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Czynniki decydujące o degradacji biowęgla w glebie w kontekście możliwości wykorzystania węgla pirogenicznego jako narzędzia sekwestracji CO_2

Factors determining biochar degradation in soil in the context of possible use of pyrogenic carbon as a CO_2 sequestration tool

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Spis treści

STRESZCZENIE

Stale rosnąca emisja dwutlenku węgla (CO_2) ze źródeł antropogenicznych wymusza opracowanie strategii umożliwiających efektywną sekwestrację węgla w środowisku. Szczególnie dużo uwagi poświęca się trwałości form węgla obecnych w glebie, a za wyjątkowo stabilny uważany jest węgiel pirogeniczny (z ang. *black carbon*). Jedną z form węgla pirogenicznego, współcześnie wprowadzanego do gleb przez człowieka jest biowęgiel (z ang. *biochar*). Biowęgiel powstający w wyniku termicznego przekształcania biomasy w warunkach ograniczonego dostępu tlenu (piroliza) charakteryzuje się wysoką zawartością węgla (> 50%) i uważany jest za skuteczne narzędzie sekwestracji CO_2. Trwałość biowęgla w środowisku glebowym zależy od jego właściwości, a także szeregu czynników abiotycznych oraz biotycznych, które mogą przyczyniać się do naruszenia jego struktury i trwałości. Rozważając możliwość wykorzystania biowęgli jako dodatku do gleb mającego duży potencjał do sekwestracji węgla, należy zbadać czynniki warunkujące jego stabilność w glebie. Jedną z metod szacowania trwałości biowęgla jest ocena jego właściwości chemicznych i wyliczenie stosunków molowych C:O i H:C. Ocena ta nie uwzględnia jednak wpływu czynników zewnętrznych, dlatego szerszym podejściem jest wykorzystanie nowych narzędzi analitycznych i badanie puli węgla labilnego, która jest aktywna chemicznie i biologicznie oraz podlega w glebie procesom zbliżonym do glebowej materii organicznej.

Głównym celem niniejszej pracy była analiza podatności biowęgli na degradację w zróżnicowanych warunkach w celu oceny ich przydatności do sekwestracji węgla. Badania prowadzono w oparciu o trwający 12 miesięcy eksperyment inkubacyjny. Uwzględniono trzy grupy zmiennych, wytypowanych jako czynniki potencjalnie istotne dla przebiegu degradacji biowęgla: (1) różnorodność biomasy wykorzystanej jako substrat do procesu pirolizy, (2) rodzaj gleby do której wprowadzony był dodatek, związany ze zmiennością uziarnienia i właściwości chemicznych, (3) obecność egzogennej materii organicznej. W celu oceny stabilności biowęgla posłużono się standardowymi metodami analitycznymi, jak również na podstawie dostępnej literatury zaproponowano zakres analiz, które miały charakteryzować biowęgiel w odniesieniu do potencjalnych czynników abiotycznych i biotycznych wpływających na jego stabilność. Realizacja badań umożliwiła weryfikację hipotez, które zakładały, że każda z trzech analizowanych grup zmiennych może mieć istotne znaczenie dla interakcji biowęgla ze środowiskiem i w konsekwencji dla jego przydatności do sekwestracji węgla.

Stwierdzono, że rodzaj biomasy przekłada się na zróżnicowanie właściwości biowęgli. Skład elementarny, zawartość labilnych frakcji węgla, przewidywana stabilność w środowisku, a w konsekwencji wpływ na respirację i aktywność enzymatyczną po wprowadzeniu do gleby wyraźnie różniły się pomiędzy badanymi biowęglami. Słabo uwęglone biowęgle z odpadów kuchennych i fusów kawy, zawierające najwięcej węgla wodnorozpuszczalnego oraz rozpuszczalnych w wodzie form węglowodanów, okazały się najbardziej podatne na procesy rozkładu, czego wyznacznikiem był wzrost emisji CO_2 oraz aktywności enzymów prowadzących rozkład materii organicznej w glebach z ich dodatkiem. Dodatek egzogennej materii organicznej modyfikował działanie biowęgli w glebie, stymulując aktywność enzymów i wpływając na wzrost strat węgla w początkowym etapie inkubacji. Zróżnicowane właściwości gleb znalazły odzwierciedlenie w intensywności respiracji – większe straty węgla wraz z CO_2 występowały w lekkiej glebie piaszczystej, jednak aktywność mikroorganizmów była wyższa na pyle gliniastym. Biorąc pod uwagę wykazaną dużą zmienność właściwości biowęgli i ich interakcji z glebą, przy ocenie podatności produktów pirolizy na degradację należy uwzględniać nie tylko ich stopień uwęglenia, ale także zawartość labilnych frakcji węgla, które mogą stanowić źródło energii dla mikroorganizmów glebowych i przyspieszać procesy rozkładu.

Na podstawie przeprowadzonych badań, w celu zapewnienia efektywnej sekwestracji węgla, rekomenduje się unikanie słabo uwęglonych produktów pirolizy odpadów spożywczych, takich jak resztki kuchenne czy fusy z kawy, które okazały się szczególnie podatne na rozkład. Należy też zwrócić uwagę na możliwość zwiększonych strat węgla z lekkich gleb wskutek emisji CO_2. Zalecane jest unikanie jednoczesnej aplikacji biowęgli z egzogenną materią organiczną, która stanowi dodatkowe źródło składników pokarmowych dla mikroorganizmów glebowych i może prowadzić do zwiększenia tempa dekompozycji wprowadzonych biowęgli.

Słowa kluczowe: biowęgiel, sekwestracja, materia organiczna, węgiel wodnorozpuszczalny, respiracja gleby

ABSTRACT

Anthropogenic emission of carbon dioxide is constantly growing, accounting for the development of effective carbon sequestration strategies. A lot of attention is paid to soil carbon pool, among which pyrogenic carbon is considered as a particularly stable. One of the contemporary forms of pyrogenic carbon applied into the soil is biochar. It is produced by thermal conversion of biomass under oxygen-limited conditions (pyrolysis), has a high carbon content (> 50%) and is considered an effective tool for CO_2 sequestration. Stability of biochar in soil depends on its properties, but also on a number of abiotic and biotic factors that can contribute to the violation of biochars structure. When considering the use of biochar as a soil amendment with high carbon sequestration potential, factors determining its stability in soil should be investigated. One of the common approaches to estimate biochar stability is evaluation of its chemical properties, including C:O and H:C molar ratios. However, this assessment does not take into account the impact of external factors. Therefore, in the presented dissertation, more complex approach is proposed, that combines new analytical tools and analysis of labile carbon pool, which is chemically and biologically active and undergoes processes similar to soil organic matter.

The main aim of this dissertation was to evaluate susceptibility of various biochars for decomposition process, in order to assess their usefulness in carbon sequestration. The research was conducted based on the 12-month incubation experiment. Three groups of variables potentially important for biochar decomposition processes were considered: (1) different feedstocks for pyrolysis process, (2) soil type with emphasis on the variability of the texture and chemical properties, (3) presence of exogenous organic matter in soil. In order to assess the stability of biochar, standard analytical methods were used as well as a range of up-to date analyses dedicated to characterize biochar response to potential abiotic and biotic factors. Obtained results verified the hypotheses that each evaluated variable has influence on the interaction of biochar with the environment and, consequently, its suitability for carbon sequestration.

It was noted that feedstock type strongly determined properties of charred biomass. The elemental composition, content of labile carbon fractions, expected stability in the environment and, consequently, the effect on respiration and enzymatic activity after introduction into the soil differed between studied biochars. Poorly carbonized biochars derived from kitchen waste and coffee grounds, the most abundant in dissolved organic carbon and water-soluble forms of

carbohydrates, proved to be the most susceptible to decomposition processes, as indicated by an increase in CO_2 emissions and the activity of extracellular enzymes. The addition of exogenous organic matter modified the effect of biochar in the soil, stimulating enzyme activity and increasing carbon losses during the initial stage of incubation. Varied properties of the soils were reflected in the intensity of respiration and enzyme activity - greater carbon loss with CO_2 occurred in sandy soil, but microbial activity was higher on the silt loam. Considering high diversity of biochars properties and their interaction with soil, in the assessment of the stability of pyrolyzates it is necessary to evaluate not only their carbonization level, but also the content of labile carbon, which can provide a source of energy for soil microorganisms and accelerate decomposition processes.

Based on the conducted research it can be concluded that to ensure effective carbon sequestration, it is recommended to avoid poorly carbonized pyrolysis products of food waste, such as kitchen residues and coffee grounds, which have been shown to be particularly susceptible to decomposition. It is also important to consider the possibility of elevated carbon losses from light-textured soils due to CO_2 emissions. Co-application of biochars with other exogenous organic amendments should be avoided, as it provides an additional source of nutrients for soil microorganisms and can lead to an increased decomposition rate of biochars.

Keywords: biochar, sequestration, organic matter, dissolved organic carbon, soil respiration

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Effect of Six Different Feedstocks on Biochar's Properties and Expected Stability

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Abstract: Biochar (BC) is often proposed as a tool for climate change mitigation, due to the expected long lifetime in the environment. However, BC's stability can vary depending on feedstock type and the presence of labile carbon fractions. In this study, we verify the recent methods with new possible tools for biochar stability assessment on six different biochars derived from commonly available Europe biomass sources. Elemental composition (CHNO), dissolved organic carbon (DOC) and water-soluble carbonates content (WSC), volatile organic compounds (VOCs) composition, and mid-infrared spectra (MIR) were performed to estimate the persistence of biochars. Under similar conditions of pyrolysis, biochar properties can vary depending on a feedstock origin. Less aromatic structure and higher contents of labile carbon fractions (DOCs and WSC) in food waste biochars affected the lower stability, while biochars derived from high lignocellulose materials (straw, wood, and grass) were strongly carbonized, with persistent, aromatic structure. Labile carbon pool content (DOC, WSC) and spectral analysis can be useful tools for biochar stability assessment, giving similar information to the standard molar ratio method. Biochars obtained from agriculture and forestry management biomass should be considered as highly stable in soil and are appropriate for long-term carbon sequestration purposes.

Keywords: biochar; stability; carbon pools; labile fractions; feedstock type

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1. Introduction

Biochar is frequently described as a final product of pyrolysis processes of agricultural leftovers, forestry biomass, or other various organic wastes [1]. As a soil amendment, it gained particular popularity in recent decades, which is reflected in numerous publications [2–4]. Potential benefits from biochar application into the soil are often described as a win-win solution, due to the positive impact on the environment and solving the problem of organic waste utilization [5,6]. Biochar used as an organic amendment is able to improve the fertility of potentially unproductive soils, increase crop yields, reduce drought stress in plants or remove pollutants from the contaminated environment [7–14].

Although the positive impact of biochar on soil properties may not be always obvious [7,12,15], the longevity of the material and its persistence in the environment is often highlighted in the literature. Many authors agree that biochar is characterized as a remarkably durable material, resistant to biotic and abiotic decomposition processes [16–18]. In general, thermally converted organic materials are characterized by a low degradation rate and longer residence time in soil, compared to unprocessed feedstocks [19–21]. Presumably, stable carbon from biochar can persist in soil for hundreds or even thousands of years [22]. Thus, biochar application into the soil is becoming a potential tool for long-term carbon sequestration in agricultural areas, which can address the current needs of mitigating anthropogenic CO_2 emissions.

Predicting the physiochemical changes in char materials deposited in the soil is crucial for understanding the extent of its benefits to C (carbon) sequestration, agriculture, and

environmental remediation in both natural and anthropogenically altered soil systems [23]. The inherent variability of biochars, coupled with that of soils to which they are applied, brings the need of determining its resistance to the degradation process in terms of its use as a carbon sequestration strategy [24]. Many different methods of biochar stability assessment have been recently proposed. In general, methods of biochar quality and longevity assessment can be divided into three groups: (I) labile/stable biochar C analysis, such as volatile compounds content and oxidation resistance [25,26], (II) carbon structure analysis, including elemental composition and molar ratios [27–29] and analysis of C-structure using instrumental methods, such as NMR, SEM, X-ray diffraction or spectroscopy [19,30], with particular emphasis on Raman spectroscopy as a new method for fast evaluation of biochars properties [31] and (III) biochar incubation and modeling [17]. Although numerous approaches for biochar quality and stability assessment are described, the evaluation of pyrolysed materials can still be problematic. Methods are often non-available in terms of time and costs for biochar producers, as well as not every analysis has equal potential to provide accurate results. Moreover, approaches based on biochar incubation are time-consuming and require additional mathematical modeling [19,32]. Due to the multiplicity of available methods, biochar stability assessment can be challenging when it comes to choosing an analytical approach, as well as may lead to varied and possibly misleading conclusions. Variety in estimated longevity can be noticed in the results reported in literature, where described biochar stability ranges from hundreds of years to even millennia [19,22,33,34]. Moreover, authors investigating biochar properties often focus more on technical aspects of pyrolysis than on the feedstock type as the determinants of biochar's characteristic [35–38].

Therefore, this work studies the influence of various types of feedstocks commonly available in Europe on biochar properties under the same conditions of the pyrolysis process, using several chosen analytical methods. An attempt was made to pre-conclude the potential stability of these biochars, based on selected parameters. This will answer the question of how the type of biomass used for pyrolysis affects the properties of biochar, including the expected lifetime of the product, and allow to recommend the most appropriate material in terms of its longevity and carbon sequestration in the environment.

2. Materials and Methods

2.1. Biochar Preparation

Biochars for this study were derived from six different types of organic waste feedstocks, commonly available in EU countries: kitchen wastes (BC1), cut green grass (BC2), coffee grounds (BC3), wheat straw (BC4), sunflower husks (BC5) and beech wood chips (BC6). Kitchen wastes were collected from households and local greengrocers, and consisted of fruit and vegetable leftovers: mainly apples, cabbage, corn cobs, and potato peels. Cut grass biomass was obtained from private home gardens located in Wrocław, Poland, and consisted of cut grass with a small admixture of varied weed species. Coffee grounds were collected from local cafes and university cafeterias. The other three types of biomasses: wheat straw, sunflower husks, and wood chips were acquired in commercially available forms—straw and wood chips were shredded to the size of several millimeters, whereas sunflower husks remained without additional preparation. All of the feedstocks are included in the Positive list of permissible biomasses for the production of biochar [27]. Prior to the pyrolysis, input materials were air-dried to avoid mold growth and to keep humidity biomasses at a similar level, which is important for the proper pyrolysis process. Materials were stored in closed containers at ambient air humidity and temperature. Biochars were produced in September 2020. Nitrogen (N_2) (to maintain the inert atmosphere) from the gas cylinder was used in the reactor chamber (90 mL·min^{-1}), constructed for semi-industrial scale biochar production (approx. 10 kg of biomass per hour) at Wroclaw University of Technology (Wrocław, Poland). The duration time of the pyrolysis process was 60 min. Obtained biochars were ground to fine particles, sieved with 2 mm mesh, and stored in closed containers in a cold place.

2.2. Biochar Properties Analysis

Biochar properties for general characteristics were determined on air-dried material with a particle diameter smaller than 2 mm. The pH was measured in triplicates in 1:5 suspension (*v/v*, 5 mL of biochar to 25 mL of distilled water), with pH-meter (Mettler-Toledo, Graifensee, Switzerland). Exchangeable cations for cation exchange capacity (CEC) were extracted with modified ammonium acetate (NH_4OAc) method at pH = 7.0 [39]. Briefly, 1 g of biochar was shaken with 20 mL of distilled water to ensure proper wetting of the sample, and the precipitate was rinsed twice more with water to avoid overestimation of exchangeable base cations. After that, base cations were extracted with 20 mL of ammonium acetate. In the end, biochar slurry was rinsed four times with 99% isopropanol and 20 mL of 2 M KCl was used to extract NH_4^+ ions. Base cations content in supernatants was analyzed by MP-AES 4200 Spectrometer (Agilent Technologies, Santa Clara, CA, USA). Ash content was measured based on mass loss after complete dry combustion in a muffle furnace at 550 °C. The analysis was considered complete when the mass of combusted material remained constant.

2.3. Analysis of Stability Assessment

Several analyses in the study were performed as indicators of the potential stability of biochars. Elemental composition was estimated according to PN-ISO 13878:2002 and PN-ISO 10694:2001 standards for carbon (C), nitrogen (N), and hydrogen (H) content. A CHNS Elemental Analyzer (CE Instruments Ltd., Wigan, UK) was used to determine the contents of C, N, and H. Ash content was defined based on the weight loss after sample combustion at 550 °C in a muffle furnace. The O content (%) was calculated using following formula: O (%) = 100 − (C% + H% + N% + Ash%) [40]. Based on elemental composition, it was possible to calculate the H:C and O:C molar ratio, often proposed as the chemical indicator of biochar quality and estimator of its stability [19,28]. A determined molar ratio was visualized on the Van Krevelen diagram, which plots H:C against O:C and allows to clearly indicate biochar's stability as a function of elemental composition. Numerical values of biochars half-life for the purpose of this paper were estimated according to the correlations between molar ratios and predicted durability from the previous literature studies, summarized in the review of Spokas [28]. Spokas compared the O:C molar ratio with the predicted half-life of biochars incubated under laboratory conditions. On that basis, he distinguished three estimated ranges of biochars half-life ($t_{1/2}$) in relation to their O:C ratio: $t_{1/2}$ > 1000 years for O:C < 0.2, 100 years < $t_{1/2}$ < 1000 years for O:C 0.2–0.6 and $t_{1/2}$ < 100 years, when O:C molar ratio exceeded 0.6. For the purposes of this study, estimates were made on the basis of the aforementioned relationships, without additional mathematical treatments or modeling. At this point, it is worth mentioning that the half-life of biochar, as the time needed to reduce to half of its initial quantity, correlates with the longevity of the material, i.e., its ability to resist degradation processes in the environment.

For qualitative insight into functional groups present in biochars and to conclude about the degree of aromaticity, spectroscopic analyses were performed. Before spectral measurements, samples were additionally dried at 37 °C to avoid interference signals from water, and the material was finely ground, which ensures proper homogenization. Mid-infrared spectra (MIR) were performed on approx. 0.1 g of biochar sample, on spectrometer Nicolet iZ10 FT-IR with the accessory Smart iTX (Thermo Fisher Scientific, Waltham, MA, USA). MIR spectra ranged from 4000–525 cm^{-1} with a 2 cm^{-1} resolution [41]. Before interpretation, the standard normal variate (SNV) method was applied to spectra, to ensure proper comparability in terms of peak intensity. Spectra were treated using Spectragryph software (Oberstdorf, Germany) [42]. Bands and corresponding functional groups revealed on spectra were identified according to Tatzber et al. [43], Le Guillou et al. [44], and Tinti et al. [45].

Volatile organic compounds (VOC) emission from biochar samples was analyzed using headspace (HS) gas chromatography coupled with mass spectrometry (MS). Briefly, solid samples were placed into 20 mL clear glass vials sealed with PTFE septum and

bored aluminum caps. Then, the material was incubated in a Shimadzu (HS-20) on a headspace system at 80 °C for 20 min. As a blank, an empty vial was used to evaluate the current experiment conditions and the possibility of interferences. The gas chromatography with mass spectrometry GC-MS Shimadzu type GCMS-QP2010 (Shimadzu, Kyoto, Japan) was used for separation, identification, and quantification (with *n*-tridecane as the internal standard) of VOCs. The resulting chromatograms were identified according to Białowiec et al. [46] through direct comparison to the commercially available mass spectra database. It was based on the interpretations of the mass spectral fragmentation patterns using the dedicated library searching NIST14 Mass Spectral Database (NIST MS Search 2.0d software, Gaithersburg, MD, USA), and Kovats indices (KI exp.), where homologous series of C7-C40 n-alkanes were used for the calculation of retention indices.

To quantify the pool of labile carbon forms in biochars, we analyzed the content of dissolved organic carbon (DOC) in water. Dissolved organic carbon was extracted with ultra-pure water in a 1:50 ratio (*w/v*, 1 g of biochar, and 50 mL of water). Briefly, samples were shaken for 1 hour at approx. 40 rpm, then extracts were pre-filtered on quantitative filter paper Filtrak/Munktell, type 390. Obtained solutions were additionally filtered using non-sterile syringe filters MCE (mixed cellulose esters) with pore size 0.45 µm, pre-washed with 10 mL of distilled water. It is assumed that the carbon fraction that remained in the solution after described treatment represents the DOC pool extracted from solid samples [47,48]. Carbon content in water extracts was determined on analyzer EnviroTOCCube (Elementar Analysensysteme GmbH, Langenselbold, Germany).

Water soluble carbohydrates (WSC) were measured quantitatively by the anthrone method. The principle of this essay is to measure the content of anthrone reactive polysaccharides in water solutions. Anthrone reagent in concentrated sulfuric acid gives a green to blue color, when combined with carbohydrates present in the solution [49,50]. To perform the analysis, biochar samples (2 g) were shaken with ultra-pure water (25 mL) per 1 h. Then, 10 mL of anthrone reagent was added to 5 mL of filtered extract, and anthrone reactive carbon content was measured colorometrically at UV-Vis Cary 60 (Agilent Technologies, Santa Clara, CA, USA) at wavelength 625 nm.

2.4. Data Management and Analysis

Microsoft Excel software for Windows was used for data management and storage (Microsoft Corporation, Redmond, WA, USA). Means and standard deviations from replicates were calculated with GraphPad Prism version 8.0.1 for Windows (GraphPad Software, San Diego, CA, USA). The figures presented in this paper were prepared using GraphPad Prism Software and CorelDraw Graphics Suite 2020 (Corel Corporation, Ottawa, ON, Canada).

3. Results

3.1. General Characteristics of Biochars

All biochars were characterized by neutral or alkaline reactions, although pH values were significantly different depending on the feedstock, and the most alkaline biochars were obtained from cut green grass (pH 10.43) and sunflower husks (pH 10.29). Cation exchange capacity strongly varied, from 7.41 cmol (+)/kg in wheat straw biochar (BC4) to even 227 cmol (+)/kg in BC1 and BC2, produced from kitchen wastes and green grass. Carbon content was between 52–78%, which fulfills the guidelines from EBC (European Biochar Certificate, Arbaz, Switzerland), according to which in the most well-pyrolysed organic feedstocks this value should exceed 50% [27]. Overall, standard biochars properties are summarized in Table 1.

Table 1. General properties of biochars.

Feedstock	Abbreviation	pH in H_2O	CEC cmol (+)/kg	TC %	TN %	Ash %
Kitchen wastes	BC1	9.41 ± 0.05	227.73	54 ± 1.1	0.98 ± 0.02	10.1 ± 1.0
Green grass	BC2	10.43 ± 0.04	227.94	52 ± 1.0	2.70 ± 0.05	31.3 ± 3.1
Coffee grounds	BC3	6.91 ± 0.07	35.07	68 ± 1.4	3.60 ± 0.07	6.2 ± 0.4
Wheat straw	BC4	7.20 ± 0.13	7.41	76 ± 1.5	0.24 ± 0.005	1.3 ± 0.1
Sunflower husks	BC5	10.29 ± 0.02	35.33	78 ± 1.6	0.63 ± 0.01	5.6 ± 0.6
Beech wood chips	BC6	6.96 ± 0.07	22.66	70 ± 1.4	1.40 ± 0.03	9.8 ± 1.0

CEC = cation exchange capacity, TC = total carbon, TN = total nitrogen. Values are means ± standard deviation (SD) from three replicates. CEC value is mean from the Agilent MP Software.

3.2. Characteristics for Biochar Stability Assessment

3.2.1. Elemental Composition and Molar Ratios

According to EBC, the H:C ratio should not exceed 0.7 and this criterion is fulfilled for all biochars in this study—four of the examined six materials have much lower results, and the other two fell within the error limits with values around 0.7. Moreover, the molar O:C ratio should be below 0.4. For 5 out of 6 tested biochars the values are between 0.12–0.20. An exception is BC1, where O:C molar ratio is remarkably higher (0.42) due to the lowest carbon content and large share of oxygen in dry mass (Table 2). Based on the calculated molar ratios, it was possible to estimate the half-life of biochars. Considering those estimations, high-cellulose feedstocks generated much more recalcitrant chars. For BC5 (sunflower husks) estimated expected half-time is exceeding over 1000 years, followed by a little less durability of green grass and wood chips derived biochars. Chars generated from kitchen waste (B1) had a much higher O:C ratio reflecting a lower half-time of decomposition, estimated for only a few centuries. Those findings were visualized on the Van Krevelen diagram, and the trend of biochars stability can be summarized as BC5 > BC2, BC6 > BC4 > BC3 >> BC1 (Figure 1).

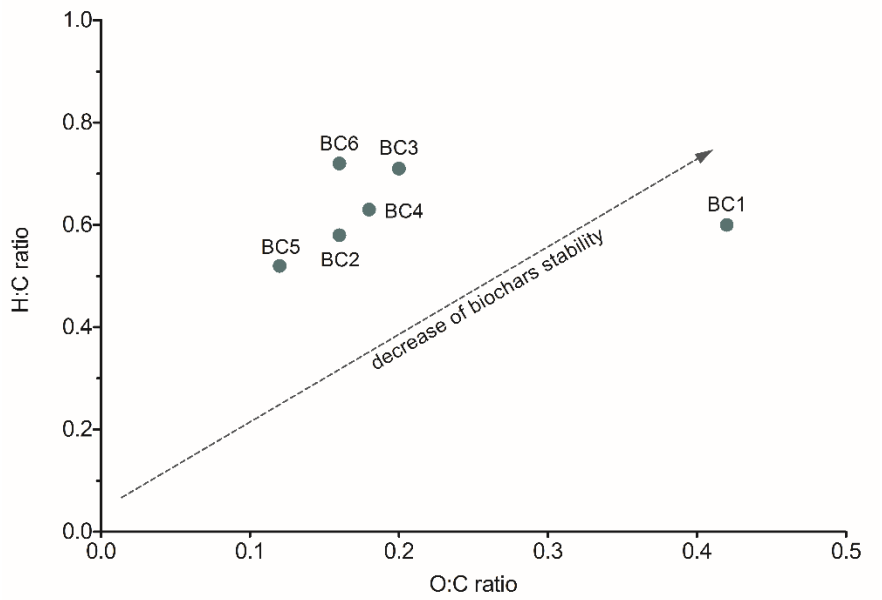

Figure 1. Van Krevelen's diagram for examined biochars. The arrow indicates direction of changes in expected biochar's stability.

Table 2. Elemental composition, molar ratios, and estimated half-life time of biochars.

Biochar	Elemental Composition [%]			Molar Ratio		Literature Half-Life $t_{1/2}$ [Years]
	TC	H	O	O:C	H:C	
BC1	54 ± 1.1	2.7 ± 0.11	30 ± 0.6	0.42	0.60	~500
BC2	52 ± 1.0	2.5 ± 0.05	11 ± 0.2	0.16	0.58	>1000
BC3	68 ± 1.4	4.0 ± 0.13	18 ± 0.4	0.20	0.71	~1000
BC4	76 ± 1.5	4.0 ± 0.08	18 ± 0.4	0.18	0.63	~1000
BC5	78 ± 1.6	3.4 ± 0.07	12 ± 0.2	0.12	0.52	>1000
BC6	70 ± 1.4	4.2 ± 0.08	15 ± 0.3	0.16	0.72	>1000

TC = total carbon, H = hydrogen, O = oxygen. Values are means ± standard deviation (SD) from three replicates. Molar ratios base on mean values. Half-life of biochar was estimated based on the literature summarized by Spokas 2010.

3.2.2. Mid-Infrared (MIR) Spectra

Standardized MIR spectra differed among tested biochars in the intensity of characteristic peaks, particularly in the region of ~1600 to 900 cm^{-1} and 2800 to 3000 cm^{-1} (Figure 2). Biochars derived from food leftovers (kitchen waste BC1, coffee grounds BC3) showed relatively high content of aliphatic compounds in their structure, reflected by the intense bands at ~2900 cm^{-1} (BC3), and ~1400 cm^{-1} (BC1). Different shapes of spectra were observed for materials produced from high lignocellulosic biomasses, which is particularly visible in the case of BC2 (green grass) and BC6 (wood chips). These biochars are characterized by a more aromatic structure, reflected by the presence of polysaccharides (~1100 cm^{-1}). Nevertheless, in the case of the BC2 sample, the mentioned band may be also due to high ash content in the sample (Table 1). Moreover, BC2 and BC6 spectra have lack of peaks in the regions responsible for aliphatic groups (Figure 2).

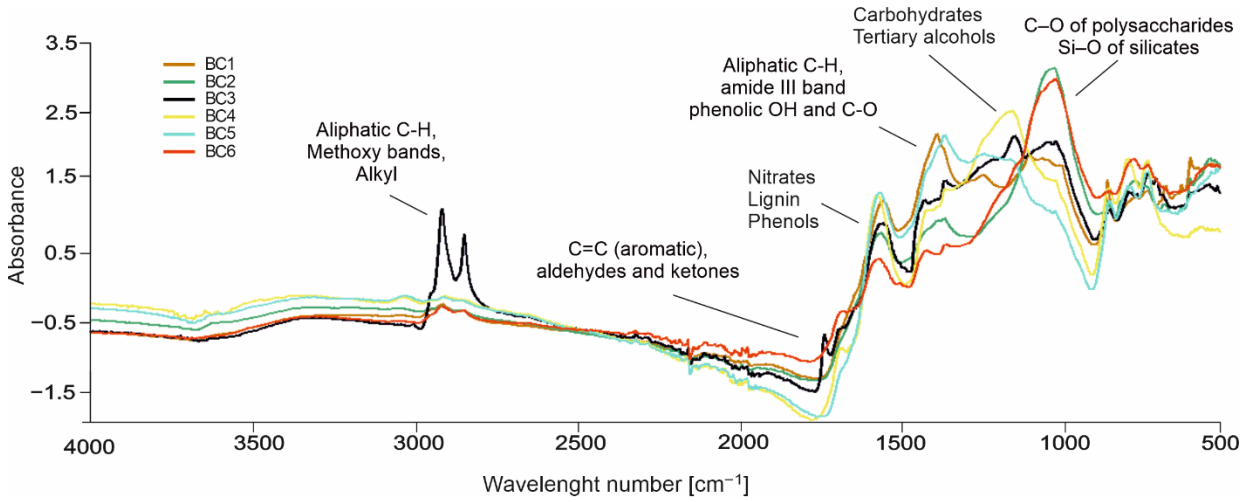

Figure 2. Mid-infrared spectra of biochars with identified bands.

3.2.3. Volatile Organic Compounds Quality

Analysis of VOC emissions revealed differences between biochars created from different feedstocks under equivalent pyrolysis temperatures and conditions. Thirty-six (36) different chemical compounds were detected as released from examined six types of biochars. The compounds and their corresponding retention times are listed in Table 3. The most prevalent volatile organic compounds were formamide in BC1, BC2, BC3, and BC5 and methane, abundant in BC4 and BC6. However, any "universal" VOC present in every sample was not identified, which may suggest that VOC sorption and quality strongly depend on feedstock type, which was the only variable during the process of biochar production. Despite equivalent pyrolysis conditions, the differences between VOCs

released from biochars created from different organic materials are substantial. Overall, the differentiation of the detected compounds was the lowest in BC2 produced from gardening wastes (only 4 VOC compounds) and in BC3 from coffee grounds (5 compounds). Wheat straw biochar BC4, in turn, revealed the greatest variation in the absorbed VOCs—from 36 compounds identified in this study, 15 were present in this particular biochar. Moreover, 13 chemical compounds were found only in BC4 and were not detected in other examined samples. The top five frequently observed VOCs were formamide, methane, n-hexane, tetra- and pentadecane, and pentanal.

Table 3. Qualitative analysis of volatile organic compounds emitted by biochars (measured with GC-MS).

Peak	Name	R_t [min]	BC1 [%]	BC2 [%]	BC3 [%]	BC4 [%]	BC5 [%]	BC6 [%]
1	Formamide	1.43	90.26	95.85	78.20	-	74.68	-
2	Methane	1.46	-	-	-	27.44	-	25.13
3	2-methyl-1-propene	1.54	-	1.78	-	-	-	-
4	Methoxycyclobutane	1.66	0.51	-	-	-	-	-
5	N′-ethyl-N,N-dimethyl-1,2-ethanediamine	1.67	-	-	-	3.55	-	-
6	2-methylpentanal	1.67	-	0.89	4.55	-	-	-
7	Dihydro-3-methylene-2,5-furandione	1.67	-	-	-	-	-	3.25
8	Propanedioic acid	1.95	-	-	-	-	-	3.41
9	2,2,3,4-tetramethylpentane	2.03	-	-	-	-	7.86	-
10	n-Hexane	2.02	2.84	1.47	-	20.98	-	11.11
11	Toluene	2.24	1.04	-	4.18	-	-	-
12	2,4-dimethylheptane	3.66	-	-	-	-	5.83	-
13	3-ethyl-2-methylheptane	7.33	1.45	-	-	-	-	-
14	6-chloro-3,4-dihydro-N,N,2,4-tetramethyl- 1,1-dioxide 2H-1,2,4-benzothiadiazine-7-sulfonamide	8.97	-	-	-	3.72	-	-
15	2-Cyclohexylamino-4-(3-hydroxybenzylidenehydrazino)-6-(4-nitroanilino)-1,3,5-triazine	9.57	-	-	-	3.86	-	-
16	Ethyl 4-amino-2-[2,4-dichlorobenzyl thio]-5-pyrimidine carboxylate	9.89	-	-	-	3.22	-	-
17	2-[2-(2-benzothiazolyl) diazenyl]-4-methoxy-6-(methylsulfonyl)phenol	10.01	-	-	-	3.18	-	-
18	6-Furfurylaminopurine	11.30	-	-	-	6.34	-	-
19	4-methyldecane	11.70	-	-	-	-	3.37	-
20	Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (R)-(D-Limonene)	11.90	2.42	-	-	-	-	-
21	Bialophos, N,O,O-tris(tert-butyldimethylsilyl)deriv.	12.01	-	-	-	6.52	-	-
22	2H-Benzocyclohepten-2-one, 3,4,4a,5,6,7,8,9-octahydro-4a-methyl-, (S)-	12.55	-	-	-	4.07	-	-
23	5-(2-methylpropyl) nonane	13.59	-	-	-	-	5.72	-
24	4-(2-ethyl-2-methyloxan-4-yl)-2-phenylthiazole	13.91	-	-	-	3.34	-	-
25	2,6,10-trimethyldodecane	14.37	-	-	-	-	2.72	-
26	Dimethylpropyl[(6a,7,8,10a-tetrahydro-6,6,9-trimethyl-3-pentyl-6H-dibenzo[b,d]pyran-1-yl)oxy]-,(6aR-trans)silane	14.72	-	-	-	4.01	-	-
27	4-methoxyphenol (Mequinol)	15.13	-	-	-	-	-	11.87
28	4-ethylphenol	19.43	-	-	-	-		5.98
29	N1-[5-hydroxy-6-(4-morpholinylsulfonyl)-1-naphthalenyl]-1,3-benzenedisulfonamide	21.80	-	-	-	3.79	-	-
30	4-ethyl-2-methoxyphenol	24.81	-	-	-	-	-	34.31
31	1-Pentyl-2-piperidinomethylnaphth [1,2-d]imidazole-4,5-dione	25.51	-	-	-	3.06	-	-
32	2-methoxy-4-propylphenol	27.79	-	-	-	-	-	3.20
33	Cyclopentanecarboxylic acid, ethenyl ester	27.94	-	-	-	3.01	-	-
34	Tetradecane	28.45	0.55	-	3.34	-	-	-
35	Pentadecane	30.05	0.92	-	6.84	-	-	-
36	Hexadecanoic acid, methyl ester	34.17	-	-	-	-	-	2.63

R_t—retention time, (-)—compound not detected.

3.2.4. Content of Labile Carbon Fractions

Dissolved organic carbon in water (DOC) content varied among biochars produced from different feedstocks, as biochars made of food waste, like BC1 and BC3, had a remarkably higher presence of this labile carbon fraction (10.91 and 11.01 mg g^{-1}, respectively). At the same time, biochars with the lowest DOC content had the tendency to reveal relatively small amounts of WSC, particularly in the case of BC2 or BC5 (Figure 3). Therefore, the impact of feedstock on biochar properties was clearly noted in labile carbon forms. Content of water-soluble carbohydrates (WSC) analyzed by reaction with anthrone ranged between 6.69–23.30 mg g^{-1}. The highest values were noted for biochars derived from coffee grounds (BC3)—23.3 mg g^{-1}, kitchen waste (BC1) 16.53 mg g^{-1}, and wood chips (BC6)—15.79 mg g^{-1}. The lowest anthrone reactive carbon content was noted in BC2 (green grass) and BC5 (sunflower husks) biochars.

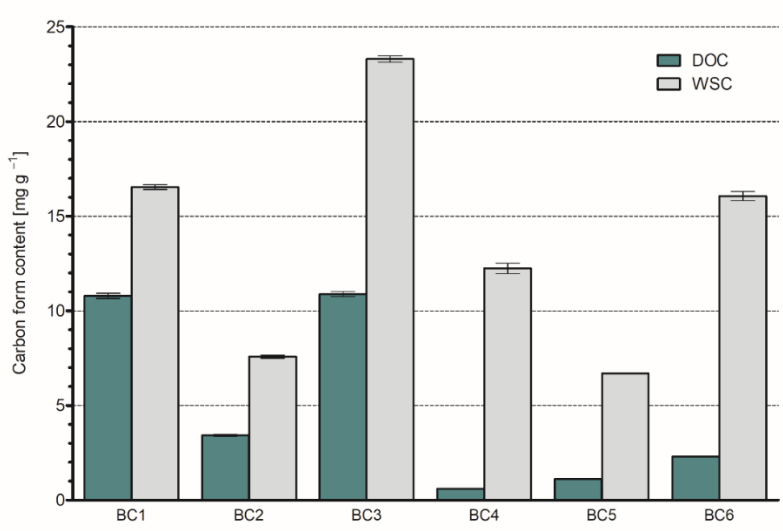

Figure 3. Dissolved organic carbon in water (DOC) and water-soluble carbohydrates (WSC) content in biochars. Values are means with error bars from three replicates.

4. Discussion

Reported results confirmed that feedstock type has a crucial impact on biochar properties, both on general characteristics and features important for expected longevity in soil. A general overview of BCs properties showed that, depending on feedstock type, the ash content can be different and was remarkably higher in BC originating from cut green grass and kitchen wastes. High ash content in these biochars corresponded with higher CEC values, which was also observed in previous studies by Mukome et al. [51] and Yang et al. [52]. It is worth noting that the biochar from wheat straw and wood had a particularly low pH. Non-alkaline biochar reaction, although not common, is not unusual and stays in agreement with studies of Tomczyk et al. [53], who claimed that wooden biochars tend to have pH lower by 2–3 units than other biomasses. Similar observations had Trigo et al. [54], who in wood biochars obtained at 550 °C recorded pH values from 6.26 to 7.11. The low pH of woody biochars can be explained by the presence of cellulose and hemicellulose, which decomposes during the thermal conversion of biomass and yields organic acids, which have an impact on the pH of the final product [53]. In the case of wheat straw, the content of CEC was remarkably low, therefore the deficiency of alkaline components resulted in the low pH of the product.

In general, feedstocks from this study can be divided into two groups: (1) high lignocellulosic biomasses from agricultural and forestry activities [55], which include cut grass, wheat straw, sunflower husks, and wood chips, and (2) less lignified food leftovers, including kitchen wastes and coffee grounds. Many authors claimed that materials rich in cellulose and lignin promote carbonization during pyrolysis [56,57]. Findings from this study follow those trends, as the highest carbon content was recorded in biochars from high lignocellulosic feedstocks—wheat straw, sunflower husks, and wood chips, whereas kitchen wastes yielded considerably less carbonized biochars, probably due to the high content of simple sugars and non-aromatic structures originated from fruits. Carbonization level is reflected in the elemental composition of the char, and thus in the molar ratios H:C and O:C, proposed by EBC and IBI as indicators of biochar stability [27,29]. In general, the more carbonized biochar is, the more aromatic C-structure is expected and more recalcitrant product can be obtained. Molar ratios are strongly correlated with the presence of aromatic ring structures and allow to distinguish biochars from deficiently carbonized materials, that are prone to the decomposition processes. It can be noted that high lignocellulosic biomasses like wheat straw, cut grass, and wood chips yielded strongly carbonized biochars with the longest expected half-life, estimated at approx. 1000 years on basis of the work by Spokas [28]. The molar ratios seem to be strongly associated with the feedstock type, as a

result of the presence and composition of functional groups [28,58]. However, it needs to be noted that despite the requirements for molar ratios stated by IBI and EBC as indicators of biochars stability, there is no widely accepted, direct correlation between them and the exact lifetime of char in the environment. Ranges proposed by Spokas [28] and presented in this article are only indicative, and this type of benchmark may not always be reliable, especially for biochars with non-typical properties. For example, the O:C ratio is unreliable for high-ash biochars like BC1 or BC2 [19], or for materials that were not sufficiently pyrolyzed and their C-content does not meet the criteria for biochars. Although the molar ratios are recommended indicators of the C-structure of biochar, a direct comparison between them and the expected lifetime of char is not widely applied.

Feedstock impact on biochar properties is also reflected in the presence and composition of VOCs—volatile organic compounds. Despite relatively little research in the literature related to the subject of volatiles in biochars, authors noticed a great uniqueness of compounds described as VOCs. Spokas [59] reported 140 different compounds, whereas Białowiec et al. [44], who examined torrefied municipal waste, noted that only phenol and acetic acid seem to be typical representatives of VOCs detected in the majority of samples. Analysis of VOCs in this study confirmed the presence of phenols, but acetic acid was not identified. Therefore, the chemical composition of volatiles seems to be unique for the pyrolysis input material, and hence, using this feature to discuss biochars stability may lead to incomplete and misleading observations. Moreover, there are assumptions that despite the effect on microbial activity, there is no long-term correlation between volatiles quality or quantity and the estimated half-life of biochars, as volatiles represent only a small, rapidly utilized pool of nutrients [60]. Although VOCs characteristics cannot be recommended in order to conclude about the half-life of biochars, the impact of volatiles on biochar—soil interaction, especially on soil biota, is a separate issue. Some VOCs, including phenols identified in examined biochars, were proven to show phytotoxic effects [61,62] or inhibit microbial activity [63]. Considering all the above, the exact effects of VOCs present on biochars surfaces on the properties of the product, and on live organisms in the environment are still unclear, due to the large diversity of this wide group of compounds and their possible interactions [64]. Therefore, further research on this topic is recommended, especially on the impact of the most common volatiles (phenols, organic acids, formamide, etc.) on soil biota after biochar application.

Considering the content of labile organic compounds present in biochars as a reliable source of knowledge about char recalcitrance, this property strongly depends on feedstock type. Much less dissolved organic carbon could be determined in woody biochars compared to other less lignocellulosic materials. High lignic agriculture feedstocks—wheat straw, sunflower husks, or wood chips are precursors of biochars with lower dissolved organic carbon content [65], due to the fact that lignin is more thermally stable than other forms of sugar present in biomass [48]. Findings of other authors support presented results—for example, Liu et al. [48] obtained 2–3 mg g^{-1} of DOC in biochars made of wood, whereas herbaceous materials contained around 10–15 mg g^{-1}. Moreover, it was noticed that DOC content is associated with molar ratios, as less carbonized biochars with the highest H:C or O:C ratio are rich in dissolved organic carbon [48,66]. Therefore, biochars with the lowest carbon content (BC1, BC3) and the shortest expected half-life in soil contained the biggest pool of DOC. It needs to be noted that high DOC content is generally unfavorable for biochar persistence in the environment. DOC constitutes the most mobile carbon pool, therefore if it is not bound into clay-humus complexes or preserved in sediments, it may easily migrate from soils with water runoff and promote carbon losses [67]. This fraction is also more prone to microbial degradation than bulk biochars [68], as it may act as a source of nutrients for microbes [69,70]. Therefore, qualitative analysis of DOC seems to be a suitable indicator of biochar resistance for further decomposition in soil and biochars made of agricultural or forestry residues such as straw, wood, or seed shells are highly recommended for long-term life in the environment. In these materials, the content of potentially leachable DOC is the lowest and simultaneously, the microbial activity shall

not be stimulated. Dissolved organic carbon content analysis may be informative for the discussion about biochars fate in the environment due to its great role in microbial turnover and correlation with C-structure, widely adopted as a stability indicator. However, it needs to be underlined that a conclusion based on DOC content should be considered supplementary, as this approach is not fully standardized and validated, contrary to molar ratios [60].

Observations of the labile carbon pool may be supported by water soluble carbohydrates (WSC) content of the studied BCs extracts. Sugars in the environment act as a source of energy for microbes, therefore high content of WSC will supply substrates for microbial communities and promote biomass turnover [70]. In this study, a positive relationship was found between DOC and WSC content—the presence of both labile carbon forms was the highest in BC1 and BC2, along with the shortest expected stability of these biochars, concluded on elemental composition. Similar findings were reported by Kwapinski et al. [71], who claimed that significant WSC content is expected in substrates rich in easily available carbohydrates such as food wastes, but also in straw or wood materials, due to the presence of lignocellulosic components. Therefore, WSC content analysis can be considered as a supplementary indicator to pre-conclude biochars fate in the environment, along with DOC content.

The chemical composition of examined biochars was additionally confirmed on MIR spectra, which revealed particularly high amounts of polysaccharides from aromatic and lignocellulosic structures in grass and woody biochars. At the same time, these materials were characterized by one of the longest expected stabilities and low DOC content. Contrary, biochars produced from food wastes revealed a higher presence of less persistent aliphatic compounds, which was associated with lower carbonization and promotion of DOC content. In general, the performed study confirmed a strong correlation between feedstock type, biochar composition, and properties, which determines further characteristics, potential interactions in the environment, and recommended application purposes [72,73]. Spectral methods have the potential to provide insight into aromatic and aliphatic structures of biochars. However, with the current state of knowledge, there is no universal relationship between spectrum shape and expected biochar lifetime. The only possibility is to identify functional groups and, optionally, semi-quantitatively analyze the intensity of the peaks. To obtain more reliable and valid results based on spectra, it will be necessary to develop widely adopted calibration models [74]. Nevertheless, qualitative spectral analysis is able to reflect the degree of biochar aromaticity and can be supplementary in the discussion on biochar stability.

5. Conclusions

Presented observations suggest that biochars from agricultural and forestry management (wood, straw, grass, or seed husks) are potentially more persistent in the environment and resistant to decomposition processes (including microbial turnovers), thus more suitable for long-term carbon sequestration than biochars produced from food wastes (kitchen leftovers or coffee grounds). Agricultural and forestry feedstocks are easily available in Europe in terms of quantity and cost; therefore, those biomasses can be recommended for the production of biochars valuable for carbon sequestration purposes. However, such recommendations should be based not only on the general properties of the BCs, but one should also consider the interactions with the environment after the application of the amendment into the soil. Therefore, to support these preliminary conclusions, the test of biochar−soil interaction, e.g., in the incubation experiment, should be performed.

Based on the results shown in this study, molar ratios H:C and O:C have maximal potential to provide accurate results in the discussion on biochar longevity. The relation between them and the expected stability of the BC is well-studied and the method is proposed by EBC and IBI as a stability indicator. Molar composition is correlated with the level of aromaticity, and the presence of aromatic ring structures is an important measure of BC recalcitrance for decomposition processes. Analysis of DOC and WSC content led to

similar conclusions as molar ratios, and they can be considered supplementary to discussing biochar-soil interaction. Qualitative spectral analysis, which reflects the degree of biochar's aromaticity, is also useful to support conclusions about the expected lifetime of the char. Biochar volatiles' composition, due to their high variability, is difficult to compare between these carbonaceous materials and hence cannot be recommended for the estimation of BCs lifespan. Nevertheless, VOCs are informative for the assessment of environmental risk and toxicity of pyrolysed materials.

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Manuksrypt 2

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Article

Biochar and Organic Fertilizer Co-Application Enhances Soil Carbon Priming Increasing CO_2 Fluxes in Two Contrasting Arable Soils

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Abstract: Biochar soil amendment along with non-tillage agriculture, are often proposed as a strategy for organic carbon sequestration in soil. How the quality of biochar might influence the priming effect on soil organic matter mineralization and whether the addition of fresh organic matter will affect its stability in soil is still questionable. In the study, six biochars of different biomass origin and three exogenous organic matter sources were added to two distinct arable soils. CO_2 emission was monitored for 100 days of incubation and CO_2 flux was estimated. Results showed that biochar application increased soil CO_2 fluxes. The highest peaks were recorded in treatments with food waste biochars, suggesting that this feedstock serves as sources of labile C fractions to soil microbes. Co-application of raw organic materials (manure and fresh clover biomass) enhanced CO_2 emission and estimated carbon losses, especially in sandy soil with low organic carbon content. Biochar properties and content of labile C fractions can stimulate CO_2 emission, however in a long-term period this contribution is negligible. Findings of our study showed that more attention should be paid to priming effects caused by addition of exogenous organic matter e.g. fertilizers or cover crops when applied to biochar amended soils.

Keywords: biochar; soil respiration; incubation experiment; CO_2 efflux

1. Introduction

Some of intensive agriculture strategies contributes to the increase of greenhouse gases (GHG) emission and biochar has been widely recommended as a soil amendment moderating global climate change. Produced by the thermochemical conversion of organic residues in oxygen–limited conditions, biochar (BC) is highly resistant to degradation due to its recalcitrant nature [1,2]. Addition of biochar to soil alters physicochemical properties, e.g. porosity, bulk density, pH, carbon (C) and nitrogen (N) content or water holding capacity, which impact soil CO_2 emissions [3–5]. BCs obtained from various feedstock, under different temperature regimes of pyrolysis, have various properties [6] and their effects after incorporation into the soil may greatly vary with local environmental conditions and cultivation systems [7,8]. In general, biochars produced from plant biomass e.g. straw or wood are rich in recalcitrant C forms and are able to sequester more C in soil in comparison with biochars derived from animal manures [9] or organic residues e.g. food wastes [10]. Biochar application to soil may increase carbon sequestration due to the inputs of recalcitrant organic C [6,11,12], however the effects of biochar application on the soil GHG emission is questionable. Results presented in meta-analysis show that biochar application significantly increased soil CO_2 fluxes by 22.14%, thus contributing to the global warming potential [13]. The mechanisms behind this process are still not well understood. The exogenous inputs of labile C sources such as fresh plant residues or dissolved organic carbon from pyrogenic organic matters (POMs) to soil induce positive priming effect with increasing CO_2 emission [14,15]. On the other hand, some studies reported negative priming and suppression of soil CO_2 emission due to reduced enzymatic activity and the precipitation of CO_2 on the biochar surface [16]. Furthermore, the direction of priming effects may

change over the time from amendment application [17]. To model the possible contribution of biochar in GHG mitigation and its stability in soil it is necessary to include many different factors, that might affect biochar behavior in soil under different climatic conditions and cultivation systems [18–20]. It is also important to answer the question whether all biochars contribute to the GHG mitigation process equally, or maybe more attention should be paid to the final product in terms of finding a proper material for effective CO_2 emission mitigation from cultivated soils. As non-tillage and organic farming strategies to increase the carbon sink in agricultural soils are receiving a lot of attention, biochar co-application with sustainable tillage practices might be a proper approach supporting greenhouse gases emission mitigation from arable soils [21,22]. The knowledge about the effects of co-application of biochar, raw crop residues and organic fertilizers e.g. manure and compost on CO_2 emission from arable soils is limited.

An increasing number of studies have demonstrated that soil organic carbon (SOC) decomposition can be influenced by exogenous organic C (EXOC) input through the priming effect. For example, Sun et al. [23] claimed that the addition of EXOC significantly enhanced native SOC decomposition by 47.5% with the highest value in cropland soils (60.9%) and the lowest value in forest soils (26.2%). SOC decomposition contributes greatly to the CO_2 emission from agricultural activities. This study gives insight into the state of knowledge about biochar CO_2 mitigation potential in soil, answering the question whether the process of carbon sequestration by biochar can be disturbed by application of other exogenous organic matter. We hypothesized that labile organic matter (LOM) from cover crops residues or organic fertilizers e.g. manures or compost may change the C-sequestration potential in biochar-amended soil as both types of C sources will contribute to the SOC priming effect. This may induce changes in native mineralization process of organic matter, which in turn will increase or decrease CO_2 flux from soil. Moreover, the presence of raw organic residues and labile C fractions may influence biochar mineralization rate and this can be indicated by CO_2 emission during respiration processes [24]. Previous studies mainly have examined biochar produced from diverse biomass streams, including forestry and agriculture wastes. As a novel approach, in this study the recalcitrance of conventional straw and wood biochar is compared with biochar produced from kitchen wastes - mainly food scraps, fruit and vegetable peels and all the wastes selectively collected for the composting process. Biochar from food waste can become a sustainable replacement of other black carbons, and food waste conversion to biochar has been widely studied as a method to sequester carbon and mitigate the substantial greenhouse gas emissions associated with wasted food signed in United States 2030 Food Loss and Reduction Goal [25]. Our previous study showed that food waste biochar contains more labile carbon compounds prone to oxidation and thus can contribute to the process of CO_2 emission from BC amended soil or enhance soil organic matter (SOM) mineralization [10], and both processes can be monitored by measuring CO_2 efflux from soil. Biochar recalcitrance is expected to last hundreds of years [1,2], but residence calculation in most of the studies do not take into account the carbon loss due to enhanced mineralization of biochar in the presence of raw organic matter delivered to soil with organic fertilization on non-tillage cultivation strategies.

The paper assesses the effects of labile carbon content in biochar on CO_2 efflux from soils amended with biochar derived from different waste materials. It also verifies the questionable effect of exogenous raw organic matter on biochar recalcitrance under conditions imitating non-tillage soil cultivation, promoted as a sustainable method of soil conservation and reduction of agriculture impact on GHG emission.

2. Materials and Methods

2.1. Incubation experiment setup

The incubation experiment was carried out with two different soil types, six biochars and three types of additional organic matter mixed with soil. Both soils used in this study are common in Central Europe and the main intended difference between them is the texture – silt loam (SiL), and loamy sand (SA) (Table 1). Samples were collected from the topsoil (0-25 cm) layer of arable land in

two locations close to Trzebnica, Poland (51°15'46.8"N 17°06'13.3"E and 51°24'13.2"N 17°06'31.6"E). Prior to the incubation experiment, moist soil samples were stored in closed containers in the refrigerator, at 4 °C to keep soil biological activity. Six different feedstocks commonly produced in urban areas and farmlands were chosen for biochar production: kitchen wastes (BC1), cut grass (BC2), coffee grounds (BC3), wheat straw (BC4), sunflower husks (BC5) and beech wood chips (BC6). All the feedstock are accepted as a permissible biomass for biochar production in Europe [26]. Before the pyrolysis, feedstock materials were air-dried and stored at ambient air humidity. Production of biochars was performed in September 2020 at Wroclaw University of Technology. Pyrolysis was conducted in the nitrogen atmosphere, at 550 °C and the conditions remained constant for every type of feedstock. The duration of the process was 60 minutes for each biomass. Organic matter applied in this experiment included compost (CO), cattle manure (MA) and fresh legume biomass (LE). Compost was produced in a home composter located in Wroclaw, Poland, from kitchen waste (vegetable and fruit peels) and garden waste (cut grass, leaves, small twigs). Dried cow manure in a form of granules was purchased from Fertigo fertilizer supplier (Suchy Las, Poland). Legume biomass consisting of whole plants of white and red clover (*Trifolium repens* L., *Trifolium pratense* L.) was collected from green areas in Wrocław, Poland.

Biochars, compost and manure were air dried and grounded in a soil mill to obtain particle sizes <2 mm. Fresh legume biomass was carefully washed with distilled water, to avoid the pollution of incubation systems and cut into pieces <2 mm with scissors to obtain materials with uniform fraction sizes. Biochars and organic materials with particle sizes <2 mm were mixed thoroughly with the soil in the following proportions: biochars 2% (v/w) (corresponding to additions of 0.565 – 0.915 t ha^{-1} depending on biochar's bulk density, assuming the thickness of plowing layer 25 cm and soil density 1.50 g cm^{-1}) and organic matter 1% (w/w) (corresponding to the dose of 37.5 t ha^{-1}). Then, 100 g of each mixed sample was placed in a 550-mL glass vessel. All treatments are summarized in Table 2. Vessels were incubated in a place protected from direct sunlight, at a constant air temperature of 22 °C. They were left open most of the time to allow soil respiration, with possibility to close tightly if needed. Moisture of the incubated material was maintained at approx. 20% by weight, by watering with distilled water when necessary.

Table 1. Summary of the treatments in incubation experiment.

Description	Abbreviation	Dose equivalent [t ha^{-1}]
Sandy soil without amendments	SA	-
Sandy soil with 6 types of biochar	SA BC1 - SA BC6 [1]	0.57 – 0.92 (2% v/w)
Sandy soil with 6 types of biochar and three types of organic matter	SA BC1- BC6 CO for compost SA BC1- BC6 MA for manure SA BC1- BC6 LE for legumes	biochar: 0.57 – 0.92 (2% v/w) organics: 37.50 (1% w/w)
Silt loam soil without amendments	SiL	-
Silt loam soil with 6 types of biochar	SiL BC1 - SiL BC6	0.57 – 0.92 (2% v/w)
Silt loam soil with 6 types of biochar and three types of organic matter	SiL BC1- BC6 CO for compost SiL BC1- BC6 MA for manure SiL BC1- BC6 LE for legumes	biochar: 0.57 – 0.92 (2% v/w) organics: 37.50 (1% w/w)

[1] - respectively for all six biochar types

2.2. Analysis of substrates

To determine standard characteristics of the substrates, samples of the soils, biochars, compost and manure were air-dried, sieved with 2 mm mesh and further prepared following the specific methodologies of analyses. The pH was determined in H_2O in 1:5 suspension (v/v) using pH-meter (Mettler-Toledo, Graifensee, Switzerland). For soil samples, particle size distribution was measured using mesh and hydrometer method, and content of calcium carbonates as an equivalent was determined using Scheibler apparatus (according to DIN 18129, ISO 10693 method) – approach frequently applied in Poland to determine $CaCO_3$ content in soil samples [27,28]. Cation exchange

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capacity was measured by MP-AES 4200 Spectrometer (Agilent Technologies, Santa Clara, CA, USA) after extraction with 1 M ammonium acetate. For biochars, a modification of the method was used, based on rinsing the samples with isopropanol, as proposed by Munera-Echeverri et al. [29]. Total organic carbon and total nitrogen was measured on enviro TOC/TN analyzer (Elementar, Langenselbold, Germany). Ash content was calculated based on mass loss after sample combustion in a muffle furnace at 550 °C (Czylok, Jastrzębie Zdrój, Poland). Characteristics of the soils, biochar and organic materials used as substrates for the experiment are summarized in Table 2.

Table 2. General properties of the 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}]	C:N	Ash [%]	$CaCO_3$ [%]
Soils	SA	Loamy sand sand silt clay [%] 81 17 2	4.62	1.62	0.72	0.04	16.9	n/a	0.25
	SiL	Silt loam sand silt clay [%] 22 64 15	6.40	11.70	0.99	0.07	13.7	n/a	0.00
Biochars	BC1	Food wastes	9.41 ± 0.05	228	53.0 ± 1.10	0.98 ± 0.02	54.1	10.1 ± 1.00	n/a
	BC2	Cut green grass	10.43 ± 0.04	228	52.0 ± 1.00	2.70 ± 0.05	19.3	31.3 ± 3.10	n/a
	BC3	Coffee grounds	6.91 ± 0.07	35.0	68.0 ± 1.40	3.60 ± 0.07	18.9	3.70 ± 0.40	n/a
	BC4	Wheat straw	7.20 ± 0.13	7.41	76.0 ± 1.50	0.24 ± 0.05	317	1.30 ± 0.1	n/a
	BC5	Sunflower husks	10.29 ± 0.02	35.3	78.0 ± 1.60	0.63 ± 0.01	124	5.60 ± 0.60	n/a
	BC6	Beech wood chips	6.96 ± 0.07	22.7	70.0 ± 1.40	1.40 ± 0.03	50.0	9.80 ± 1.00	n/a
Organic matter	CO	Compost	5.66	10.8	17.6	2.01	8.77	n/a	n/a
	MA	Manure	7.00	n/a	28.0	4.00	7.00	n/a	n/a
	LE	Legume biomass	n/a	n/a	51.8	n/a	n/a	12.20	n/a

[1] in table: Abbr. = abbreviation, CEC = cation exchange capacity, TOC = total organic carbon, TN = total nitrogen, n/a = not applicable. Values are means ± standard deviation (SD) from three replicates.

2.3. Respiration measurements

Soil respiration was measured during the incubation as an amount of CO_2 released by the unit of soil + treatment in the unit of time. To determine this value, a portable gas detector with infrared (IR) sensor dedicated for CO_2 concentration measurements (GasHunter II, Alter S.A., Tarnowo Podgórne, Poland) was used. Measuring range of the device was 0-5000 ppm with a resolution of 50 ppm. The assumption was that in closed vessels concentration of CO_2 will increase over time only as an effect of the soil respiration. The test began with the sealing of the jars for one hour, to allow CO_2 to accumulate. Then, carbon dioxide concentrations were measured in the air inside the vessel, by inserting the probe with the pump through a dedicated valve in the cap and collecting the sample for 60 seconds (please see the scheme below – Figure 1).

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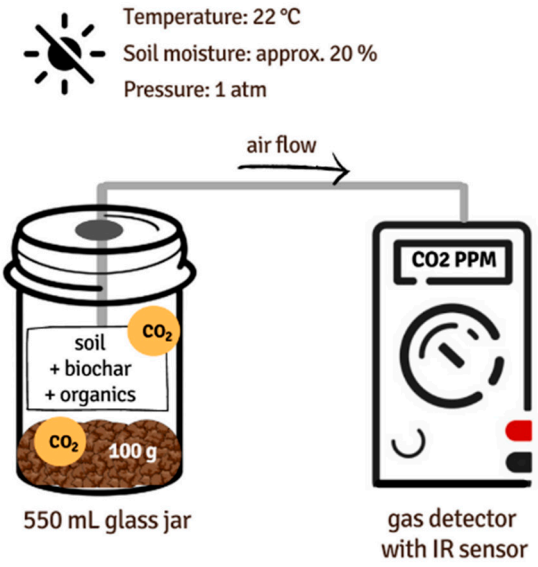

Figure 1. Scheme of the soil respiration measurements.

Measurements were conducted in three replicates and the final value is an average. Zero value as a reference point was CO_2 concentration in the air in the laboratory. Measurements were carried out at 1, 3, 5, 7, 14, 35, 42, 55, 70, 84 and 98 days of an incubation. After this time the CO_2 was constant and at a very low rate, close to the detection limit of the device. The temperature and air humidity in the room were constant during the measurements (22 °C, humidity approx. 50%). To control these conditions, automatic sensors of air parameters were used and conditions in the incubation room were adjusted by air-conditioning if needed.

Values recorded by CO_2 sensor were displayed in ppm, therefore it was necessary to perform some calculations. We adapted protocol proposed by Fierer [30], based on the universal gas law to convert ppm CO_2 to C-CO_2 [µg]. We assumed that the pressure and temperature were constant during all the measurements (1 atm and 22 °C), and volume of free space in the vessel was 490 mL (the remaining volume from 550 mL was taken up by soil). To calculate the number of moles of air in the vessel (n) the modified ideal gas law was applied:

$$n = \frac{pV}{RT}$$

Where V = volume of air in the vessel (490 mL), p = pressure (1 atm), R = const. [82.05 mL atm mol^{-1} K^{-1}], T = temperature in K = 273 + °C [273 + 22 = 293 K]. According to the calculations each vessel contained 20.38 mmol of air. To determine the exact amount of C-CO_2 released by the incubated mixture, the following equation was applied, on the basis of laboratory protocol by Fierer [30].

$$\mu g\ C - CO_2 = mmol\ air\ \times ppm\ CO_2\ \times 10^{-3}\ \frac{mol}{mmol} \times 12\ \frac{\mu gC}{\mu molC}$$

Calculated values relate to µg of C-CO_2, released by 100 g of soil in one hour.

Graphs and figures were prepared with GraphPad Prism 5 Software for Windows (GraphPad Software Inc., San Diego, CA, USA). Calculations of results were performed using MS Excel Software (Microsoft, Redmond, WA, USA) and GraphPad Prism 5 Software for Windows.

2.4. Carbon loss estimation

Carbon loss was balanced as a percentage of carbon released during the respiration measurements in relation to whole carbon pool present in incubated vessels, originated from native soil organic matter, biochars and organic amendments (compost, manure or legume biomass). Results

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of cumulative respiration were calculated to obtain mass of released carbon. Carbon content in soil, biochar and organic amendment was determined before the experiment, in dry substrates. Then, the amount of C introduced with biochar and organic amendment required the following calculations.

For biochars, calculations were based on carbon content in dry substrates and bulk density of biochars, using the formula:

$$BCC = \rho \times V \times \%C\ [g]$$

Where BCC = carbon originating from biochars, ϱ = bulk density of biochars [g cm^{-3}], V = amount of biochar in vessel (2 cm^3), %C = carbon content in biochars.

For compost, manure and legume biomass, calculations included dry mass and carbon content in the substrate:

$$COA = m \times d.m. \times \%C\ [g]$$

Where COA = carbon originating from organic amendment, m = mass of amendment in vessel (1 g), d.m. = content of dry mass [%], %C = carbon content in dry mass of the amendment.

Then, carbon pool introduced from organic amendments, biochars and present in soil was summarized to obtain total C content in incubated vessels (g 100 g^{-1} soil). Total carbon content was compared with carbon losses during the respiration, what allowed to express the loss as a percentage of carbon pool. Results were summarized in the Table S1 – supplementary.

3. Results

3.1. Effect of soil and biochar type on respiration

During 100 days of incubation, in each variant, regardless of soil characteristics and type of biochar, the highest respiratory activity was indicated during the first 7 days. After the initial peaks and some fluctuations of respiration, strong decreases of CO_2 release were noted and after about 10 days of the experiment recorded values were definitely lower and stable. Despite similar trends over time (the highest CO2 evolution in the first week of incubation, followed by sharp decrease and stabilization of the values), meas-ured values differed between sandy (SA) and loamy (SiL) soil. Carbon dioxide evolu-tion tended to be higher on SA, compared with SiL amended with analogous doses and types of biochars (Figure 2 and Figure 3). The largest CO2 emission was from sandy soil with BC1, up to 162 μg C-CO2 h−1 100 g−1 of soil (mean value), recorded on the 1st day of in-cubation with biochar made from food waste (Figure 2). The treatment with BC1 was as-sociated with the highest respiration also in loamy soil (Fig 3). The second biochar that led to remarkably higher respiration rate on both soil types was derived from coffee grounds (BC3). The pattern between all the treatments reveals that the non-amended control soils had lower respiration rate than samples incubated with biochars. Moreo-ver, CO2 evolution from SiL tends to be lower than from sand mixed with the same bi-ochar types, regardless of the fact that loamy soil had higher initial carbon content (0.99 g 100 g−1 vs. 0.72 g 100 g−1 on SA) (Table 1).

Carbon losses were calculated on the basis of TOC in soil and amendments, com-pared with the amount of carbon lost as C-CO2 during the respiration. Although in none of the treatments calculated C depletion exceeded 1%, there were some clear dif-ferences between variants of the experiment. During 100 days of incubation, sandy soil (SA) amended only with biochars lost from 0.22% (SA + BC5) to 1.01% (SA + BC1) of the total organic carbon present in incubated mixture, whereas silt loam (SiL) mixed with the same biochar types exhibited lower declines of C content in the range of 0.21% (SiL + BC5) to 0.52% (SiL + BC1) (Table S1 – supplementary).

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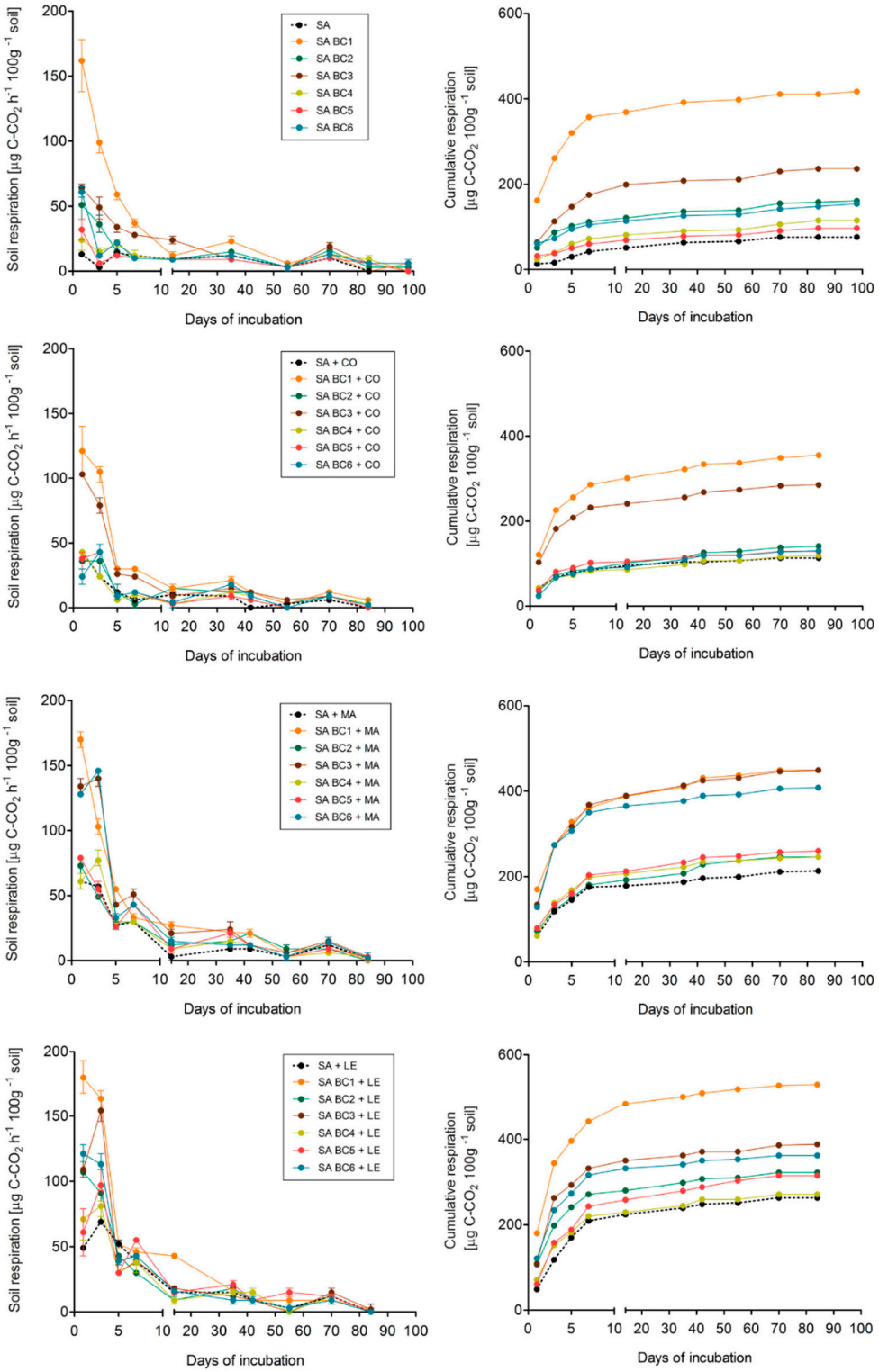

Figure 2. Respiration of sandy soil (SA) amended with biochars (BC1-BC6) and organic materials: compost (CO), cattle manure (MA) and legume biomass (LE). Point = mean, bars = minimum and maximum. BCs origins: BC1 = kitchen waste, BC2 = cut grass, BC3 = coffee grounds, BC4 = wheat straw, BC5 = sunflower husks, BC6 = beech wood chips.

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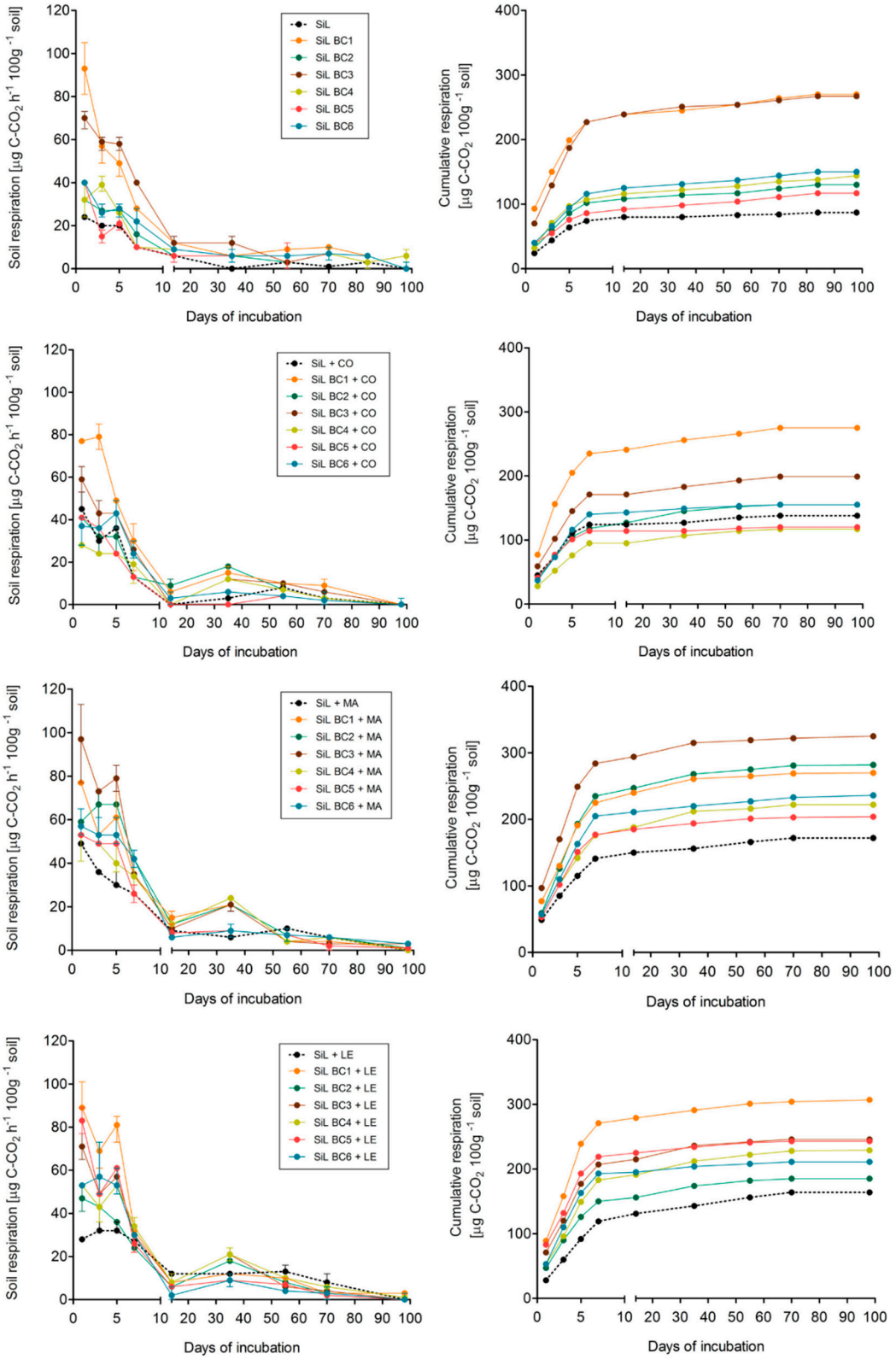

Figure 3. Respiration of silt loam (SiL) amended with biochars (BC1 – BC6) and organic materials: compost (CO), cattle manure (MA) and legume biomass (LE). Point = mean, bars = minimum and maximum. BCs origins: BC1 = kitchen waste, BC2 = cut grass, BC3 = coffee grounds, BC4 = wheat straw, BC5 = sunflower husks, BC6 = beech wood chips.

3.2. Effect of exogenous organic matter on soil respiration

Considering variants with additional organic amendments – compost, manure and legume biomass, higher CO_2 evolution rates were obtained from soils treated with BCs + manure and legume biomass than with compost. For sandy soils, in variants with manure, respiration day-by-day reached up to 145–170 μg C-CO_2 h^{-1} 100 g^{-1} in 3 out of 7 tested combinations, whereas legume biomass addition caused even higher CO_2 release, with the maximum of 180 μg C-CO_2 h^{-1} 100 g^{-1} (SA BC1 + LE). In addition, it was noted that SA + MA and SA + LE treatments showed values of respiration around 30-40 μg C-CO_2 h^{-1} 100 g^{-1} for longer time (approx. 30 days) than soils amended with compost or with biochars only (Figure 2). Treatments with SiL follow the same trend. As within the sandy soils, respiration rate increased in silt loams amended with biochars and legume biomass or manure, whereas compost had lesser effect on CO_2 evolution. Moreover, organic amendments, especially legumes and manure resulted in longer persistence of high soil respiration values – after 14 days of incubation the CO_2 evolution in SiL + MA or SiL + LE treatments rate was still relatively high, around 20-40 μg C-CO_2 h^{-1} 100 g^{-1} (Figure 3). Considering carbon loss percentage in biochar + organic amended soils, addition of easily decomposable organic matter generally increased percentage of carbon loss, especially in manure treated soil with BC (up to 0.85% in SA BC1 + MA) and legume biomass (1.10% in SA BC1 + LE). Effect of compost on the dynamics of C losses during respiration was less evident, as the maximum reached 0.73% (SA BC1 + CO), whereas most values in compost-amended soil were 0.2-0.3%, both in sandy and silty soils (Table S1 – supplementary).

To sum up, initial soil carbon status (native organic carbon pool) had no effect on observed CO_2 efflux – respiration rate was even lower on SiL than on SA soil. Regardless of the soil type, BCs showed similar patterns of respiration among tested treatments. Carbon dioxide emissions were the highest directly after application of the amendment and maximum values were observed for BCs rich in labile organic compounds, such as BC1 or BC3. This strongly suggests that initial peak of CO_2 evolution was a result of labiles decomposition from biochars. Moreover, among tested exogenous organic matter sources, raw materials (legume biomass and manure) had greater impact on C mineralization rates than biologically stable OM from compost, what was reflected in remarkably lower respiration in CO_2 amended treatments, compared with LE or MA. Generally, the trend in CO_2 evolution due to the type of biochar is: BC1 > BC3 > BC6, BC2 > BC5, BC4, and due to the additional exogenous organic matter source is: legume biomass > manure > compost.

4. Discussion

Results of study showed that under controlled environmental conditions biochar amendments affected GHG emission increasing CO_2 release from soils. The stimulating effects of biochar application on soil CO_2 fluxes can be ascribed to higher labile C mineralization and inorganic C release from biochar [13]. Furthermore, biochar application supports labile soil organic carbon pools enhancing microbial activity [31]. Microbial available C and nutrients in biochar are strongly correlated with temperature of pyrolysis [1,32], however findings of our study support the thesis that also biomass origin and properties of biochar, especially the content of labile C fractions, will contribute to the process of SOC mineralization, stimulating CO_2 emission from soil. Addition of biochar with more labile C fractions e.g. derived from kitchen wastes contributed to the process of CO_2 emission from soil more prominently than biochars derived from high lignocellulose biomass e.g. wood chips or straw. This observation is in agreement with our previous findings described by Bednik et al. [10]. Higher content of water-soluble carbohydrates (WSC) or dissolved organic carbon (DOC), and also less aliphatic structure of biochars derived from kitchen wastes such as coffee grounds (BC3) or vegetable and fruit peels (BC1) serve as labile C sources for microbes when applied to soil. Similar patterns in BCs mineralization were observed by Farrell et al. [33], showing that soil microbes rapidly utilize easily-available carbon pools delivered with biochar in forms of carbohydrates, dissolved organic carbon or volatile solids, but also a wide range of other organic compounds. Our results indicated that CO_2 fluxes varied over time after biochar application, which is in an agreement with previous findings [34–36]. However, mechanisms involved in soil CO_2 stimulation after biochar application may differ in the short term compared to long term study. The

effect of breakdown of organic C and release of DOC from biochar is stimulating CO_2 emission from soil in a very short term of time after biochar application (up to 7 days). After sources of readily available carbon are utilized, CO_2 flux in biochar amended soils was stable, however higher compared to un-amended soils. This confirms that biochar can cause priming effect to native soil organic matter, but in the long time the process of CO_2 emission directly from biochar transformations becomes negligible thus not contributing to the GHG emission in global scale [17,36]. Application of biochar to tested soils also affected carbon pools, causing carbon losses, probably due to disturbance of soil environment (input of nutrients and labile carbon source) [34]. Usually, SOC content increases in the short term are due to the application and incorporation of fresh and C-rich biochar into the soil. This initial exposition of fresh biochar leads to a high microbial response and the turnover of the labile C fractions, often referred to as a positive priming effect [37].

Carbon losses can be also correlated with soil texture and this phenomenon was observed in the study. According to Gross et al. [38] in meta-analysis, biochar applications to clay soils resulted in the highest SOC stock increase, followed by silty soils and loamy soils. The lowest increases were observed for sandy soils. In general, higher clay mineral content in finer textured soils not only provides physical protection of SOC to enzymatic activity and thus turnover, but also increases SOC stability in the form of aggregates [39]. The effects of biochar application on soil CO_2 fluxes can be different depending on experimental design and conditions. Usually, very simple experiments with only biochar and unfertilized soils are preferred, however distinct effects can be observed when inorganic or organic fertilization is performed on biochar – amended soil.

In our study, we hypothesized that labile organic matter (LOM) from cover crops residues or organic fertilizers e.g. manures or compost may change the C-sequestration potential in biochar-amended soil. Both types of C sources will contribute to the SOC priming effect and this may induce changes in native mineralization process of organic matter, which in turn will increase or decrease CO_2 flux from soil [40]. The results of the experiment showed that introduction of EXOC to biochar amended soil enhances CO_2 fluxes from soil, however not equally, and raw materials e.g. cover crop residues will contribute to the process more actively than stable forms of organics like compost. The effect will vary also depending on soil type and properties. More prominent stimulating effect of EXOC on CO_2 emission was observed in sandy soil with biochar amendment. Faster BC-C mineralization on soil with low organic matter content is associated with good adaptation of microbes for the limited nutrients, and more effective utilization of available labile compounds, in comparison with soils rich in native organic matter [1,2,14,41–43]. In terms of loamy soil, lower CO_2 emission can be explained by organo-mineral interactions and protection of organic matter against mineralization process, which is claimed as a main factor of reduced GHG emission from soils with high clay minerals content [44,45].

Food waste is one of the society's highest volumes and most environmentally impactful waste streams. Upcycling of food waste into usable materials can be integral to mitigate the substantial greenhouse gas emissions associated with wasted food [25]. Inference on the high stability of biochar in the soil environment is limited to a very narrow group of biochars produced from basic and generally available types of biomasses, more attention should be paid to 'new biochars' obtained by utilizing household and food waste. As a very valuable source of nutrients and active compounds its utilization as a soil amendment seems to be a natural way of waste upcycling. This work highlights the problem of future implications related to incorporation to soil new types of black carbon. Variability of soil CO_2 fluxes in biochar amended soils can be attributed to biochar and soil properties, but also inputs of exogenous organic matter from soil fertilization and other agronomic practices.

5. Conclusions

Performed study confirms that biochars, when applied into the soil, are the subject of slow mineralization process with CO_2 release. The key factor that affects CO_2 efflux from amended soil is feedstock type used for biochar production, that determines further properties of biochar. CO_2 efflux was the highest for food waste biochars containing more labile C fraction and consequently more susceptible for decomposition processes, compared to high-cellulose biochar. Application of

exogenous organic matter, especially raw organic plant residues and cow manure to biochar amended soils enhanced CO_2 release and carbon losses, however in the long – term contribution of the process might be negligible. To predict biochar behavior in soil under different farming practices it is necessary to develop field trials and provide data from long-term observation under natural conditions.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Table S1: Carbon balance in incubated treatments.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, (M.B., A.M.-J.); methodology, (M.B.); software, (M.B.); validation, (M.B.); formal analysis, (M.B.); investigation, (M.B., A.M.-J.); resources, (A.M.-J.); data curation, (M.B.); writing—original draft preparation, (M.B.); writing—review and editing, (A.M.-J., I.Ć.-P.); visualization, (M.B.); supervision, (A.M.-J., I.Ć.-P.); project administration, (M.B., A.M.-J. and I.Ć.-P.); funding acquisition, (M.B.). All authors have read and agreed to the published version of the manuscript.

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Supplementary data for the manuscript

Table S1. Carbon balance in incubated treatments.

Treatment	C from soil [g/100 g]	C from biochar [g/ 100 g soil]	C from organic amendment [g/100 g soil]	Total C content [g/ 100g soil]	Total measured C-CO_2 loss [g/ 100g soil]	Measured C loss in respiration [%]
SA	0.721	n/a	n/a	0.721	0.0018	0.25
SA BC1	0.721	0.268	n/a	0.989	0.0100	1.01
SA BC2	0.721	0.210	n/a	0.931	0.0039	0.42
SA BC3	0.721	0.366	n/a	1.087	0.0057	0.52
SA BC4	0.721	0.350	n/a	1.071	0.0028	0.26
SA BC5	0.721	0.353	n/a	1.074	0.0023	0.22
SA BC6	0.721	0.512	n/a	1.233	0.0037	0.30
SiL	0.986	n/a	n/a	0.986	0.0021	0.21
SiL BC1	0.986	0.268	n/a	1.254	0.0065	0.52
SiL BC2	0.986	0.210	n/a	1.196	0.0031	0.26
SiL BC3	0.986	0.366	n/a	1.352	0.0064	0.47
SiL BC4	0.986	0.350	n/a	1.336	0.0035	0.26
SiL BC5	0.986	0.353	n/a	1.339	0.0028	0.21
SiL BC6	0.986	0.512	n/a	1.498	0.0036	0.24
SA + CO	0.721	n/a	0.176	0.90	0.0027	0.30
SA + MA	0.721	n/a	0.280	1.00	0.0051	0.51
SA + LE	0.721	n/a	0.163	0.88	0.0063	0.71
SA BC1 + CO	0.721	0.268	0.176	1.17	0.0085	0.73
SA BC1 + MA	0.721	0.268	0.280	1.27	0.0108	0.85
SA BC1 + LE	0.721	0.268	0.163	1.15	0.0127	1.10
SA BC2 + CO	0.721	0.210	0.176	1.11	0.0034	0.31
SA BC2 + MA	0.721	0.210	0.280	1.21	0.0059	0.49
SA BC2 + LE	0.721	0.210	0.163	1.09	0.0077	0.71
SA BC3 + CO	0.721	0.366	0.176	1.26	0.0068	0.54
SA BC3 + MA	0.721	0.366	0.280	1.37	0.0108	0.79
SA BC3 + LE	0.721	0.366	0.163	1.25	0.0093	0.75
SA BC4 + CO	0.721	0.350	0.176	1.25	0.0029	0.23
SA BC4 + MA	0.721	0.350	0.280	1.35	0.0059	0.44
SA BC4 + LE	0.721	0.350	0.163	1.23	0.0065	0.53
SA BC5 + CO	0.721	0.353	0.176	1.25	0.0031	0.25
SA BC5 + MA	0.721	0.353	0.280	1.35	0.0062	0.46
SA BC5 + LE	0.721	0.353	0.163	1.24	0.0076	0.61
SA BC6 + CO	0.721	0.512	0.176	1.41	0.0031	0.22
SA BC6 + MA	0.721	0.512	0.280	1.51	0.0098	0.65
SA BC6 + LE	0.721	0.512	0.163	1.40	0.0087	0.62
SiL + CO	0.986	n/a	0.176	1.162	0.0033	0.28
SiL + MA	0.986	n/a	0.280	1.266	0.0041	0.33

SiL + LE	0.986	n/a	0.163	1.149	0.0039	0.34
SiL BC1 + CO	0.986	0.268	0.176	1.430	0.0066	0.46
SiL BC1 + MA	0.986	0.268	0.280	1.534	0.0065	0.42
SiL BC1 + LE	0.986	0.268	0.163	1.417	0.0074	0.52
SiL BC2 + CO	0.986	0.210	0.176	1.372	0.0037	0.27
SiL BC2 + MA	0.986	0.210	0.280	1.476	0.0068	0.46
SiL BC2 + LE	0.986	0.210	0.163	1.358	0.0044	0.33
SiL BC3 + CO	0.986	0.366	0.176	1.528	0.0048	0.31
SiL BC3 + MA	0.986	0.366	0.280	1.632	0.0078	0.48
SiL BC3 + LE	0.986	0.366	0.163	1.514	0.0059	0.39
SiL BC4 + CO	0.986	0.350	0.176	1.512	0.0028	0.19
SiL BC4 + MA	0.986	0.350	0.280	1.616	0.0053	0.33
SiL BC4 + LE	0.986	0.350	0.163	1.498	0.0055	0.37
SiL BC5 + CO	0.986	0.353	0.176	1.515	0.0029	0.19
SiL BC5 + MA	0.986	0.353	0.280	1.619	0.0049	0.30
SiL BC5 + LE	0.986	0.353	0.163	1.501	0.0058	0.39
SiL BC6 + CO	0.986	0.512	0.176	1.675	0.0037	0.22
SiL BC6 + MA	0.986	0.512	0.280	1.778	0.0057	0.32
SiL BC6 + LE	0.986	0.512	0.163	1.661	0.0051	0.30

n/a = not applicable.

Manuksrypt 3

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Article

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.

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.

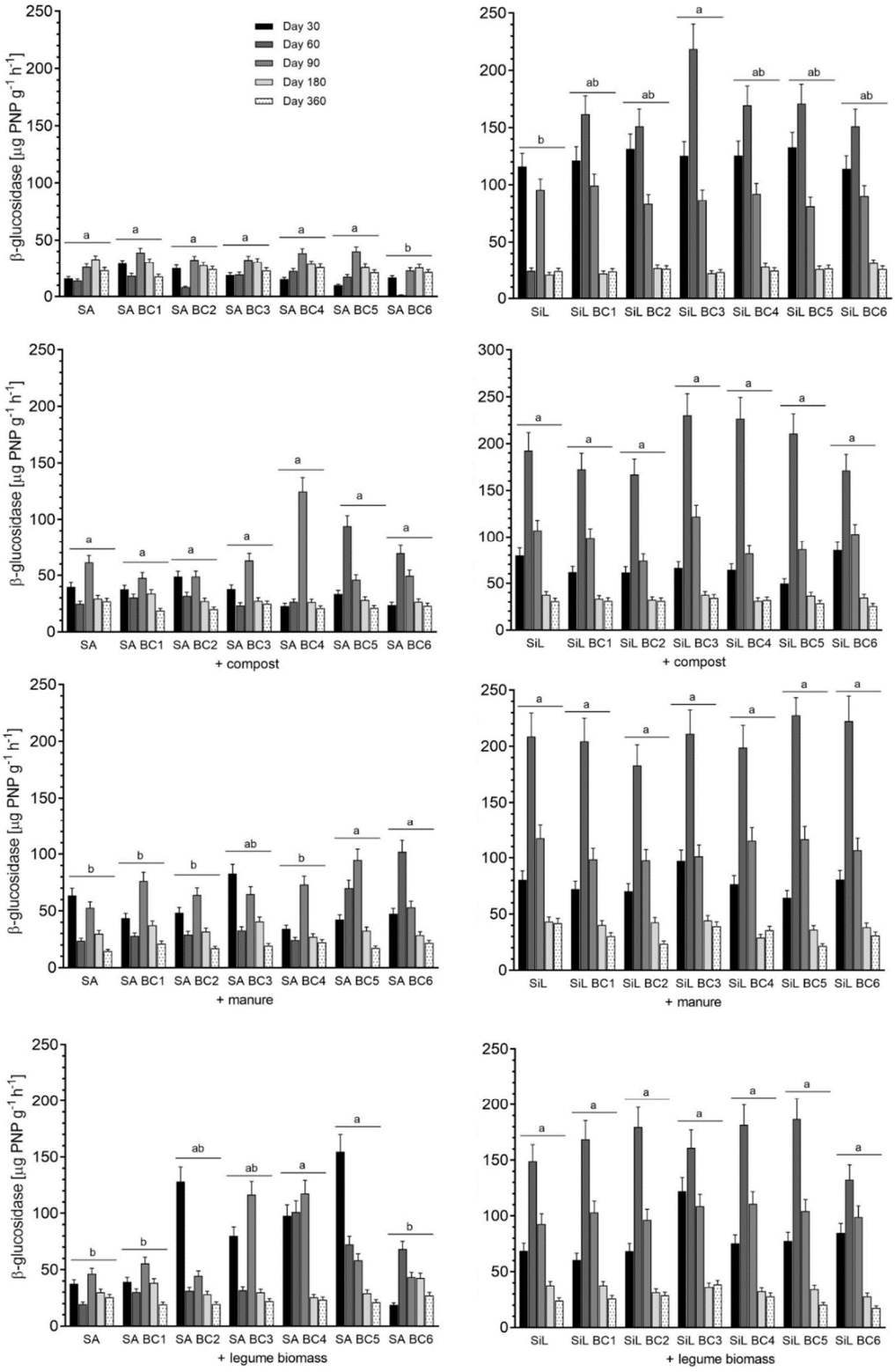

Figure 1. β-glucosidase activity among tested treatments. SA = sandy soil, SiL = silt loam soil, BC1-6 = biochars (see Table 1). Values are means ± SD from three replicates. Letters indicate homogenous groups considering biochar type as a main factor ($p < 0.05$).

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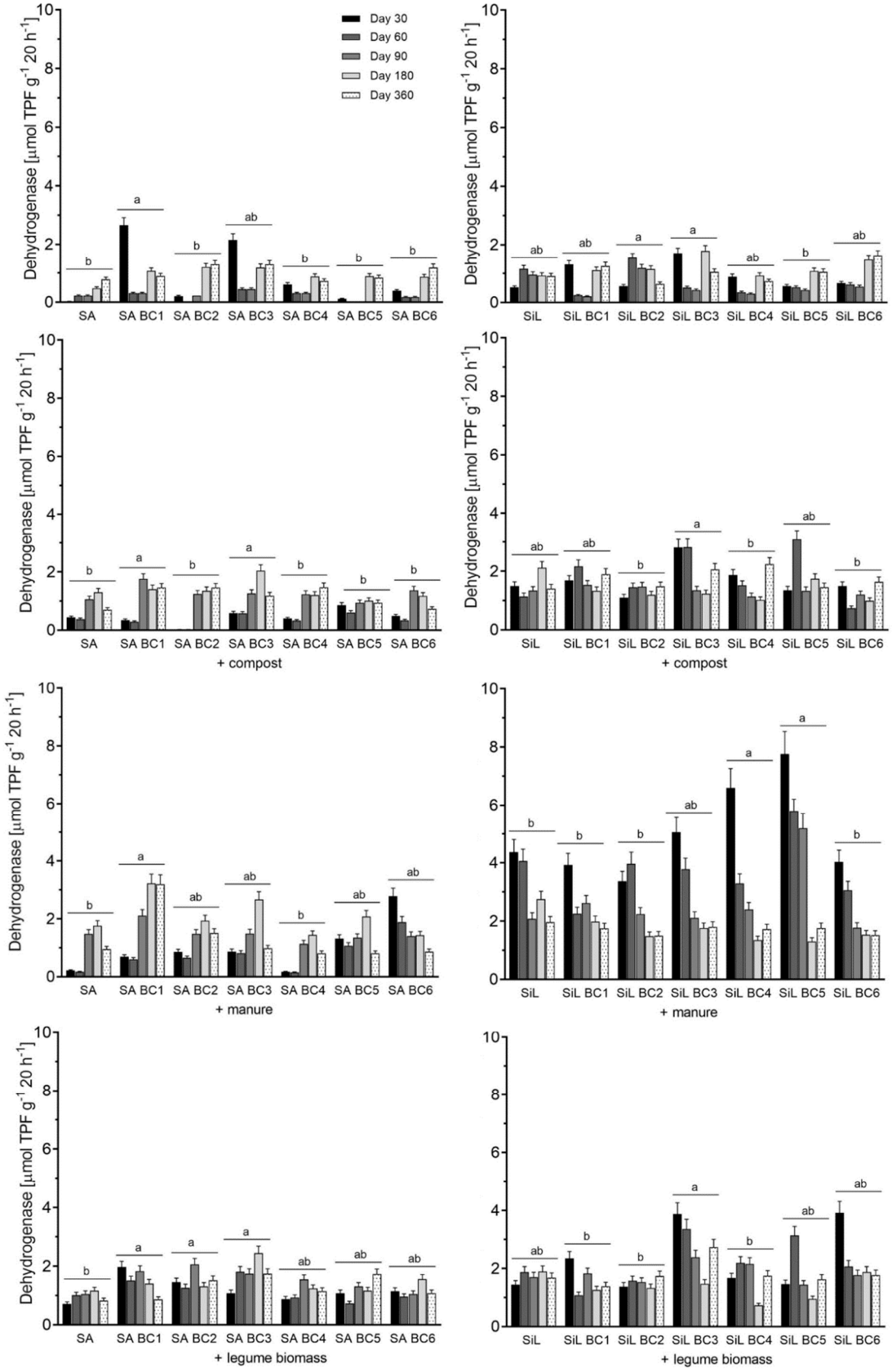

Figure 2. Dehydrogenase activity among tested treatments. SA = sandy soil, SiL = silt loam soil, BC1-6 = biochars (see Table 1). Values are means ± SD from three replicates. Letters indicate homogenous group considering biochar type as a main factor ($p < 0.05$).

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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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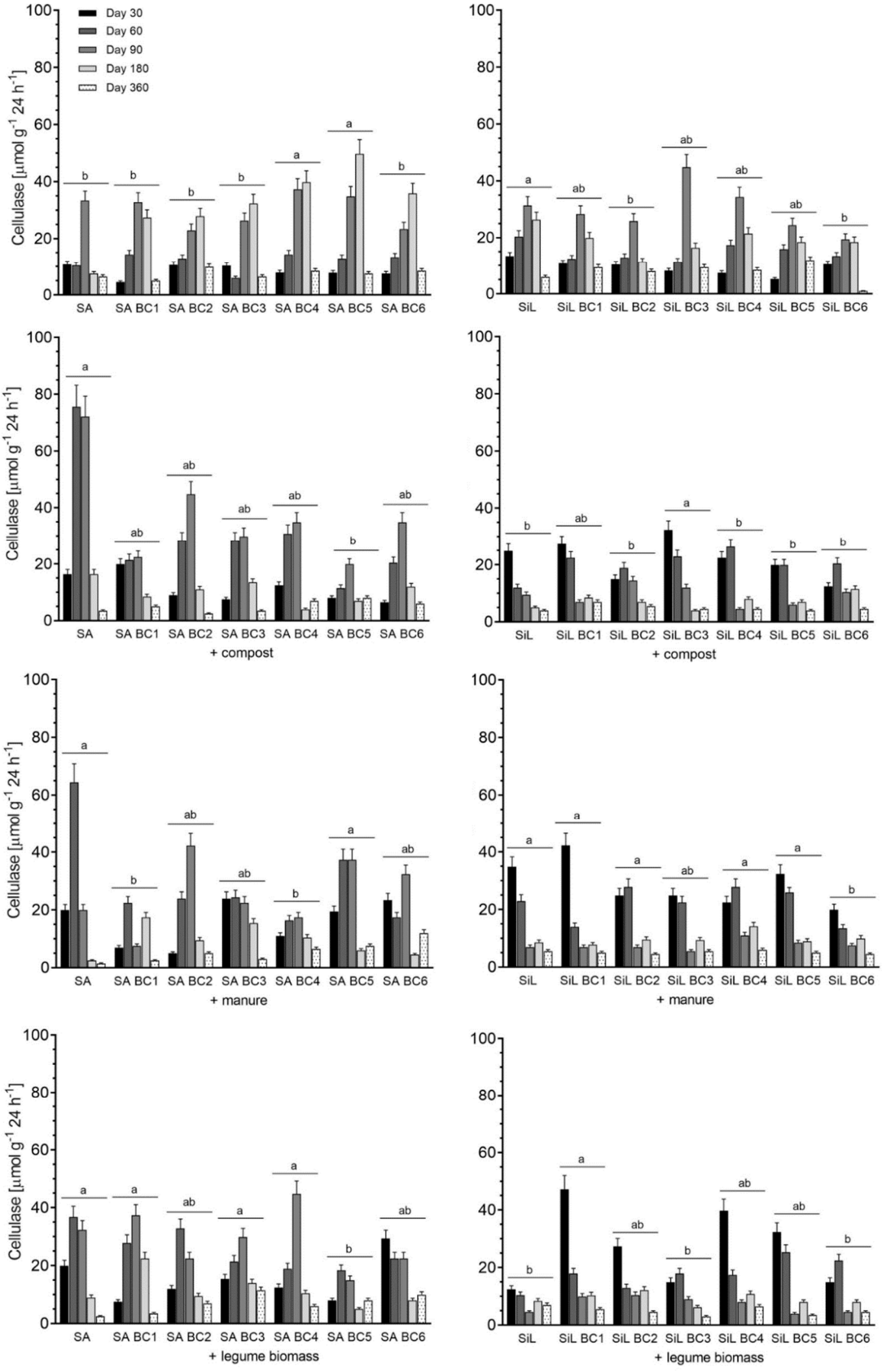

Figure 3. Cellulase activity among tested treatments. SA = sandy soil, SiL = silt loam soil, BC1-6 = biochars (see Table 1). Values are means ± SD from three replicates. Letters indicate homogenous group considering biochar type as a main factor ($p < 0.05$).

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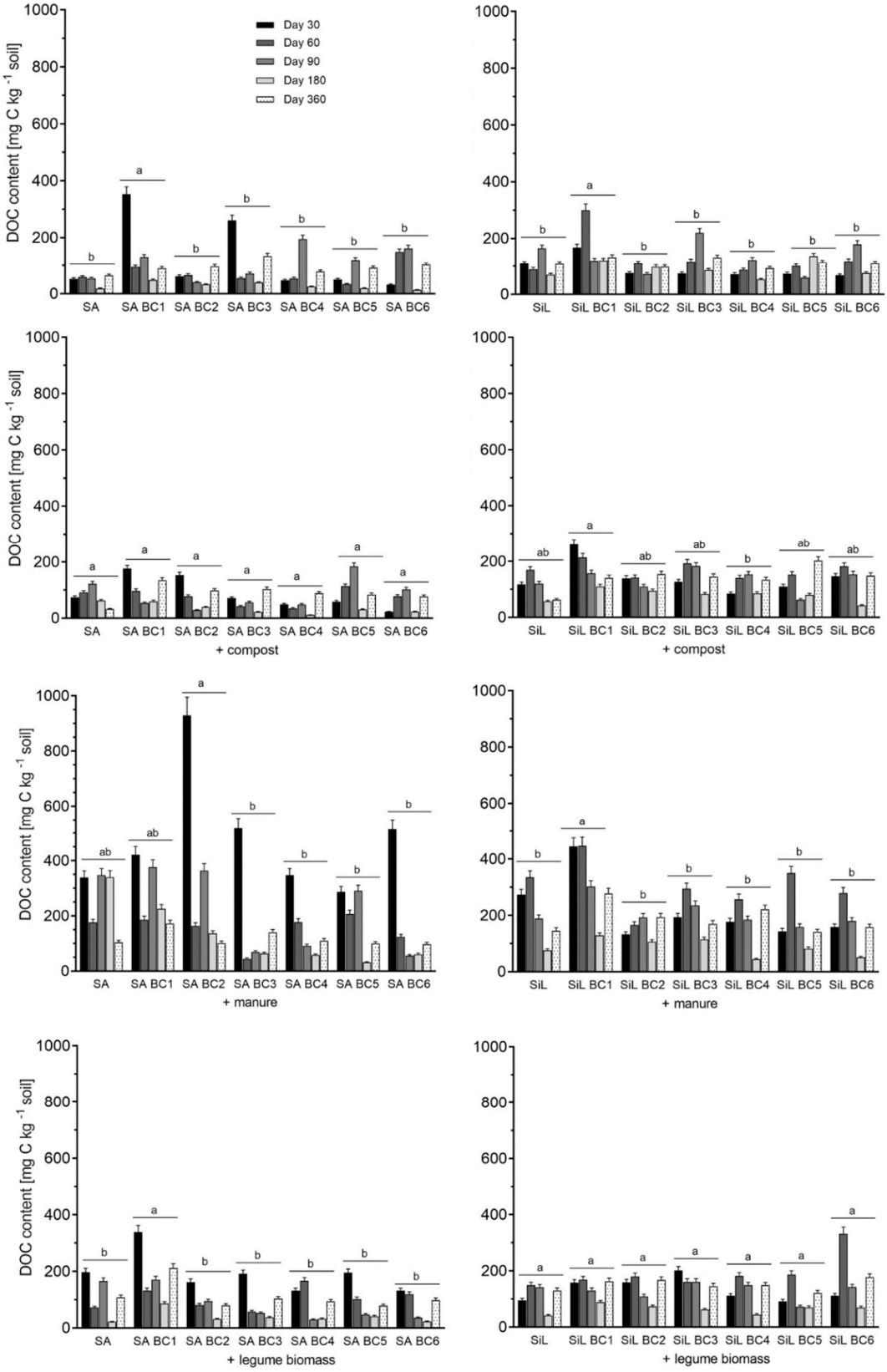

Figure 4. Dissolved organic carbon content among tested treatments. SA = sandy soil, SiL = silt loam soil, BC1-6 = biochars (see Table 1). Values are means ± SD from three replicates. Letters indicate homogenous group considering biochar type as a main factor ($p < 0.05$).

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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

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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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POTWIERDZENIA Z REDAKCJI O WPŁYNIĘCIU MANUKSRYPTÓW

Manuksrypt 2

Bednik M., Medyńska – Juraszek A., Ćwieląg – Piasecka I., 2023.
Biochar and Organic Fertilizer Co-Application Enhance Soil Carbon Priming, Increasing CO_2 Fluxes in Two Contrasting Arable Soils

Manuskrypt nieopublikowany, wysłany do redakcji *Materials*

Dostępny jako preprint:
10.20944/preprints202309.1124.v1

UNIWERSYTET PRZYRODNICZY WE WROCŁAWIU

Magdalena Bednik <magdalena.bednik@upwr.edu.pl>

[Materials] Manuscript ID: materials-2642629 - Co-Authorship Confirmation

1 wiadomość

Materials Editorial Office <materials@mdpi.com> 16 września 2023 20:02
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Do: Magdalena Bednik <magdalena.bednik@upwr.edu.pl>
CC: Materials Editorial Office <materials@mdpi.com>

Dear Ms. Bednik,

We are writing to let you know that we have received the below submission to Materials for which you are listed as a co-author.

Manuscript ID: materials-2642629
Type of manuscript: Article
Title: Biochar and organic fertilizer co-application enhances soil carbon priming increasing CO2 fluxes in two contrasting arable soils
Authors: Magdalena Bednik, Agnieszka Medyńska-Juraszek *, Irmina Ćwieląg-Piasecka
Received: 16 Sep 2023

In order to confirm your connection to this submission, please click here to confirm your co-authorship:
https://susy.mdpi.com/author/confirm/10497/F7vmWKBL

Kind regards,
Materials Editorial Office

Manuksrypt 3

Bednik M., Medyńska – Juraszek A., Ćwieląg – Piasecka I., Dudek M., 2023. Enzyme Activity and Dissolved Organic Carbon Content in Soils Amended with Different Types of Biochar and Exogenous Organic Matter

Manuskrypt nieopublikowany, wysłany do redakcji *Sustainability*

Dostępny jako preprint:
doi.org/10.20944/preprints202309.1603.v1

UNIWERSYTET PRZYRODNICZY WE WROCŁAWIU

Magdalena Bednik <magdalena.bednik@upwr.edu.pl>

[Sustainability] Manuscript ID: sustainability-2652519 - Co-Authorship Confirmation

1 wiadomość

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Do: Magdalena Bednik <magdalena.bednik@upwr.edu.pl>
CC: Sustainability Editorial Office <sustainability@mdpi.com>

Dear Ms. Bednik,

We are writing to let you know that we have received the below submission to Sustainability for which you are listed as a co-author.

Manuscript ID: sustainability-2652519
Type of manuscript: Article
Title: Enzyme Activity and Dissolved Organic Carbon Content in Soils Amended with Different Types of Biochar and Exogenous Organic Matter
Authors: Magdalena Bednik, Agnieszka Medyńska-Juraszek *, Irmina Ćwieląg-Piasecka, Michał Dudek
Received: 22 Sep 2023

In order to confirm your connection to this submission, please click here to confirm your co-authorship:
https://susy.mdpi.com/author/confirm/63163/2BGkjsfR

Kind regards,
Sustainability Editorial Office

OŚWIADCZENIA WSPÓŁAUTORÓW

Publikacja 1

Bednik M., Medyńska – Juraszek A., Ćwieląg – Piasecka I., 2022.
Effect of Six Different Feedstocks on Biochar's Properties and Expected Stability.
Agronomy, 12(7), 1525.
10.3390/agronomy12071525

Załącznik nr 3 do Trybu postępowania w sprawie nadania stopnia naukowego doktora

mgr inż. Magdalena Bednik
imię i nazwisko

Wrocław, 06.09.2023
miejscowość i data

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OŚWIADCZENIE

Oświadczam, że w pracy:

Bednik M., Medyńska-Juraszek A., Ćwieląg-Piasecka I., 2022. Effect of Six Different Feedstocks on Biochar's Properties and Expected Stability, *Agronomy*, vol. 12, nr 7, s.1-14, Numer artykułu:1525. DOI:10.3390/agronomy12071525

mój udział polegał na opracowaniu koncepcji oraz metodyki prezentowanych badań, przeprowadzeniu analiz laboratoryjnych, opracowaniu wyników i ich wizualizacji w formie wykresów, poddaniu wyników interpretacji i dyskusji, przygotowaniu draftu manuskryptu i dalszej edycji jego treści zgodnie z sugestiami współautorów, a także na przygotowaniu odpowiedzi na recenzje i kontakcie z edytorami czasopisma. Kierowałam również projektem naukowym "Innowacyjny Doktorat" nr N070/0009/20, obejmującym badania opisane w niniejszej pracy. Mój całościowy wkład w powstanie publikacji był dominujący i został oszacowany na 65%.

25.09.2023 M. Bednik
data i podpis

Potwierdzam treść oświadczenia.

25.09.23 A. Medyńska-Juraszek
data i podpis promotora

Załącznik nr 3 do Trybu postępowania w sprawie nadania stopnia naukowego doktora

dr hab. inż. Agnieszka Medyńska-Juraszek, prof. uczelni
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OŚWIADCZENIE

Oświadczam, że w pracy:

Bednik M., **Medyńska-Juraszek A.,** Ćwieląg-Piasecka I., 2022. Effect of Six Different Feedstocks on Biochar's Properties and Expected Stability, *Agronomy*, vol. 12, nr 7, s.1-14, Numer artykułu:1525. DOI:10.3390/agronomy12071525

mój udział polegał na nadzorowaniu koncepcji oraz poprawności zaproponowanej metodyki badań, opiece merytorycznej nad wykonywaniem analiz laboratoryjnych, uczestnictwie w interpretacji uzyskanych danych, edycji treści manuskryptu oraz sprawdzeniu odpowiedzi dla recenzentów publikacji. Mój całościowy wkład w powstanie publikacji został oszacowany na 25%.

25.09.23 A. Medyńska-Juraszek
data i podpis

Załącznik nr 3 do Trybu postępowania w sprawie nadania stopnia naukowego doktora

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OŚWIADCZENIE

Oświadczam, że w pracy:

Bednik M., Medyńska-Juraszek A., **Ćwieląg-Piasecka I.,** 2022. Effect of Six Different Feedstocks on Biochar's Properties and Expected Stability, *Agronomy*, vol. 12, nr 7, s.1-14, Numer artykułu:1525. DOI:10.3390/agronomy12071525

mój udział polegał na sprawdzeniu oraz edycji treści manuskryptu oraz sprawowaniu nadzoru merytorycznego nad pracą doktorantki. Mój całościowy wkład w powstanie publikacji został oszacowany na 10%.

25.09.2023
Irmina Ćwieląg-Piasecka
data i podpis

Manuksrypt 2

Bednik M., Medyńska – Juraszek A., Ćwieląg – Piasecka I., 2023.
Biochar And Organic Fertilizer Co-Application Enhance Soil Carbon Priming, Increasing CO_2 Fluxes in Two Contrasting Arable Soils

Załącznik nr 3 do Trybu postępowania w sprawie nadania stopnia naukowego doktora

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OŚWIADCZENIE

Oświadczam, że w pracy:

Bednik M., Medyńska-Juraszek A., Ćwieląg-Piasecka I., 2023. Biochar and organic fertilizer co-application enhance soil carbon priming, increasing CO_2 fluxes in two contrasting arable soils (manuskrypt wysłany do czasopisma *Materials)*

mój udział polegał na zaproponowaniu schematu doświadczenia laboratoryjnego, założeniu i prowadzeniu doświadczenia, przeprowadzaniu pomiarów respiracji gleby oraz opracowaniu metody obliczania wyników na podstawie dostępnej literatury. Przeprowadziłam analizę, wizualizację na rysunkach oraz wykresach, a także dyskusję uzyskanych danych. Przygotowałam oryginalny draft manuskryptu, a następnie edytowałam go zgodnie z komentarzami współautorów. Kierowałam również projektem naukowym "Innowacyjny Doktorat" nr N070/0009/20, obejmującym badania opisane w niniejszej pracy. Mój całościowy wkład
w powstanie manuskryptu był dominujący i został oszacowany na 60%.

25.09.2023 M. Bednik

data i podpis

Potwierdzam treść oświadczenia.

25.09.23 A. Medyńska-Juraszek

data i podpis promotora

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dr hab. inż. Agnieszka Medyńska-Juraszek, prof. uczelni
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OŚWIADCZENIE

Oświadczam, że w pracy:

Bednik M., **Medyńska-Juraszek A.,** Ćwieląg-Piasecka I., 2023. Biochar and organic fertilizer co-application enhance soil carbon priming, increasing CO_2 fluxes in two contrasting arable soils (manuskrypt wysłany do czasopisma *Materials*)

mój udział polegał na merytorycznej ocenie koncepcji doświadczenia zaproponowanej przez doktorantkę, ocenie poprawności uzyskanych wyników oraz sprawdzaniu i edycji treści manuskryptu, a także sprawowaniu nadzoru nad przebiegiem pracy doktorantki i pełnieniu roli autora korespondencyjnego w niniejszej pracy. Mój całościowy wkład w powstanie manuksryptu został oszacowany na 30%.

25.09.23 A. Medyńska-Juraszek

data i podpis

Załącznik nr 3 do Trybu postępowania w sprawie nadania stopnia naukowego doktora

dr Irmina Ćwieląg-Piasecka
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OŚWIADCZENIE

Oświadczam, że w pracy:

Bednik M., Medyńska-Juraszek A., **Ćwieląg-Piasecka I.**, 2023. Biochar and organic fertilizer co-application enhance soil carbon priming, increasing CO_2 fluxes in two contrasting arable soils (manuskrypt wysłany do czasopisma *Materials*)

mój udział polegał na sprawdzaniu oraz edycji treści manuskryptu oraz sprawowaniu nadzoru merytorycznego nad pracą doktorantki i został oszacowany na 10%.

25.09.2023
Irmina Ćwieląg-Piasecka
data i podpis

Manuksrypt 3

Bednik M., Medyńska – Juraszek A., Ćwieląg – Piasecka I., Dudek M., 2023. Enzyme Activity and Dissolved Organic Carbon Content in Soils Amended with Different Types of Biochar and Exogenous Organic Matter

Załącznik nr 3 do Trybu postępowania w sprawie nadania stopnia naukowego doktora

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Wrocław, 06.09.2023
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OŚWIADCZENIE

Oświadczam, że w pracy:

Bednik M., Medyńska-Juraszek A., Ćwieląg-Piasecka I., Dudek M., 2023. Enzyme activity and dissolved organic carbon content in soils amended with different types of biochar and exogenous organic matter (manuskrypt wysłany do czasopisma *Sustainability*)

mój udział polegał na zaproponowaniu na podstawie literatury i przetestowaniu metodyki kolorymetrycznych oznaczeń enzymów oraz ekstrakcji DOC, przeprowadzaniu opisanych w pracy analiz laboratoryjnych oraz obliczeniu wyników. Przeprowadziłam wizualizację uzyskanych wyników oraz odniosłam je do obecnego stanu wiedzy, przygotowując oryginalny draft manuskryptu i edytując go zgodnie z komentarzami współautorów. Kierowałam także projektem naukowym "Innowacyjny Doktorat" nr N070/0009/20, obejmującym badania opisane w niniejszej pracy. Mój całościowy wkład w powstanie manuskryptu był dominujący i został oszacowany na 50%.

25.09.2023 M. Bednik
data i podpis

Potwierdzam treść oświadczenia.

25.09.2023 A. Medyńska-Juraszek
data i podpis promotora

Załącznik nr 3 do Trybu postępowania w sprawie nadania stopnia naukowego doktora

dr hab. inż. Agnieszka Medyńska-Juraszek, prof. uczelni
imię i nazwisko

Wrocław, 06.09.2023
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OŚWIADCZENIE

Oświadczam, że w pracy:

Bednik M., **Medyńska-Juraszek A.,** Ćwieląg-Piasecka I., Dudek M., 2023. Enzyme activity and dissolved organic carbon content in soils amended with different types of biochar and exogenous organic matter (manuskrypt wysłany do czasopisma *Sustainability*)

mój udział polegał na merytorycznym nadzorze nad przebiegiem prac doktorantki, opiniowaniu proponowanych metodyk oznaczeń laboratoryjnych oraz weryfikacji poprawności uzyskanych wyników. Dyskutowałam z doktorantką koncepcję i zakres publikacji, a następnie edytowałam przygotowany draft manuskryptu. Mój całościowy wkład w powstanie manuksryptu został oszacowany na 25%.

25.09.23 A. Medyńska-Juraszek

data i podpis

Załącznik nr 3 do Trybu postępowania w sprawie nadania stopnia naukowego doktora

dr Irmina Ćwieląg-Piasecka
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Wrocław, 06.09.2023
miejscowość i data

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OŚWIADCZENIE

Oświadczam, że w pracy:

Bednik M., Medyńska-Juraszek A., **Ćwieląg-Piasecka I.**, Dudek M., 2023. Enzyme activity and dissolved organic carbon content in soils amended with different types of biochar and exogenous organic matter (manuskrypt wysłany do czasopisma *Sustainability*)

mój udział polegał na konsultowaniu z doktorantką zakresu danych prezentowanych w manuskrypcie oraz sprawdzaniu i nanoszeniu poprawek do przygotowanego draftu i został oszacowany na 10%.

25.09.2023
Irmina Ćwieląg-Piasecka
data i podpis

Załącznik nr 3 do Trybu postępowania w sprawie nadania stopnia naukowego doktora

mgr inż. Michał Dudek
imię i nazwisko

Wrocław, 06.09.2023
miejscowość i data

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OŚWIADCZENIE

Oświadczam, że w pracy:

Bednik M., Medyńska-Juraszek A., Ćwieląg-Piasecka I., **Dudek M.**, 2023. Enzyme activity and dissolved organic carbon content in soils amended with different types of biochar and exogenous organic matter (manuskrypt wysłany do czasopisma *Sustainability*)

mój udział polegał na uczestnictwie w zakładaniu doświadczenia inkubacyjnego, udzielaniu mgr inż. Magdalenie Bednik pomocy w wykonywaniu analiz aktywności enzymatycznej i węgla wodnorozpuszczalnego oraz na formatowaniu tekstu manuksryptu zgodnie z wytycznymi czasopisma. Mój całościowy wkład w powstanie manuksryptu został oszacowany na 15%.

25.09.2023 Dudek M.
data i podpis