Effect of Coapplication of Biochar and Nutrients on Microbiocenotic Composition, Dehydrogenase Activity Index and Chemical Properties of Sandy Soil

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Abstract

Biochar improves soil physical, chemical and biological properties. However, there is a very limited number of studies comparing the effect of various doses of biochar and wheat straw with nutrients on microbiocenotic composition of soil and their connection with selected biochemical and chemical parameters of soil. The aim of this study was to determine the effect of the addition to the soil of wheat straw (WS) and wheat straw biochar (WSB) (300 °C) at 0.2%, 0.5%, 1%, and 2% doses and the addition of nutrients (MF) on microbial community composition (bacteria, fungi, actinobacteria, *Azotobacter* spp., ammonifiers, nitrifiers, denitrifiers, *C. pasteurianum*), dehydrogenase activity index, carbon and nitrogen fractions contents and the content of water soluble Cu, Cd, Zn, and Pb. It was demonstrated that coapplication of WS and WSB with MF at 1% and 2% doses increased carbon and nitrogen contents in soil and, in particular, their water soluble fractions (DOC and DON). The synergistic effect of biochar and MF contributed to the increase in the population of soil microorganisms. Dehydrogenase activity index in treatments with the addition of WS, WSB and MF was 1.6–4 times higher compared to the control. The content of heavy metals significantly ($p \leq 0.05$) affected dehydrogenase activity and the number of nitrifiers and ammonifiers. It was demonstrated that the content of C and N measured for soil microbial biomass in treatments amended with biochar and MF was much greater than in control treatment and MF. However, our studies suggest that the microorganisms' response to the addition of biochar with nutrients increased the number and intensified the activity of soil microorganisms.

Graphic Abstract

Keywords Dissolved organic carbon · Microorganisms · Heavy metals · Dehydrogenase activity · Biochar · Soil

Statement of Novelty

Our manuscript describes the issues concerning many aspects of the environment and waste management: fertility and relationships between chemical properties, enzymatic activity and microbiocenotic composition of soil with addition of biochar or wheat straw and nutrients. The novelty of this manuscript is comparison of quantitative and qualitative parameters of microbiocenotic composition of soil, changes in dissolved organic C, N and heavy metals contents in soil with biochar and NPK. This direction of research, particularly with reference to the microbial parameters of soils, provides great potential for progress not only in agricultural sciences, but also in chemistry and biology. Identification of changes to biochar, taking account of the above aspects may play a key role in improve of soil properties.

Introduction

Quantitative and qualitative changes in microbial populations have a significant impact on soil functional integrity, and soil microbial diversity is of fundamental importance for sustainable environmental management. Soil microorganisms are key indicators of soil quality due to their participation in many biochemical processes, essential for the environment and ecological functions of the soil [1–5]. The addition of organic matter to the soil may lead to significant changes in the structural and functional diversity of microbial population, and thus changes in the intensity of microbial degradation of organic connections [6, 7]. Biochar is considered a source of nutrients in long time because it is produced in enclosed combustion of nutrient rich biomass. It can also improve soil nutrients' bioavailability through incorporating with soil pH, cations exchange, redox potential, and soils biotic commotion. Additionally, due to its structure and high porosity, it improves the physical properties of soil, which also can increase the number of microorganisms [8]. Another factor by which biochar effectively influences microbial activity is soil pH [6, 9, 10]. Hale et al. [11] demonstrated a positive relationship between the population of microorganisms and pH of soil (in the range from 3.7 to 8.3) with the addition of biochar. However, this relationship was not confirmed by the studies of Ippolito et al. [12] and Khadem and Raiesi [13], who showed that the increase of the pH value to 7 results in a drastic reduction in the population of bacteria and fungi. These discrepancies probably resulted from the interaction between the properties of biochars and soil used by the authors for the

study, which consequently diversified the conditions for the growth and development of microorganisms. However, it is obvious that any qualitative and quantitative changes in the population of microorganisms, as well as an increase or decrease in their activity, affect the remaining components of the ecosystem [14]. In general, in the light of the currently changing scientific knowledge, (due to different production conditions and various feedstocks used for biochar production, as well as the conditions in which experiments are carried out) it is difficult to clearly describe the nature and effect of the addition of biochar on the soil biological indicators, including the diversity and number of soil microorganisms [5, 15–17]. Many authors emphasise that laboratory tests eliminating the impact of environmental factors are better to understand the biochar's effect and to determine the aspects responsible for changes in the structure and abundance of soil microorganisms [5, 7, 16, 18, 19]. Thus, interest in maximising the potential benefits of biochar for the stimulation of the biological and enzymatic activity of soil is constantly increasing [6, 13, 20]. Dehydrogenase activity, among all soil biological indicators, is considered to be one of the most important parameters useful for the general assessment of soil condition, because dehydrogenases are intracellular enzymes, and therefore their number is directly related to the number of active cells/living microorganisms [5]. Determination of dehydrogenase activity is also of interest for assessing the stability of organic matter in soils amended with biochar [21].

It is expected that, due to the long persistence of biochar in soil, which lasts from 100 to 1000 of years, changes in the composition and abundance of soil microorganisms may persist for similar long period. Studies carried out by Tian et al. [7] and Gul et al. [6] indicated that biochar properties, such as pH, EC, CEC, specific surface area, and porosity have the greatest impact on the microbiological parameters of soil. The number of studies assessing the effect of biochar production conditions and biochar types on the activity of soil microorganisms is also limited. This is essential, because the feedstock from which biochar was produced and the pyrolysis conditions determine the degree of biochar aromaticity [6, 16]. It is believed that pyrolysis at up to 400 °C promotes the formation of amorphous C which is more susceptible to microbial degradation. This is a very valuable observation, because it is known that soil microorganisms are actively involved in the processes of organic matter transformation (SOM) [8, 22]. Studies carried out by Farrell et al. [23] and Lu et al. [24] showed that a more intensive decomposition of carbon-containing compounds was observed after applying biochar to the soil, which was accompanied by greater microbial activity. However, Lu

et al. [24] noted that difficulties in determining unambiguous structural changes in the population of microorganisms after biochar application result from the varied rate of mineralisation of carbon contained in this material. For this reason, particular attention is paid to the analysis of SOM fraction, especially the dissolved organic C content, which is the basic source of energy for microorganisms [7, 25, 26]. The positive effect of the pyrolysis process on reducing the content of available forms of trace elements is an important element when applying various types of biochars [27]. In addition to numerous known benefits of the use of biochar, the literature is also rich in examples of negative effects of BC application. For example, Muhammad et al. [28] reported that due to its high C:N ratio, biochar can reduce the availability of nitrogen not only for microorganisms, but also for plants, limiting their yield. For this reason, the most recent proposal is to co-apply nutrients, such as N, P and K with biochar [7, 29]. The use of NPK fertilizer can increase the nutrient content in the soil that can be used directly by plants and soil microorganisms. In our studies, the use of NPK with biochar gives also the opportunity to create comparable conditions between individual experimental treatments because it is known that the deficiency of nutrients could limit the course of biochemical processes. On the other hand, Jindo et al. [30] informed about the immobilisation of nutrients by biochar, which can also negatively affect the soil microbial diversity. Considering the contradictory positive and negative effects of biochar, the study was carried out with the aim to compare the effect of different doses of wheat straw and wheat straw biochar with addition of nutrients on changes in the number of selected groups of microorganisms, dehydrogenase activity and some chemical properties of sandy soil.

Materials and Methods

Soil Collection and Characterisation

The laboratory experiment was carried out on typical brown soil with the granulometric composition of loamy sand (85% sand, 9% silt, and 6% clay) Eutric Cambisol (CM-eu) (WRB 2015). The soil was collected from 0 to 0.2 m layer in southern Poland. Clay, loam and sand contents were determined by the areometric method. Water holding capacity (WHC) was determined in the laboratory with undisturbed and disturbed soil by saturating a soil column with water by capillary transport. The soil was dried and 2 mm sieved. The following were determined in the soil before incubation: soil pH was measured at a soil to water ratio of 1:2.5 (w/v) electrochemically using pH meter (pH-meter CP-505), electrical conductivity (material:water = 1:2.5) was determined using conductivity meter (Conductivity/Oxygen meter CCO-501) [27], the total nitrogen, carbon and sulphur contents

were determined using CNS analyser (Vario MAX Cube, Elementar Analysensysteme, GmbH, Germany). The total Cd, Cu, Pb and Zn contents were determined after incinerating the sample in chamber furnace at 450 °C for 12 h and mineralising its residues in a mixture of concentrated nitric and perchloric acids (3:2) (v/v). The concentration of analysed elements in the resulting solutions was determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin Elmer Optima 7300 DV) [31].

Biochar Production and Characterisation

The study material was wheat straw from an agricultural holding located in the Malopolska Province (southern Poland) and biochar produced from it. Wheat straw was dried at 65 °C, ground in a laboratory mill (mesh size of 4 mm) and mixed to ensure homogeneity. The plant material was pyrolysed in an electric laboratory furnace (equipped with a temperature controller) at 300 °C, for 15 min, under a limited supply of air [32]. The rate of heating the combustion chamber was 10 °C min⁻¹. The process parameters were configured so as to obtain the lowest possible losses of carbon.

In order to identify the properties of wheat straw (WS) and wheat straw biochar (WSB), all of which were used in the study, the materials were ground in a laboratory mill (with 1 mm sieve mesh diameter), and then dried at 105 °C for 12 h [30], and subjected to analyses. The pH of materials (material:water = 1:5) was determined electrochemically using pH meter (pH-meter CP-505), electrical conductivity (material:water = 1:5) was determined using conductivity meter (Conductivity/Oxygen meter CCO-501) [33], and the total nitrogen, carbon and sulphur contents were determined using CNS analyser (Vario MAX Cube, Elementar Analysensysteme, GmbH, Germany).

The total forms of Cd, Cu, Pb and Zn contents were determined using the inductively coupled plasma optical emission spectrometry technique (ICP-OES, Perkin Elmer Optima 7300 DV apparatus) after the previous extraction of the sample in a microwave system (Multiwave 3000, Anton-Paar) in a mixture of concentrated HCl and HNO₃ acids (3:1) (v/v). Specific surface area (S_{BET}) of WS and WSB, as well as pore volume and diameter were determined using multi-function accelerated surface area and porosimetry analyser ASAP 2010 (Micrometrics, the USA). Specific surface area was determined by physical nitrogen adsorption at liquid nitrogen temperature (77°K) using Brunauer–Emmet–Teller equation [34]. Before measuring specific surface area (S_{BET}), test samples were subjected to desorption at 105 °C under vacuum conditions and rinsed with pure helium. Sample degassing time was 16 h. The status of surface degassing was controlled in an automatic manner [34]. The selected

physical and chemical properties of the materials used in the incubation experiment are shown in Table 2.

Experimental Design and Soil Sample Collection

Prior to application of the wheat straw (WS) and wheat straw biochar (WSB), soil moisture was adjusted to 50% of WHC and the soil was incubated at 25 °C for 1 week to activate the existing microbial communities. Then, the soil (except for the control soil) was mixed with nutrients: for N (0.10 g N kg⁻¹ D.M. of soil—ammonium nitrate—NH₄NO₃), P (0.04 g P kg⁻¹ D.M. of soil—monocalcium phosphate monohydrate—Ca(H₂PO₄)₂·H₂O) and K (0.12 g K kg⁻¹ D.M. of soil—potassium chloride—KCl), and four doses of straw or wheat straw biochar (0.2%, 0.5%, 1% and 2%), thoroughly mixed and then placed in plastic containers (able to contain 250 g of soil), transferred to a thermostatic cabinet, and incubated at 24 °C. The experiment comprised 10 treatments carried out in three replications. The control treatments were: soil without nutrients and without organic fertilisers (C) and soil with the addition of nutrients (MF), which allowed to demonstrate the real effect of organic materials. During the experiment, the humidity of the incubated samples was maintained at a constant level of 45% of the maximum soil water capacity. Soil samples were collected from each treatment on the first day of the experiment and after 254 days, and stored at 4 °C for biological analysis and at 25 °C for physio-chemical analysis.

Selected Analyses of Control and Amended Soils After Incubation

The pH and EC were measured at a 1:2.5 (soil:redistilled water) ratio. The total nitrogen and carbon contents were determined using CNS analyser (Vario MAX Cube, Elementar Analysensysteme, GmbH, Germany). All measurements are the means of three replicate determinations and are expressed on an oven-dried soil basis (105 °C, 12 h). The contents of water-extracted forms of the studied elements were determined after 24-h extraction with distilled water (sample:solution = 1:10). The Cd, Cu and Pb contents in the resulting solutions and extracts were determined by ICP-OES using the Perkin Elmer Optima 7300 DV instrument, and the obtained results were converted into the absolute dry mass of soil.

Dissolved Organic Carbon (DOC) and Dissolved Organic Nitrogen (DON)

The DOC and DON contents in soil were determined by water extraction according to the methods described by Jones and Willett [35], respectively. Organic carbon and nitrogen soluble in water were determined by shaking 5 g

of the soil sample with 50 ml of redistilled water at 20 °C for 24 h. The obtained suspension was centrifuged (20 min, 3500 rpm), and the solution was decanted and filtered through a Buchner funnel equipped with a sterile membrane filter of 0.45 µm pore size. All extracts were kept at the refrigerator before analysis. DOC and DON contents were determined with CN Multi N/C 2100/2100S analyser (Analytik Jena).

Dehydrogenase Activity

Dehydrogenase activity (DhA) was determined using the method of Thalmann [36] after 24-h incubation of the soil samples at 37 °C with TTC solution, and expressed by the amount of the formed 2,3,5-triphenylformazan (µg TPF g⁻¹ h⁻¹ D.M. of soil). In order to determine the effect of the applied fertilisation with organic materials on the dehydrogenase activity, the enzyme activity change index (Iz) was calculated according to the following formula [37]:

$$DAI = \frac{DA}{DAMF}$$

where DAI—dehydrogenase activity change index, DA—dehydrogenase activity in soil fertilised with an organic material, DAMF—dehydrogenase activity in soil fertilised with nutrients.

The results were interpreted based on the following criteria:

DAI = 1, if DA = DAMF (no stimulation and inhibition), DAI > 1 dehydrogenase activity stimulation, DAI < 1 dehydrogenase activity inhibition.

Microbiocenotic Composition of Soil

The evaluation of the effect of the applied fertilisation on the number of selected groups of soil microorganisms was carried out on 1 mm sieved soil samples with natural humidity. Using the serial dilution method developed by Koch with a number of microbiological substrates, the following groups of microorganisms were determined: vegetative and endospore bacteria (Trypticasein Soy Lab Agar, BTL, Poland, grown at 37 °C, for 24 h), mould fungi (Malt Extract Agar, BTL, Poland, grown at 28 °C, for 5 days), actinomycetes (Actinomycete Isolation Lab Agar, Biocorp, Poland, grown at 28 °C, for 7 days). The tube method was used to determine the titres of microorganisms involved in nitrogen transformations: nitrifiers (medium according to Winogradski [38], grown at 28 °C, for 7 days), denitrifiers (medium according to Giltay [38], grown at 28 °C, for 7 days), *Clostridium pasteurianum* (medium according to Rougieux [38], grown at 28 °C, for 7 days), as well as the number of colony forming units for *Azotobacter* spp. Ashby's agar [39], grown at 28 °C, for 7 days) and for

ammonifying bacteria (medium according to Rougieux [38], grown at 28 °C, for 7 days). The number of colony forming units (CFU) of microorganisms was determined by the dilution culture method. The result was converted into 1 g D.M. of soil or the titre was determined for microorganisms involved in nitrification and denitrification processes.

Biological Index of Soil Fertility (BISF) In order to compare the effect of organic-mineral and mineral fertilisation, the biological index of soil fertility (BISF) was estimated based on the equation [40] after own modification as follows:

$$\text{BISF} = \sqrt{M^2 + \text{Corg}^2 + T^2} \times 100\%$$

where M—soil biological activity including dehydrogenase activity ($\mu\text{g TPF kg}^{-1}$ D.M. of soil 24 h^{-1}) and the number of microbial groups referred to in the article (CFU g^{-1} or titre in soil), Corg – organic carbon content (g kg^{-1} D.M. of soil), T—soil sorption capacity ($\text{mmol}(+) \text{ kg}^{-1}$ D.M. of soil).

Due to different units, the above parameters were transformed into ‘weights’ with values from 0 to 1, by comparing the initial values with the maximum values in the soil of individual treatments.

Statistical Analysis

The differences between each treatment and the control as well as between treatments were evaluated using two-way analysis of variance (ANOVA, Duncan test, $p \leq 0.05$).

Variation within treatments was determined by calculating the values of standard deviation ($\pm \text{SD}$). Spearman’s correlation coefficient was used for evaluation of relationship between the selective soil chemical and biological properties (for each pair of variables). All statistical analyses were performed using Statistica PL 13 software (StatSoft Inc.).

Results and Discussion

Characterisation of Organic Materials

Biochar used in the experiment (WSB) had higher pH and EC values than wheat straw (WS) from which it was produced (Table 1). Pyrolysis of WS increased the concentration of trace elements and decreased the contents of S, H and O in WSB. Biochar also had greater specific surface area and higher porosity than wheat straw.

pH, Soil C and N Fractions

Before it was amended with organic materials, the soil used in the experiment had slightly acidic reaction (pH in $\text{H}_2\text{O} = 5.67$), $\text{EC} = 32.2 \mu\text{S cm}^{-1}$ and natural content of trace elements (0.19 mg, 1.85 mg, 16.5 mg and 37.9 mg kg^{-1} D.M. for Cd, Cu, Zn and Pb, respectively). The total content of nitrogen was 1.28 g kg^{-1} D.M., and carbon 9.84 g kg^{-1} D.M.

Table 1 Chemical and physical properties of wheat straw and wheat straw biochar

Determination	Unit	Wheat straw (WS)	Wheat straw biochar (WSB)
pH in H_2O	—	5.84 ± 0.15	6.52 ± 0.60
EC	mS cm^{-1}	4.48 ± 0.21	3.78 ± 21
Dry matter	g kg^{-1}	952 ± 0.2	966 ± 2
Ash	g kg^{-1} DM	55.8 ± 3.1	118 ± 1
Carbon	%	46.1 ± 1.0	62.9 ± 1.8
Nitrogen	%	0.37 ± 0.04	1.00 ± 0.05
Sulphur	%	0.06 ± 0.01	0.05 ± 0.01
Hydrogen	%	6.39 ± 0.20	4.58 ± 0.09
Oxygen	%	41.0 ± 1.1	18.0 ± 1.9
Copper	mg kg^{-1} DM	1.32 ± 0.08	3.19 ± 0.15
Zinc	mg kg^{-1} DM	32.9 ± 4.78	48.8 ± 1.26
Lead	mg kg^{-1} DM	0.73 ± 0.09	1.62 ± 0.24
Cadmium	mg kg^{-1} DM	0.56 ± 0.04	1.20 ± 0.02
Specific surface area (S_{BET})	$\text{m}^2 \text{ g}^{-1}$	0.55 ± 0.02	0.67 ± 0.09
Pore volume	$\text{cm}^3 \text{ g}^{-1}$	0.0009 ± 0.000	0.0016 ± 0.002
Pore diameter	nm	6 ± 2	12 ± 3
Maximum pore volume	nm	67	123

$\pm$ Standard deviation, $n = 3$

Table 2 Selected properties of soil in 0 day and 254 day

Treatment	pH H ₂ O		EC		C _{total}		N _{total}	
			mS cm ⁻¹		g kg ⁻¹ D.M.		g kg ⁻¹ D.M.	
	0 days	254 days	0 days	254 days	0 days	254 days	0 days	254 days
C	4.68a	4.65a	0.054a	0.130b	6.297a	6.173a	0.502a	0.613abcd
MF	5.13efg	4.52ab	0.200bc	0.343fg	6.092a	6.347a	0.598abc	0.670abcd
WS 0.2%	5.12efg	4.57ab	0.197bc	0.321efg	6.295a	6.437a	0.580ab	0.677abcd
WS 0.5%	5.20fgh	4.68a	0.211cd	0.363g	9.087bc	7.030ab	0.653abcd	0.700abcd
WS 1%	5.29gh	4.88cd	0.211cd	0.439h	11.49cde	7.713ab	0.675abcd	0.743bcd
WS 2%	5.42hi	5.11efg	0.222cd	0.283def	18.09g	8.113ab	0.755bcd	0.750bcd
WSB 0.2%	5.23fgh	4.70bc	0.202bc	0.617i	7.232ab	6.733a	0.613abcd	0.593ab
WSB 0.5%	5.57ij	4.83cd	0.224cd	0.627i	9.075bc	9.107bc	0.630bcd	0.670abcd
WSB 1%	5.68j	4.97de	0.237cd	0.651ij	12.94de	12.23de	0.755bcd	0.707abcd
WSB 2%	6.05k	5.29gh	0.223cd	0.748j	14.74ef	16.50fg	0.783bcd	0.813cd

Each value represents the mean of three replicates. The different letters within a column indicate a significant difference at $p \leq 0.05$ according to Duncan's multiple range tests, factors: treatment $\times$ analysis date

Our results suggest that the addition of WS and WSB to the soil proportionally increased the pH value in all treatments with the increased dose of organic material (0.2%, 0.5%, 1%, and 2%) (Table 2). However, pH values determined after 254 days were smaller than pH values determined at the beginning of the experiment (0 days). We compared pH of organic materials with pH of the soil used in the experiment and discovered that WSB had higher (6.52), and WS lower (5.48) pH. For this reason, in both analysed terms, pH values of soil in treatments with the addition of WSB were higher than those in treatments with the addition of WS. The increase of the soil pH value after biochar application is a very common case and results from its generally alkaline pH [7, 41, 42]. According to Stewart et al. [43] and Gul et al. [6], the positive effect of biochar on the increase of the soil pH value is more clear in acidic soils with low SOM content. This is probably due to the fact that the SOM is closely related to the soil buffering capacity. A significant positive relationship between pH and DOC was also demonstrated in our studies ($r=0.635$, $p \leq 0.05$).

The decrease in soil pH observed after 254 days could be caused by slow oxidation and decomposition of biochar. Intensive transformations of organic materials added to the soil occur as a result of the chemical and microbiological processes, and this can lead to the release of acid functional groups that partially neutralise the alkalinity of soil. The formation of acid functional groups can be a factor limiting soil salinity and ultimately lowering soil pH. We demonstrated no increase in the electric conductivity (EC) value in relation to the applied dose of organic material (Table 2). However, the application of WS and WSB significantly increased the EC value, which was particularly visible after 254 days of incubation (Table 2).

The addition of WS and WSB to the soil affected the total carbon and dissolved organic carbon (DOC) contents

in both analysed terms: 0 days and after 254 days (Table 2; Fig. 1). The total carbon content (C_{total}) in soil in both terms increased relative to the applied doses of organic materials. After 254 days of incubation, the C_{total} content in treatments amended with WS increased by 3–26%, and in treatments amended with WSB by 5–156% compared to the MF treatment. At the same time, the concentration of DOC increased by 4–78% in treatments amended with wheat straw and by 2–40% in treatments amended with biochar. Compared to the MF treatment, significant increase in the C_{total} and DOC contents after 254 days of incubation was determined in treatments with the addition of WSB at 0.5%, 1%, and 2% doses. The addition of the same doses of straw only caused a significant increase of the dissolved organic carbon content (Fig. 1). The share of DOC in C_{total} in treatments with the addition of WS in both analysed terms was comparable regardless of the applied dose and amounted to 21% (0 days) and 16.9% (254 days) on average. Higher diversification of the DOC share in C_{total} was found after using different doses of wheat straw biochar (from 14.6 to 21%—0 days, from 8 to 14%—254 days). Our results confirm that biochar is a carbon-rich material which, due to its aromatic structure, is resistant to decomposition. Studies carried out by Kuzyakov et al. [41] proved that after 8.5 years of incubation, only 6% of biochar was mineralised to CO₂, which could be directly influenced by the diversity and number of microorganisms. However, the mechanisms responsible for the dynamics of SOM changes with the participation of microorganisms after biochar application remain unclear [6, 7]. This is due not only to the diversity of SOM, but also the heterogeneity of organic matter introduced into the soil which degrades at different times. For this reason, it has been suggested in recent years to extract a labile fraction of organic matter as the most sensitive indicator

allowing a better understanding of the biochar's impact on the population of soil microorganisms [7, 10, 41].

The application of biochar to the soil not only increases the SOC content and affects the C cycle [16, 41], but also accelerate the nitrogen (N) transformation or, in some cases, even reduces organic N turnover [44, 45]. Latest study results show that, due to the relatively low N content in biochar, the best solution is to use this material together with nutrients [7, 8, 44]. Coapplication of biochar and nutrients in this study has a synergistic effect on the yielding of plants and the functioning of soil microorganisms [7]. Our results show that the N_{total} content increased relative to the amount of the organic material applied (WS, WSB); however, there were no significant differences compared to control treatments C and MF (Table 2). The N_{total} content was significantly affected by: pH ($r=0.394$; $p \leq 0.05$), EC ($r=0.373$; $p \leq 0.05$), and C_{total} ($r=0.658$; $p \leq 0.05$). As shown in Fig. 1, much greater variation after the application of organic materials was found in the content of dissolved organic nitrogen (DON). The addition of WS and WSB at 0.2%, 0.5%, 1%, and 2% doses significantly increased the DON content in all the analysed treatments compared to the treatment with no fertilisation applied. It was shown that, after 254 days of incubation, the DON content increased significantly only in treatments where WSB was used and in the MF treatment. The share of DON in N_{total} was comparable in both terms and reached 14% on average, regardless of the material and dose applied. Our results correspond to the results obtained by Tian et al. [7] who also demonstrated more beneficial effect of biochar used together with nutrients on the N_{total} and DON contents compared to the MF treatment and treatment with no fertilisation applied.

Dehydrogenase Activity

Extracellular enzymes are direct factors of the mineralisation of organic matter and the circulation of nutrients in the soil [6, 46]. The available data present a varied effect of biochar on soil enzymatic activity [8, 47]. The effect of adding external organic matter such as biochar on the dehydrogenase activity depends on the interaction of substrate and enzyme with that material (e.g. sorption and desorption of substrates on the biochar surface, biochar porosity and specific surface area [48]. Studies carried out by Lammirato et al. [48] and Ameloot et al. [49] confirmed that the addition to the soil of biochar produced at a high temperature and with higher porosity and specific surface area reduces extracellular enzymatic activity. This is due to the presence of functional groups on the biochar surface, which tend to bind substrates and extracellular enzymes, thus changing the rate of substrate diffusion to the site of active enzymatic catalysis [48]. For this reason, many authors recommend to use biochar produced at up to 400 °C in order to improve

soil quality [7, 8, 44, 47, 49]. Our results showed that the addition of wheat straw and biochar produced at 300 °C to the soil significantly increased the dehydrogenase activity at the beginning of the experiment (Fig. 2). Compared to the MF treatment, the dehydrogenase activity in treatments with 0.2%, 0.5%, 1%, and 2% additions of WS increased by 33%, 65%, 59%, and 110%, respectively, and in treatments amended with biochar by: 170%, 173%, 245%, and 425%, respectively. After 254 days of incubation, this effect was significantly reduced, while the tendency of increasing DhA activity with the increasing dose of WS and WSB was maintained. Compared to the MF treatment, the DhA in treatments with the addition of WS was higher by 10%, 15%, 19%, and 45%, and in treatments with the addition of WSB, by 1.5%, 6%, 13%, and 33%, after 254 days of incubation. Our results correspond with the recently published study of Beheshti et al. [5]. These authors also demonstrated that, after 120 days of incubation, the highest DhA activity was present in the soil amended with wheat straw biochar produced at 300 °C, and this activity decreased over time with depletion of nutrients. In the present study, the DhA activity was significantly influenced by the pH ($r=0.658$; $p \leq 0.05$), the DOC content ($r=0.657$; $p \leq 0.05$), as well as the contents of all analysed heavy metals Cd, Cu, Zn, and Pb ($r=-0.461$; $r=0.511$; $r=-0.588$; $r=0.533$, $p \leq 0.05$). Studies carried out by Wyszowska et al. [1] showed that the DhA sensitivity to the analysed heavy metals is as follows: $\text{Cd}^{2+} > \text{Zn}^{2+} > \text{Pb}^{2+} > \text{Cu}^{2+}$. However, these authors emphasised that sensitivity to Cd, Zn, Pb, and Cu may be affected by the content of SOM and granulometric composition of the soil. The above information clearly shows that excessive contents of Cd, Cu, Zn, Pb may interfere with soil homeostasis and disturb the mechanisms responsible for proper biochemical changes at the cellular level [1, 6, 50–53].

The dehydrogenase activity change index confirmed that the increased dose of organic material (WS and WSB) had a positive effect on the stimulation of dehydrogenase activity (Fig. 2). In none of the analysed treatments, we discovered the negative effect of applied fertilisation, and thus inhibition of the activity of the tested enzymes. The largest stimulation of DhA after 254 days of incubation was determined in treatments amended with 2% of WS and WSB. As it can be concluded from the results presented in Fig. 2, the largest decrease in dehydrogenase activity was observed in treatments with the addition of biochar. This was probably due to the exhaustion of carbon available for microorganisms, whose content correlated with the number of populations of the tested groups of microorganisms and with the DhA activity (Table 5). However, in our studies, nitrogen could have a greater importance, because its content did not significantly increase after the application of organic materials (Table 2).

Environmental hazards related to the presence of heavy metals in soil depend mainly on the bioavailability of these

elements. Contamination of soil with heavy metals affects the number and diversity of physiological groups of soil microorganisms, and also changes their enzymatic activity [8, 47]. The effect of application of organic materials at the following doses: 0.2%, 0.5%, 1%, and 2% on the content of water-extracted forms of Cd, Cu, Zn, and Pb is shown in Table 3. After 254 days of incubation, the Cd content undergone no clear changes. After the same time, the application of WS and WSB resulted in a significant decrease in the Cu and Pb contents. However, the content of water extracted forms of Cu was determined only in treatments amended with 2% of WS and WSB. In both analysed terms, the Pb content decreased with the increased dose of organic materials. Once the addition of WSB to the soil was increased, the efficiency in immobilising water-extracted Zn forms increased as well. Our results indicate that the use of higher doses of biochar increased soil pH, which, in turn, reduced the contents of trace elements available for plants and soil microorganisms. Correlation analysis confirmed a significant effect of pH on the content of water-extracted forms of: Cd, Cu, Zn, and Pb ($r = -0.606$; $r = 0.424$; $r = -0.626$; $r = 0.654$, $p \leq 0.05$). This relationship is confirmed by the results obtained by many authors [27, 47, 54].

Microbiocenotic Composition

Wheat straw and wheat straw biochar application significantly affected the abundance of microbial groups and the patterns of the microbial community (Table 4). However, the number of soil microorganisms was very diverse and varied depending on the type and amount of organic material used. Although the pH of the soils was slightly acidic and acidic, the most numerous group of microorganisms in both terms were vegetative bacteria. The number of vegetative bacteria (Vb) and mould fungi (Mf) generally increased significantly

with the increasing dose of WS and WSB compared to the MF treatment. Analysis of the correlation between the number of Vb and Mf and soil chemical parameters showed that the number of Vb significantly depended on the content of N_{total} and DOC ($r = 0.755$; $r = 0.656$; $p \leq 0.05$), and the content of Mf depended on the soil pH, N_{total} and DOC ($r = 0.610$; $r = 0.595$, $r = 0.756$; $p \leq 0.05$). In turn, the number of ammonifiers in soils before incubation increased only in treatments with the addition of WS. The number of this group of microorganisms was influenced not only by soil pH and DOC, but also by the content of all analysed heavy metals (Table 3). In none of the terms, *Azotobacter* bacteria were found in the soil. After 254 days of incubation, we observed a clear decrease in the number of vegetative bacteria, mould fungi, actinomycetes and ammonifiers, as well as lower titres of nitrifying and denitrifying bacteria. Also, after 254 days of incubation, a significant reduction in the number of nitrate (nitrifying, denitrifying, ammonifying) bacteria in soil was noticed. The titre of nitrifying and denitrifying bacteria before incubation ranged from 10^{-3} to 10^{-6} , and, after 254 days of incubation, from 10^{-2} to 10^{-4} . On the other hand, the titre of *C. pasteurianum* bacteria did not change and ranged from 10^{-2} to 10^{-3} . The predominance of vegetative bacteria over endospore bacteria, as well as numerous fungi, actinomyces and microorganisms binding atmospheric nitrogen (*C. pasteurianum*) in the initial stage of the experiment proved the richness of the microorganisms' living environment in nutrients easily digestible for microorganisms. Lower number of the analysed microorganisms after 254 days of incubation also indicates the depletion of nutrients and the deterioration of conditions for their growth and development of microbes (soil acidification, depletion of an easily accessible source of C and N). Results obtained by Tian et al. [7] demonstrated that mineral fertilisation was the main factor influencing the number and

Table 3 Contents of water-extracted forms of Cd, Cu, Zn, and Pb in soil after organic material application

Treatment	Cd (mg kg ⁻¹ D.M.)		Cu (mg kg ⁻¹ D.M.)		Zn (mg kg ⁻¹ D.M.)		Pb (mg kg ⁻¹ D.M.)	
	0 days	254 days	0 days	254 days	0 days	254 days	0 days	254 days
C	0.020bcd	0.017abc	0.043cde	0.040cde	0.313a	0.350ab	0.210a	0.213a
MF	0.020bcd	0.027de	0.047cde	0.010a	0.587b	1.327e	0.360cd	0.210a
WS 0.2%	0.020bcd	0.023cde	0.048cde	nd	0.630b	1.080d	0.433de	0.220ab
WS 0.5%	0.020bcd	0.023cde	0.060ef	nd	0.577b	1.330e	0.440e	0.187a
WS 1%	0.020bcd	0.027de	0.067ef	nd	0.617b	1.200de	0.420de	0.243ab
WS 2%	0.023cde	0.010a	0.093g	0.030c	0.707bc	0.557b	0.493ef	0.320c
WSB 0.2%	0.020bcd	0.027de	0.033cde	nd	0.633b	1.263de	0.487ef	0.197a
WSB 0.5%	0.020bcd	0.020bcd	0.043cde	nd	0.590b	1.173de	0.483ef	0.200a
WSB 1%	0.020bcd	0.020bcd	0.050def	nd	0.707bc	0.877c	0.547fg	0.293bc
WSB 2%	0.013ab	0.010a	0.050def	0.013b	0.600b	0.577b	0.577g	0.323c

Each value represents the mean of three replicates. The different letters within a column indicate a significant difference at $p \leq 0.05$ according to Duncan's multiple range tests, factors: treatment $\times$ analysis date
nd: not determined

Table 4 Average number of microorganisms ($\times 10^{-3}$ CFU g^{-1}) and values of nitrification and denitrification titre in soil after application of organic materials

Treatment	Microorganisms								
	Vegetative bacteria	Bacterial endospores	Mould fungi	Actinobacteria	Azoto-bacter spp.	Ammonifiers	Nitrifiers	Denitrifiers	<i>C. pasteurianum</i>
On the start day the experiment (0 day)									
C	273a	nd	16a	15d	nd	228b	$10^{-3}a$	$10^{-3}a$	$10^{-2}a$
MF	361ab	nd	18a	3ab	nd	430c	$10^{-3}a$	$10^{-3}a$	$10^{-2}a$
WS 0.2%	462bc	nd	50d	1a	nd	204b	$10^{-5}c$	$10^{-5}c$	$10^{-3}b$
WS 0.5%	610d	nd	56e	5bc	nd	605d	$10^{-5}c$	$10^{-5}c$	$10^{-2}a$
WS 1%	903e	nd	68f	5bc	nd	862f	$10^{-4}b$	$10^{-4}b$	$10^{-3}b$
WS 2%	2404g	nd	88g	6bc	nd	675e	$10^{-6}d$	$10^{-5}c$	$10^{-3}b$
WSB 0.2%	285a	nd	25b	0a	nd	196b	$10^{-6}d$	$10^{-6}d$	$10^{-2}a$
WSB 0.5%	260a	nd	39c	4ab	nd	92a	$10^{-4}b$	$10^{-6}d$	$10^{-3}b$
WSB 1%	557cd	nd	43c	8c	nd	132a	$10^{-6}d$	$10^{-4}b$	$10^{-3}b$
WSB 2%	1255f	nd	61e	25e	nd	98a	$10^{-6}d$	$10^{-6}d$	$10^{-3}b$
After 254 days of the experiment									
C	188a	nd	15c	nd	nd	4c	$10^{-2}a$	$10^{-2}a$	$10^{-2}a$
MF	206a	nd	16c	nd	nd	nd	$10^{-2}a$	$10^{-3}b$	$10^{-2}a$
WS 0.2%	231ab	nd	14c	nd	nd	nd	$10^{-3}b$	$10^{-3}b$	$10^{-3}b$
WS 0.5%	525e	nd	5a	nd	nd	nd	$10^{-4}c$	$10^{-3}b$	$10^{-2}a$
WS 1%	897f	nd	38e	nd	nd	nd	$10^{-2}a$	$10^{-2}a$	$10^{-3}b$
WS 2%	1174g	33b	48f	nd	nd	3b	$10^{-2}a$	$10^{-2}a$	$10^{-2}a$
WSB 0.2%	256b	nd	10b	nd	nd	nd	$10^{-3}b$	$10^{-2}a$	$10^{-2}a$
WSB 0.5%	227ab	nd	18cd	nd	nd	nd	$10^{-4}c$	$10^{-4}c$	$10^{-3}b$
WSB 1%	441d	325c	21d	nd	nd	3b	$10^{-4}c$	$10^{-4}c$	$10^{-3}b$
WSB 2%	341c	nd	21d	nd	nd	4c	$10^{-3}b$	$10^{-3}b$	$10^{-3}b$

Each value represents the mean of three replicates. The different letters within a column indicate a significant difference at $p \leq 0.05$ according to Duncan's multiple range tests, factors: treatment $\times$ analysis date

nd: not determined

Fig. 1 Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) contents in soil after the application of organic materials. Each value represents the mean of three replicates. The different let-

ters indicate a significant difference at $p \leq 0.05$ according to Duncan's multiple range tests, factors: treatment $\times$ analysis date

Fig. 2 Dehydrogenase activity in soil. Each value represents the mean of three replicates. The different letters indicate a significant difference at $p \leq 0.05$ according to Duncan's multiple range tests, factors: treatment $\times$ analysis date

Table 5 Spearman's correlation coefficients between the selective soil chemical and biological properties

	Vegetative bacteria	Bacterial endospores	Mould fungi	Nitrifiers	Denitrifiers	<i>C. pasteurianum</i>	Ammonifiers	DhA
N_{total}	0.755***	0.368*	0.595***	0.037	−0.068	−0.449*	0.249	0.340
C_{total}	0.493**	0.440*	0.520**	−0.416*	−0.432*	−0.635***	0.298	0.163
DOC	0.656***	0.470**	0.756***	0.307	0.274	−0.207	0.625***	0.643***
DON	−0.087	−0.178	0.015	−0.295	−0.360	−0.437*	−0.168	−0.209
pH	0.179	0.418*	0.610***	0.326	0.157	−0.094	0.763***	0.657***
EC	0.207	0.134	0.198	−0.567**	−0.484**	−0.657***	−0.006	−0.155
Cu	−0.014	0.295	0.192	0.395*	0.270	0.358	0.579***	0.511**
Zn	0.110	−0.103	−0.210	−0.249	−0.128	−0.150	−0.543**	−0.588***
Cd	0.214	−0.174	−0.208	−0.221	−0.038	−0.153	−0.518**	−0.461*
Pb	−0.171	0.052	0.277	0.467**	0.279	0.249	0.634***	0.533**
DhA	0.381*	0.316	0.418*	0.256	0.349	−0.008	0.610***	—

*Significant at the $p \leq 0.05$ level

**Significant at the $p \leq 0.01$ level

***Significant at the $p \leq 0.001$ level

community structure of microorganisms. The observations of Tian et al. [7] are contradictory to our results. Our studies showed that the number of microorganisms in the control soil (C) and in the soil with nutrients (MF) applied did not change significantly. This confirms the fact that the organic materials used had a much greater effect on the diversity and abundance of microorganisms. Also previously published studies indicate that mineral fertilisation enhances biochar's action and positively affects the population of microorganisms [29, 55]. For example, Doan et al. [29] showed that the fertilisation of soil with biochar and mineral fertilisers increased the total number of bacteria by 59%. Our results showed that the synergistic effect of biochar and nutrients applied at 0.2%, 0.5%, 1%, and 2% doses increased the bacteria number after 254 days of incubation by: 36%, 21%, 135%, and 81%, respectively. However, it is known that the number and biodiversity of soil microorganisms are

constantly changing [3]. According to Lehmann and Joseph [8], the addition of biochar to the soil has a much greater impact on its physio-chemical properties than on microorganisms that inhabit it. Lehmann et al. [16] observed an increase in the number of microorganisms in soils with the addition of biochar, but, at the same time, they paid attention to the fact that it is difficult to explain the mechanisms and processes that directly caused these changes. There is still a lack of comprehensive reports on the dynamics of changes and the effect of biochar addition on quantitative and qualitative changes of soil microorganisms. Nevertheless, it is clear that the porous structure and physical and chemical properties of biochar and, consequently, its impact on sorptive phenomena occurring in soil, soil pH and mineral matter content play an important role in the formation of soil microbial populations [16].

Fig. 3 The values of biological index of soil fertility (BISF) after 254 days of incubation

After 254 days of incubation, the biological index of soil fertility (BISF) formulated based on the analytical values ranged from 71 to 165 (Fig. 3). Compared to the BISF value calculated for the MF treatment, fertilisation of soil with WSB at 0.2%, 0.5%, 1%, and 2% doses increased the index value by: 43%, 44%, 71%, and 78%, respectively. The BISF index estimated on the basis of organic carbon content, sorption capacity, dehydrogenase activity, and the number of 9 groups of soil microorganisms allowed us to rank the studied treatments in the following order: MF < C < WSB 0.2% < WS 0.2% < WSB 0.5% < WSB 1% < WS 0.5% < WS 1% < WS 2% < WSB 2%. The study revealed that the organic-mineral fertilisation positively affected the BISF index. The application of nutrients and 1% and 2% doses of WS or WSB together increased the BISF value from 71 to 131%.

Conclusions

Thermal conversion of wheat straw into biochar can be an excellent way to manage waste agricultural biomass, whose application contributes to a significant improvement of soil quality. The addition to soil of both wheat straw and biochar at 1% and 2% doses significantly increased the pH value and the C and N contents in soil. The DOC and DON contents were higher on the day of starting the experiment and, after 254 days of studies, this fraction of both elements was clearly reduced. We demonstrated a close relationship between the number of soil microorganisms and the C and N contents in soil. Our results suggest that coapplication of biochar and nutrients increases the number and intensifies the activity of soil microorganisms without causing any visible disturbance. The addition of wheat straw and biochar to the soil significantly increased dehydrogenase activity on the

day of starting the experiment. After 254 days of incubation, this effect was significantly reduced, while the tendency of increasing DhA activity with the increasing dose of WS and WSB was maintained. However, the application of WS and WSB did not allow to draw unambiguous conclusions and determine the tendency of changes in the number and community structure of soil microorganisms. For this reason, further monitoring of microbiological processes in the soil with the addition of biochar is required.

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