that are utilized by microbes as a source of energy. Due to that, GA is considered a reliable indicator of organic matter turnover [27]. Activity of the enzyme was measured colorimetrically, based on the estimations of p-nitrophenol (PNP). The principle of this method is to determine the quantity of PNP, produced in hydrolysis of p-nitrophenyl-beta-D-glucopyranoside. Briefly, 1 g of moist sample was incubated for 1 hour in 37 °C with buffer and toluene. Then, the yellow color was developed by the addition of 0.5 M $CaCl_2$ and TRIS buffer with pH = 12 [27]. Measurements of absorbance were conducted in three replicates at 400 nm wavelength. Activity of β-glucosidase was expressed as micrograms of PNP released by 1 g of dry soil sample in one hour [28].

Dehydrogenase

Dehydrogenase (DHA) is often proposed as an indicator of microbial activity as well as changes in soil quality. The enzyme is crucial in biological decomposition of organic matter, by transferring the electrons and protons in the oxidative degradation (dehydrogenation) process. Assay applied in this study assumes the reduction of 2,3,5- triphenyltetrazolium chloride (TTC) to red-colored formazan (TPF), that can be measured colorimetrically. In this method, 6 g of moist sample was incubated for 20 hours in 30 °C with TTC solution, with the addition of $CaCO_3$. After the incubation, 25 mL of ethanol was added to the suspension to extract produced TFP. Red solution was filtered and concentration of TPF was measured at wavelength of 485 nm. Activity of dehydrogenase was expressed as millimoles of TPF released by 1 g of soil dry mass, during 20 hours of incubation [29,30].

Cellulase

Cellulases (CA) are a group of enzymes responsible for the degradation of cellulose, one of the most abundant organic components in the biosphere, that can be transformed by microorganisms into oligosaccharides. Since cellulose is the most common biopolymer in the environment, activity of cellulase is crucial to understand soil C cycle and organic matter turnover [31]. Activity of this enzyme was estimated following the principles of methodology described in detail by Zhang et al. [18] (in supplementary materials), based on the anthrone colorimetry [32]. 1 g of moist soil sample was treated with toluene and then incubated with carboxymethyl-cellulose solution and acetate buffer. Samples were incubated in 37 °C for 3 h, and then the temperature was increased to 90 °C for 15 minutes. The suspension was filtered and anthrone reagent was added to the clear filtrate. Samples were left for 10 minutes to develop the blue color. Cellulase activity was measured at 620 nm wavelength and expressed as micromoles of the enzyme per 1 g of dry soil mass per 1 day.

Dissolved organic carbon

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Dissolved organic carbon (DOC) extraction methods described in the literature differ in terms of the main reagent and assume the use of distilled water, diluted NaOH or HCl, as well as neutral salts, mainly KCl and K_2SO_4 [20,33]. Considering the advantages and drawbacks of available approaches, it was chosen to extract DOC with water that reflects natural conditions in soil without changes in pH [34]. Time of extraction is also a subject of discussion, however, as a result of our own observations, no significant differences were noted between the amount of DOC determined after 1 h and 24 h of extraction. Protocol applied in this study assumed extraction of soil samples with ultrapure water in 1:20 ratio. Samples were shaken on the rotary stirrer for 1 hour, then the suspension was pre-filtered with a cellulose filter. To ensure that fraction remained in the solution is DOC (particles smaller than 0.45 µm), extracts were additionally filtered with MCE (mixed cellulose esters) syringe filters, pre-washed with 5 mL of distilled water, with pore diameters of 0.45 µm. Organic carbon content in extracts, that reflect the DOC content, was determined on sample TOC/TN analyzer (Elementar, Langenselbold, Germany).

2.4. Data analysis and visualization

Results of the experiment were stored and calculated using MS Excel Software (Microsoft, Redmond, WA, USA). Statistical tool used to compare effect of biochar on enzyme activity and DOC content was ANOVA, applied on cumulative results, in order to consider the whole incubation period, not only the varied observations of particular measurements. ANOVA analysis was performed using R software for Windows. Figures were prepared in GraphPad Prism 5 Software for Windows (GraphPad Software Inc., San Diego, CA, USA), along with the calculations of standard deviation. The charts were combined into collective graphics using the Canva application (Perth, Australia).

3. Results

3.1. β-glucosidase activity

The results indicated that biochar application to soil had a relevant effect on the β-glucosidase activity (GA) (Figure 1). In sandy soil (SA), biochar application increased β-glucosidase activity between 60th and 180th day of incubation, however no significant differences ($p < 0.05$) were observed between tested biochars originating from different biomass. The effect of the feedstock was more pronounced in SiL BC3 treatment indicating significantly ($p < 0.05$) higher values of GA in coffee ground biochar treated soil (up to 230.2 µg PNP g^{-1} h^{-1}). Application of organic matter also contributed to the process, nonetheless better response to exogenous organic matter was indicated on silt loam soil (SiL). On sandy soil, the highest peak of β-glucosidase activity was determined on treatments SA BC4 (wheat straw BC) and SA BC5 (sunflower husk BC) with additional compost application and SA BC2 (cut grass BC) along with SA BC5 for legume biomass treated soil. In SiL BC treatments application of CO, MA or LE caused an increase of GA after 60 days from amendment application, however changes between different SiL BC treatments were not statistically significant ($p < 0.05$). The β-glucosidase activity decreased with time reaching the lowest values at the 12th month of the incubation experiment.

3.2. Dehydrogenase activity

In all treatments soils dehydrogenase activity (DHA) was higher in SiL compared to SA (Figure 2). Biochar presence in tested soils affected microbial activity with respect to untreated soil. Significant ($p < 0.05$) changes were indicated in SA BC1 (food waste biochar) and SA BC3 (coffee ground biochar) treatments, while for SA BC5 and SA BC6 higher than detectable by method DHA values were registered after 180 days from BC application, showing that less carbonized biochars with high TN content are more prone to microbial degradation compared with high lignocellulose biochars obtained from biomass with low TN values (Table 1). Considering the impact of additional organic amendments, there was a positive effect of manure (MA) on DHA in both soil types. Dehydrogenase activity reached up to 6.60 µmol TPF g^{-1} 20 h^{-1} on SiL BC4 + MA or 7.76 µmol TPF g^{-}

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^{1} 20 h^{-1} on SiL BC5 + MA, being several times higher than in other tested variants. The lowest values were noted on compost-amendment soils, up to 2-3 μmol TPF g^{-1} 20 h^{-1}, nonetheless they were higher than on soils with solely biochar addition (without organic fertilizers). In general, the effect of organic amendment on dehydrogenase activity was similar for both tested soil types. The greatest impact on DHA was observed for manure, followed by legume biomass and the lowest for compost.

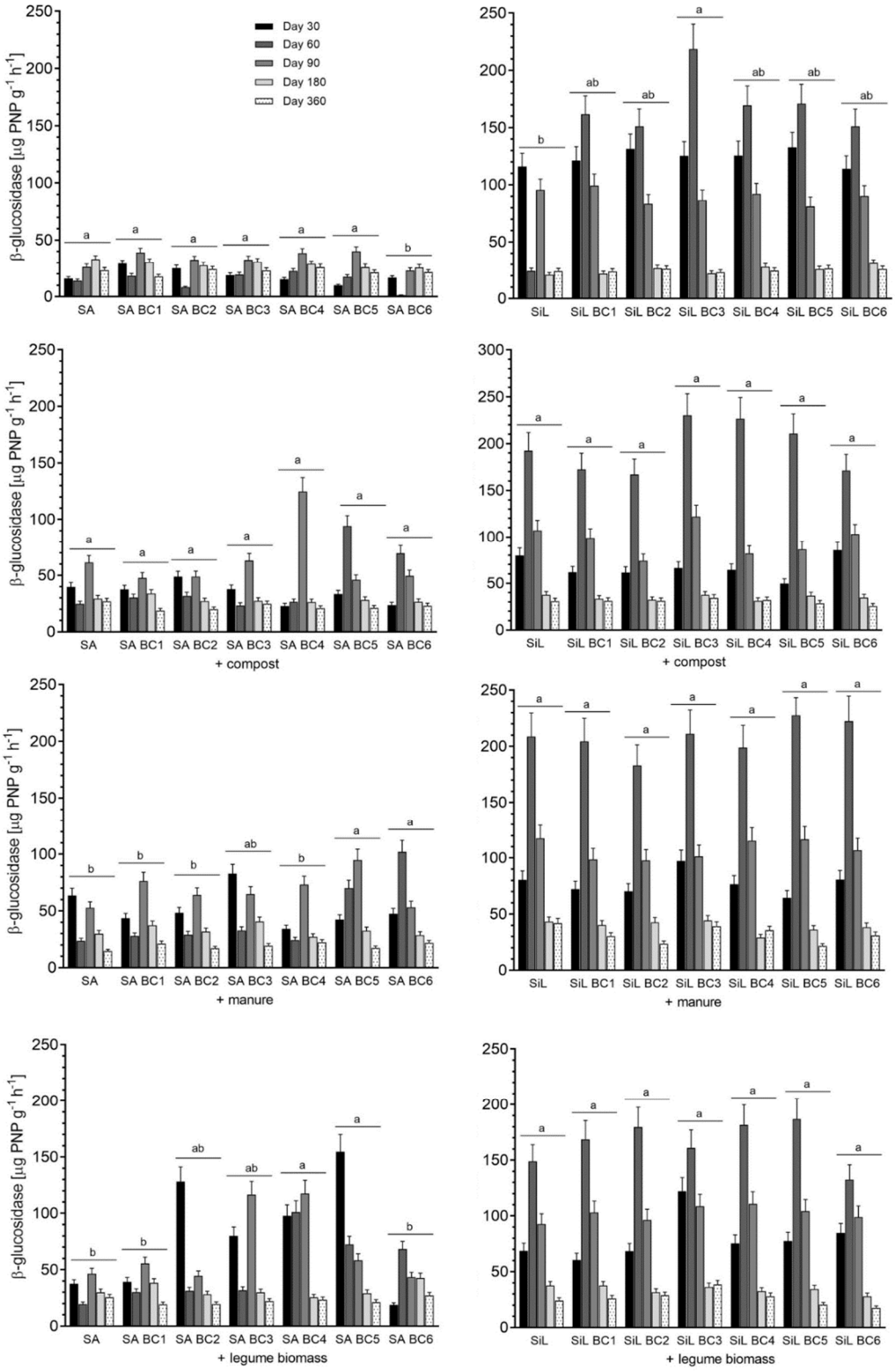

Figure 1. β-glucosidase activity among tested treatments. SA = sandy soil, SiL = silt loam soil, BC1-6 = biochars (see Table 1). Values are means ± SD from three replicates. Letters indicate homogenous groups considering biochar type as a main factor ($p < 0.05$).

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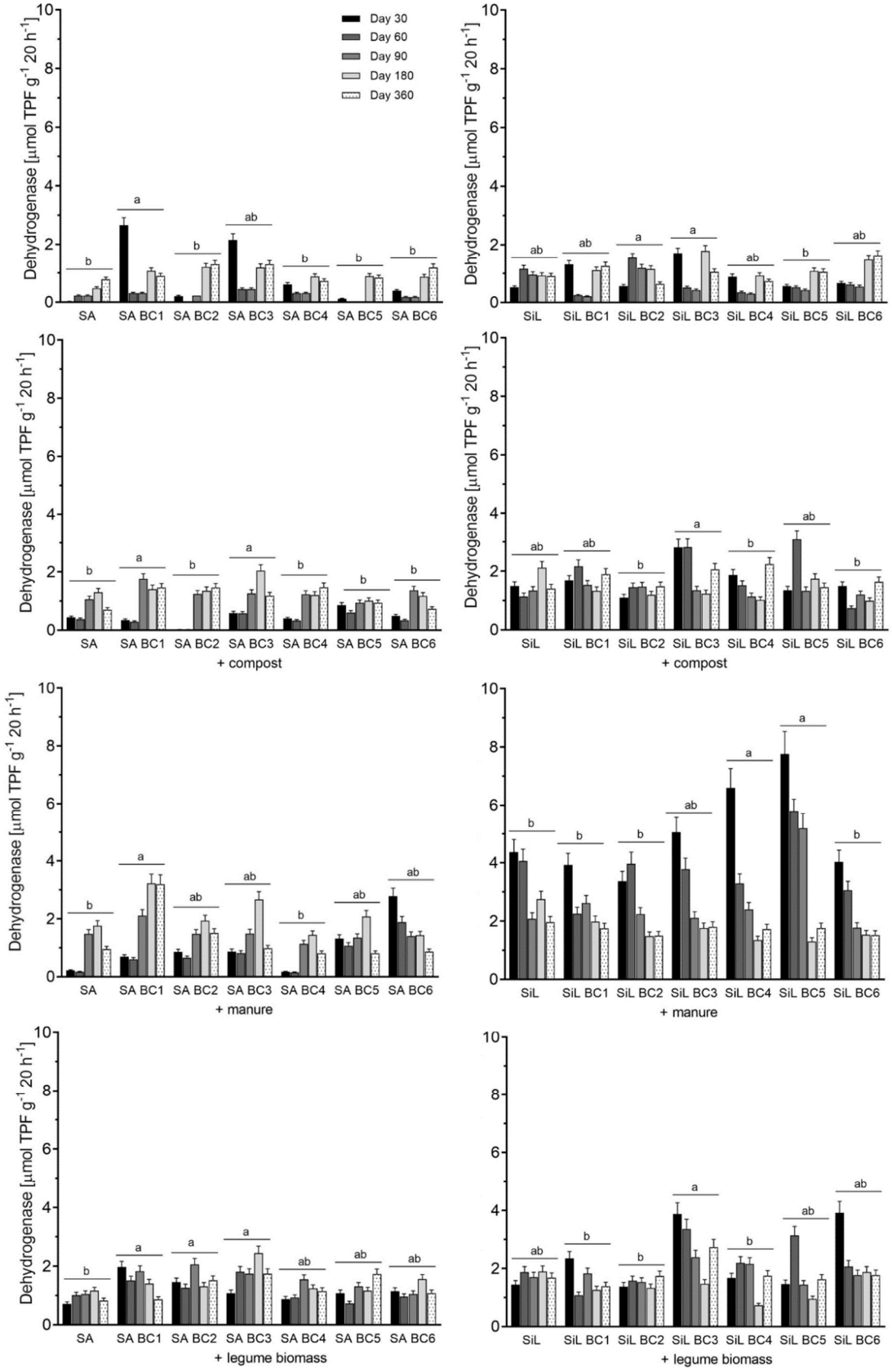

Figure 2. Dehydrogenase activity among tested treatments. SA = sandy soil, SiL = silt loam soil, BC1-6 = biochars (see Table 1). Values are means ± SD from three replicates. Letters indicate homogenous group considering biochar type as a main factor ($p < 0.05$).

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3.3. Cellulase activity

Opposite effect compared to GA and DHA was noticed for cellulase activity (CA), indicating higher values on sandy soil (SA) compared to silt loam soil (SiL) during the whole incubation period (Figure 3). In biochar-amended treatments, increase of enzyme activity was observed between 90th and 180th day of incubation, decreasing rapidly with time. The highest peaks of CA were detected on the 180th day of incubation. Compost and manure application to sandy soils with biochar decreased CA compared with treatments without biochar addition. The highest values were measured in control soil without biochar combined with manure or compost - peak 75.60 μmol g^{-1} 24 h^{-1} in SA + CO treatment (Figure 3). Opposite effect of enhanced CA activity was observed in SA BC treatments with addition of fresh legume biomass, however on SA BC5, CA values were the lowest at significant level ($p < 0.05$). In silt loam soil CA was the highest in SiL BC3 and SiL BC4, however application of compost or manure did not enhance enzymatic activity. Co-application of biochar with organic amendments in some cases resulted in significant inhibition of CA activity, compared with non-biochar-amended treatments (SiL BC3 + MA, SiL BC6 + MA). Increased CA was observed for both tested soils after co-application of raw legume biomass with food waste biochar (BC1), wheat straw (BC4) and sunflower husk (BC5) (Figure 3).

3.4. Dissolved organic carbon

Dissolved organic carbon (DOC) represents the mobile pool of organic matter, easily available to microbes. Application of biochar impacted the content of DOC, however the effect was distinct in both tested soils. In SA treatments the DOC content increased rapidly after BC application up to the first 90 days of incubation. The highest content of DOC was observed in SA BC1 and SA BC3, while some biochars e.g., SA BC2 did not contribute to the process (Figure 4). In SA BC soils treated with compost no significant changes were observed between the treatments, while application of manure to SA BC soils increased DOC content and surprisingly the highest peak was observed on SA BC2 with the lowest initial content of DOC. Application of raw organic matter in the form of legumes along with biochars did not significantly affect the DOC, with exception of SA BC1 treatment (Figure 4). In SiL treatments significant ($p < 0.05$) increase of DOC after biochar application was only observed for SiL BC1, and similarly to SA the effect of biochar application on DOC content was observed after 60th to 90th day of incubation. Co-application of biochars with compost and legume biomass did not significantly affect the DOC in silty soil, while the greatest significant ($p < 0.05$) effect was observed in SiL BC1 + MA treatment. Depending on the variant of the experiment, the maximum concentrations of DOC were observed at different stages of incubation. In treatments with BC1 (kitchen waste biochar) DOC content was particularly high at the beginning (day 30th and 60th). In soils amended with BC4 (wheat straw), BC5 (sunflower husks) or BC6 (wood chips biochar), maxima of DOC concentration were observed at 60th and 90th day of incubation. Moreover, after 360 days the DOC concentrations were in almost every treatment higher than at previous measurement at day 180, probably due to the decomposition of tested organic amendments. Considering the effect of biochar type on the DOC content among the treatments, kitchen waste biochar (BC1) significantly ($p < 0.05$) increased the labile carbon pool in almost every tested combination. In most cases, however, no significant differences at ($p < 0.05$) were noted between studied biochars, considering the entire incubation period.

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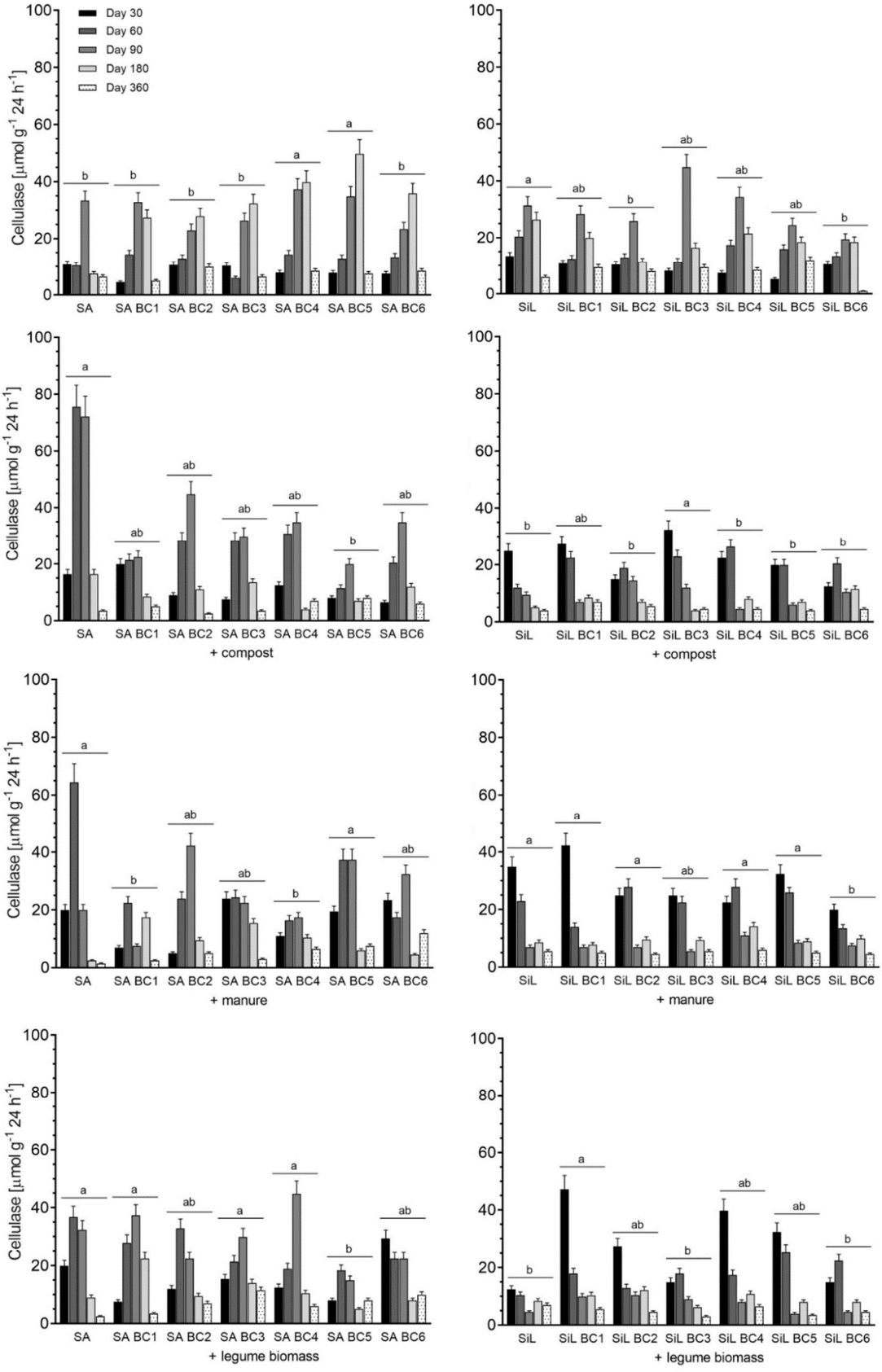

Figure 3. Cellulase activity among tested treatments. SA = sandy soil, SiL = silt loam soil, BC1-6 = biochars (see Table 1). Values are means ± SD from three replicates. Letters indicate homogenous group considering biochar type as a main factor ($p < 0.05$).

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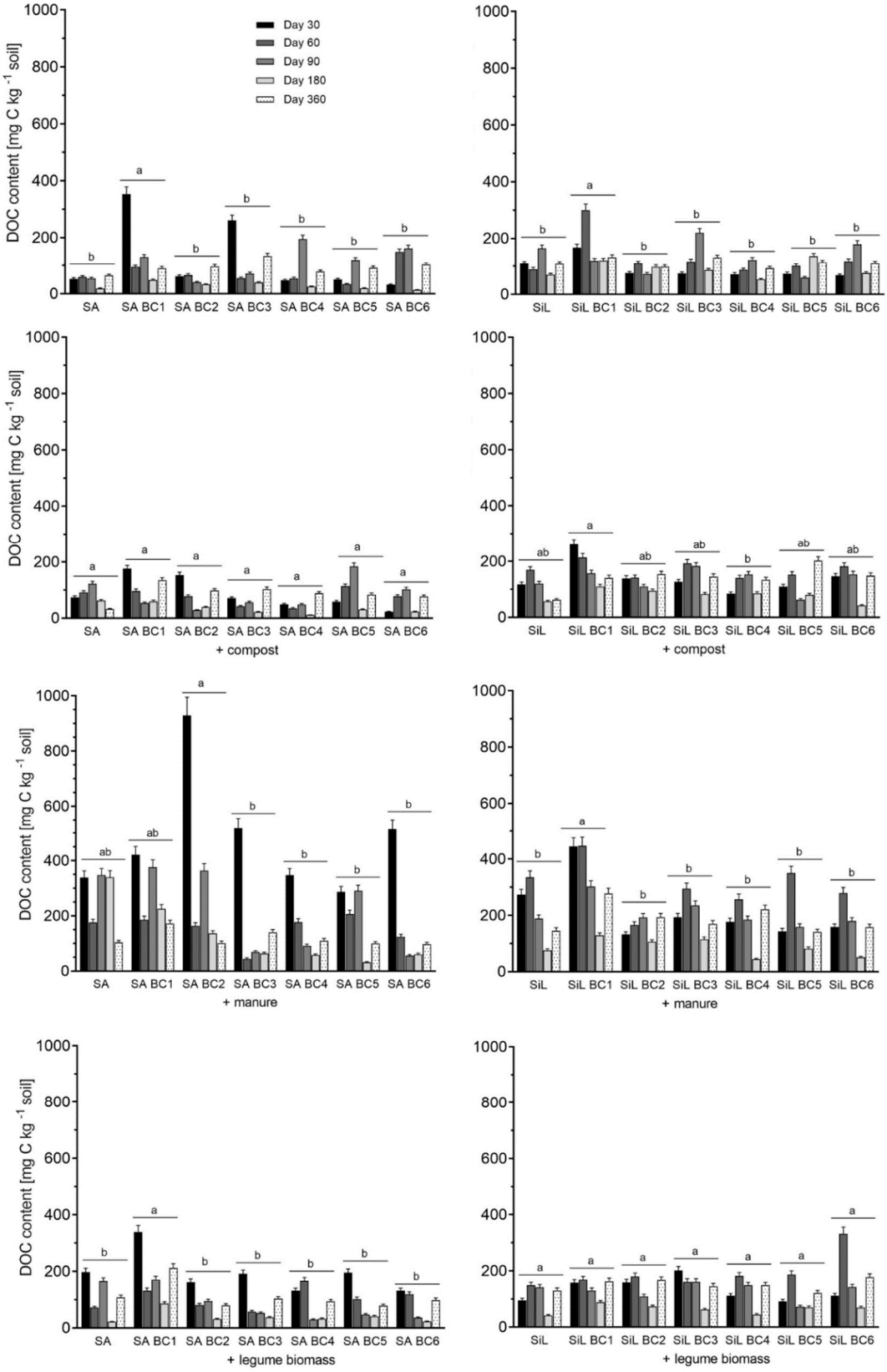

Figure 4. Dissolved organic carbon content among tested treatments. SA = sandy soil, SiL = silt loam soil, BC1-6 = biochars (see Table 1). Values are means ± SD from three replicates. Letters indicate homogenous group considering biochar type as a main factor ($p < 0.05$).

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4. Discussion

Although the biochar effect on soil properties has been recently studied and discussed by researchers, the knowledge about BCs role in C turnover and sequestration of CO_2 is largely unknown. Microbial activity is crucial for the process of soil organic matter (SOM) mineralization. The addition of exogenous organic amendments like biochar, manure or fresh biomass can affect decomposition of SOM, mainly by becoming an additional source of C, nutrients and moisture to soil microbes. Based on our previous research, the content of potentially available to microbes forms of C in biochars, e.g., DOC or polysaccharides, depends on biochar origin. Some biochars, due to their properties, can be more prone to microbial degradation, contributing to the process of C turnover in soil [35]. The addition of organic amendments influences the physical and chemical environment of the soil, and therefore affects soil microorganisms [36]. Enzymatic activity helps to identify the main drivers of the C, N and P biogeochemical cycles and extracellular enzyme activity is considered as one of the most important indicators for assessing the stability of organic matter in soils amended with biochar [37,38]. One of the objectives of this research was to determine the effect of biochar derived from different feedstock on soil enzyme activity and to justify if soil enzymes are useful indicators of biochar impact on C cycle. For better understanding the effects of biochar addition on CO_2 sequestration under field conditions, we compared enzymatic activity from biochar-amended soils with soils amended with biochar and exogenous forms of organic matter (manure, compost and fresh legume biomass), commonly applied to soil due to agriculture practices.

Presented results confirm that enzymes are sensitive indicators of changes in soil environment caused by the addition of biochar or organic matter [39]. However, the effect of biochar and biochar co-application with unprocessed organic matter on soil enzyme activity was inconsistent. As our data showed, these responses vary depending on biochar origin soil type, presence of exogenous organic carbon (EXOC) or even tested extracellular enzyme. For example, biochar and EXOC application tended to increase activity of dehydrogenase and β-glucosidase, while cellulase activity was inhibited compared with non-amended soils. Similar findings on C-cycle enzymes were reported by Wang et al., [40] or Khadem and Raiesi [41]. The effect of biochar on the extracellular enzymes activity is known to depend on the interaction of substrate and enzyme (e.g., in sorption and desorption processes), and could be affected by biochar porosity or specific surface area [42]. Biochars produced at high temperature, with more aromatic structure and well developed functional groups on the surface tend to bind nutrients and extracellular enzymes, thus reducing soil enzyme activity. In our study biochars obtained at 550°C did not reduce β-glucosidase and dehydrogenase activity, however lower carbonization rate, higher total nitrogen content and more aliphatic properties of biochars derived from kitchen wastes and coffee grounds seems to have more pronounced impact on soil microbial activity [43]. The highest enzymatic activity in soils amended with kitchen waste (BC1) and coffee grounds (BC3) biochar confirmed findings of our previous analysis [35]. Biochars characterized with the high content of labile carbon fractions, such as DOC or water soluble carbohydrates (WSC) are more prone to degradation processes, becoming a source of easily-utilized carbon for soil microbes, thus enhancing microbial activity [44,45]. Comparing the data regarding chemical characteristics of biochars with microbial activity after their application into the soil, we can conclude that biochar carbonization rate and H:C or O:C ratios are useful predictors of their recalcitrance in soil [41,46].

Increase of β-glucosidase and dehydrogenase activity in soils amended with BCs and EXOC stays in agreement with findings of other studies [47,48], and can be explained as a consequence of increased soil organic carbon content, which is a source of energy for microorganisms and promotes microbial activity [49–51]. Mierzwa – Hersztek et. al. [52] indicated that application of wheat straw biochar with co-application of nutrients increased the population of soil microorganism, thus increasing dehydrogenase activity. Bailey et al.,. studying effects of biochar made from fast pyrolysis of switchgrass described increased β-glucosidase activity (up to 7 folds) in shrub-steppe soil [53]. Opposite effect of biochar application to soils was indicated in terms of cellulase activity. Suppression of cellulase activity caused by biochar was reported by Feng et al., [54], who performed comprehensive meta-analysis of data from 130 research papers. Several factors were indicated as

responsible for cellulase activity inhibition e.g., biochar feedstock type, pyrolysis temperature or soil texture. It was noted that herb and wood biochars (BC2 and BC6 in this study) tended to significantly reduce cellulase activity, along with sandy and clayey soil texture [54]. The effect of suppressed cellulase activity can be attributed to the properties of biochar or changes in microbial community after amendment application. Biochar addition by introducing additional phenolic and lignin-like compounds, can alter the chemical composition of soil organic matter, reducing the bioavailability of C compounds decomposable with cellulase [54,55]. Li et. al. [56] in meta-analysis pointed out that biochar causes a shift towards a fungi-dominant microbial community, promoting ligninase activity and suppressing cellulase in biochar amended soils. Suppressed activity of the enzyme is beneficial for long-term carbon sequestration in soil, reducing the biodegradation of polysaccharides [57]. However, the response of cellulase to BC amendment often varies between short-term (<1 year) and long-term experiment, which may cause misleading conclusions regarding C-sequestration potential based on this parameter [58].

The response of soil enzymes to biochars was highly variable, and not only depended on biochar origin and properties, but also on the soil properties e.g., texture, pH, carbon and nitrogen content. In the study, higher activity of extracellular enzymes was observed on less acidic SiL soil with higher carbon and nitrogen content. Also clay minerals can contribute to the process [59], increasing the availability of mineral N [60] and promoting the production of C-decomposing enzymes [61]. Manure, compost and legume biomass impacted biochar amended soil differently compared to application of solely biochar. We assumed that partly decomposed organic matter from manure and compost was easily available to microorganisms. Organic manure and compost are known to have a great impact on the carbon content and microbial activity, compared with mineral fertilizers [62]. The effect of manure and compost application on enzyme activity enhancement was often the greatest between day 60 and 180 from application, while microbes were able to utilize carbon and nitrogen from fresh legume immediately after biomass application to soil. Results of the study indicated that co-application of biochar with fresh biomass on non-tillage agronomic practices accelerates turnover of C in soil, thus limiting efficiency of C sequestration process in biochar amended soils.

DOC analysis in soil can be also a useful tool in predicting the potential of organic amendment to increase/decrease soil microbial activity. In the study, we have used this indicator to identify which of tested biochars are potentially more prone to degradation processes. The DOC content in BCs corresponded well with changes of enzymatic activity after biochar application. For example, the highest DOC content in soils with BC1 and BC 3, was in line with the initial high DOC content in these biochars and enhanced enzyme activity in amended soils. Karimi et al., [63] and Wojewódzki et al., [39] reported that biochar application to soil increases DOC content, along with dehydrogenase activity, and described positive correlation between DOC and enzyme concentration. Positive correlation was also found between DOC and β-glucosidase, suggesting that labile carbon pool introduced into the soil provide energy for microbes and support their activity [64]. In this context, it should be explained why the content of DOC was quite equal between soil types, despite the higher enzyme activity in SiL soil. Dissolved organic carbon is mobile and easily-leachable. Accumulation and stabilization of organic compounds is affected by the presence of soil clay minerals [65]. As the clay content was higher in SiL soil, DOC was adsorbed and could be utilized as a source of energy for microbes, contrary to sandy substrate, where labile carbon fractions were easily leached in the first months of incubation.

Responses of enzyme activity and DOC to biochar and EXOC addition could have an effect on carbon sequestration. As EXOC acts as a source of carbon for microbes, what was expressed by enhanced DOC content along with increased microbial activity in treatments with compost, manure or legumes, co-application of BCs and EXOC may cause positive priming effect and reduce the carbon sequestration potential. However, literature meta-analysis of available data on the correlation between enzymes activity and carbon sequestration potential of biochar indicates that short-term and long-term results are often contradictory [54], and during the incubation period some fluctuations were observed. Moreover, it is underlined that simple shifts in mobile carbon pool and microbial activity cannot fully explain BCs carbon sequestration potential, as other soil properties and processes

could also significantly influence this process [66]. However, described relationships between biochar properties such as molar ratio, labile carbon content and enzyme activity allow certain conclusions to be drawn about the factors that promote biochar degradation in soils and about potential of tested biochars for carbon sequestration. Results showed that weakly carbonized biochars, such as those from food biomass, will be more susceptible to microbial attack and decompose faster in the soil than more carbonized pyrolyzed high lignocellulose biomass.

5. Conclusions

Presented observations showed that activity of the enzymes along with dissolved organic carbon content differ depending on the soil type, biomass used as a feedstock for biochar production or presence and type of exogenous organic matter. Considering soil type, enzyme activity tended to be enhanced on silt loam, compared to loamy sand, as a result of greater content and availability of organic C and N, acting as a source of energy for microbes. Addition of EXOC promoted microbial activity due to the incorporation of DOC and nutrients, causing short-term priming effect. The response of enzymatic activity varied between treatments and analyzed enzymes. Application of biochar increased β-glucosidase and dehydrogenase activity, similarly to the introduction of raw legume biomass, manure or compost, while cellulase activity was suppressed, what can be explained by changes of soil organic matter composition and presence of lignin more prone to degradation by fungi and with other enzyme - ligninase. Low-carbonized food waste biochars, containing larger pool of labile compounds, were more susceptible for microbial attack than well-charred wood or grass biomass. Our findings support the hypothesis that biochar properties and presence of additional organic matter greatly affect microbial response in soil and thus are important for carbon sequestration potential. Application of well-carbonized biochars in soils with low organic matter content may prevent organic carbon losses, thus contributing to C sequestration and maintaining soil quality. However, long-term studies are highly recommended to fully understand the mechanisms that determine response of soil biota to biochar addition.

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