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Biochar changes in soil based on quantitative and qualitative humus compounds parameters

Abstract: Due to the indisputable significance of humus in many biochemical processes as well as its increasing deficit particularly in light soils, alternative sources of substrates for the reproduction of this constituent should be sought. The aim of this study was to evaluate the effect of the addition of wheat straw and wheat straw biochar (in four rates) on quantitative and qualitative humus parameters. The following properties were determined in soil: pH, organic carbon, total nitrogen, humic and fulvic acids, carbon in the extract, non-hydrolysing carbon and spectrophotometric indexes for solution of humic acids including A2/6, A2/4, A4/6. After applying 1% and 2% additions of biochar to the soil, the C_{org} soil content significantly increased compared to the same doses of thermally unconverted straw. After 254 days of incubation, the addition of biochar to soil at higher doses, decreased the share of humic acid carbon (CHA, CFA) in the C_{org} content compared to treatments without organic additions and WS treatment. The non-hydrolysing carbon soil content was significantly increased by treatments with 1% and 2% additions of WSB, which indicates greater stabilisation of humus compounds and, at the same time, lower CO_2 emission. Soil humic acids amended by treatment with biochar, especially at 1% and 2% doses, were characterised by lower A2/6 and A2/4 ratios. Recognition of changes that may occur in the quantitative and qualitative composition of soil humus after the application of biochar may in the future be helpful information for determining appropriate biochar dose.

Keywords: biochar, humus compounds, soil, wheat straw, organic carbon

INTRODUCTION

The role of soil as the most important natural resource is expressed in the European Soil Charter (2003). Learning morphological features as well as biological, physical and chemical soil processes is essential for rational management of its resources (Bastida et al. 2008). According to a directive of the European Parliament and of the Council establishing a framework for soil protection, soil is defined as a depleting resource of which the formation and regeneration processes are very slow. A thematic strategy on soil protection, aiming to stop and reverse its degradation is formulated in the Sixth Community Environment Action Programme. Additionally, in the communication "Towards a Thematic Strategy for Soil Protection" of 2002, the decrease in soil organic matter content was listed as the most important of the eight threats to soil in the European Union. The recently observed decrease in soil organic matter content is a result of intensifying soil degradation processes. For example, in Poland, up to 90% of agricultural use soils have a low organic carbon

content, and losses of carbon in the balance of soil organic matter are greater than its accumulation.

Due to the indisputable significance of humus in many biochemical processes as well as its increasing deficit particularly in light soils, alternative sources of substrates for the reproduction of this constituent should be sought. Considering the physical, chemical and biological stability, one source may be the solid product of thermal biomass transformation called biochar (Kwapinski et al. 2010, Allaire et al. 2015, Mierzwa-Hersztek et al. 2018). Biochar is a carbon-rich solid product obtained from pyrolising biomass under low oxygen conditions (Lehmann 2007, Kwapinski et al. 2010) and is defined by its intentional application to the soil for environmental applications. Biochar contains highly condensed aromatic structures that resist decomposition in soil. For this reason, it can effectively reduce atmospheric CO_2 concentration, because biochar slows the rate at which fixed carbon is returned to the atmosphere (Xu et al. 2012). As reported by Woolf et al. (2010), the use of biochar could mitigate up to 12% of current anthropogenic CO_2 emissions.

The use of different types of exogenous organic matter (EOM), such as biochar, has a great potential in stabilising humus content in soil (Kwapinski et al. 2010, Gondek and Mierzwa-Hersztek 2017). However, frequent literature references to quality standards of humus and the soil itself do not describe individual factors such as quantitative and qualitative humus compounds parameters. Its production involves substrates of different origins and physicochemical and biochemical properties. These include not only agricultural biomass (Sánchez et al. 2009), but also energetic plants (Kwapinski et al. 2010). Due to the diversity of substrates and parameters of the pyrolysis process, the obtained solid and stable products differ significantly in chemical composition, for example, in the carbon content (Jindo et al. 2014). Biochar is believed to be an attractive product with a great potential for long-term storage of carbon in soil. Unfortunately, the as yet poorly recognised effect on the humification rate and humus compounds heterogeneity of application of varied doses of biochar to soil does not allow a full evaluation of the biochar's effect on humus quality.

The latest literature review brings up many doubts regarding the influence of biochar on soil properties, biological activity, or changes of organic compounds, depending on the material used for the production of biochar, temperature of the process and period of application or dose (Lehmann 2007, Jha et al. 2010). Moreover, there is no information about quantitative and qualitative parameters of humus compounds in soil after biochar application. In consequence, it is difficult to unambiguously show the positive and negative aspects of biochar changes, making it impossible to properly assess the effect of its application on physical, chemical and biological soil properties. Worse still, the question of the extent of biochar's influence on quantitative and qualitative humus compounds composition remains unanswered. That is why it is essential to identify potential changes in the quantitative and qualitative composition of humus after biochar application. This direction of research, particularly with reference to the qualitative composition of humus, provides great potential for progress not only in agricultural sciences, but also in chemistry, biology, or even geoscience. In addition, identification of changes to biochar, taking account the above aspects, may play a key role in detoxification and restoration of soil properties. The aim of this study was to evaluate the effect of the addition of wheat straw (*Triticum aestivum* L.) and biochar obtained from this biomass on quantitative and qualitative humus parameters.

MATERIAL AND METHODS

The research material was wheat straw from an agricultural holding located in the Małopolska Province (southern Poland) and biochar produced from from wheat straw. Wheat straw was dried at 65°C, ground down 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 minutes under a limited supply of air (IBI 2015). The rate of heating the combustion chamber was 10°C min^{-1}. The process parameters were configured so as to obtain the lowest possible carbon losses. The selected physical and chemical properties of the materials used in the incubation experiment are shown in Table 1.

The laboratory experiment was carried out on soil with a loamy sand texture (PTG 2008), collected from 0–20 cm layer in southern Poland. By assumption, the experiment was supposed to reflect the processes taking place in cultivated soil after the application of mineral fertilisers and organic materials. The soil was mixed with mineral salts (0.10 g N·kg^{-1} DM of soil, 0.04 g P·kg^{-1} DM of soil, and 0.12 g K·kg^{-1} DM of soil) and organic materials (straw, wheat straw biochar) and then placed in plastic containers (able to contain 250 g of soil material), transferred to a thermostatic cabinet, and incubated at 24°C for 254 days. During the experiment, the humidity of the incubated samples was maintained at a constant

TABLE 1. Chemical and physical properties of wheat straw and biochar

Determination	Unit	Wheat straw (WS)	Wheat straw biochar (WSB)
pH in H_2O	–	5.84±0.15	6.52±0.60
EC	µS·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	g·kg^{-1} DM	461±104	629±183
Nitrogen	g·kg^{-1} DM	3.70±0.42	10.0±0.52
Sulphur	g·kg^{-1} DM	0.62±0.11	0.54±0.11
Hydrogen	g·kg^{-1} DM	63.9±2.02	45.8±0.92
Oxygen	g·kg^{-1} DM	410±11	180±19
Specic surface area (S_{BET})	m^2·g^{-1}	0.55±0.02	0.67±0.09
Pore volume	cm^3·g^{-1}	0.0009±0.000	0.0016±0.002
Pore diameter	nm	6±2	12±3
Maximum pore volume	nm	67	123

±standard deviation, n=3.

level of 50% of the maximum soil water capacity. The properties of the soil used in the studies are presented in Table 2.

The experimental diagram included two control treatments: soil without mineral and organic fertilisers (C) and soil with the addition of mineral salts (MF), which allowed us to demonstrate the real effect of organic materials. The experiment comprised 10 treatments carried out in 3 replications: control – soil without fertilisation (C); soil + NPK (MF); soil + NPK + wheat straw (WS) in a doses of 0.2%, 0.5%, 1% and 2% (WS 0.2%, WS 0.5%, WS 1%, WS 2%), soil + NPK + wheat straw biochar (WSB) in a doses of 0.2%, 0.5%, 1% and 2% (WSB 0.2%, WSB 0.5%, WSB 1%, WSB 2%).

Soil samples were collected on the day of starting the experiment (0 day) and, after 245 days of incubation, dried in the open air, ground down in a porcelain mortar and 1 mm sieved. Then, organic carbon was assessed by oxidative-titrating method. Humus compounds content was extracted from soil by mixture of 0.1 mol·dm^{-3} $Na_4P_2O_7$ solution + 0.1 mol·dm^{-3} NaOH. Humic acid carbon (CHA) was isolated in the extract of sodium pyrophosphate and sodium base, whereas fulvic acid carbon (CFA) was calculated from the difference between the amount of carbon in the extract (C_{ext}) and the amount of humic acid carbon (CHA) in the extract (Kononowa 1968). The non-hydrolysing carbon (CNH) extraction residue was computed from the difference between the organic carbon content (C_{org}) and the amount of carbon in the extract (Gondek and Mierzwa 2014). Light absorbance was then measured in the obtained solutions of humic acids at the wavelength of 280, 465 and 665

nm, and colour ratio (A2/6, A2/4, A4/6) was computed.

Statistical analysis

The differences between each treatment and the control as well as between treatments were evaluated using one–way analysis of variance (ANOVA, Duncan test, $\alpha \le 0.05$). Variation within treatments was determined by calculating the values of standard deviation (±SD). All statistical analyses were performed using Statistica PL 13 software (StatSoft Inc.).

RESULTS

Wheat straw (WS) had lower values of pH, dry matter and ash content compared to biochar obtained from this material (WSB) (Table 1). The contents of sulphur, hydrogen, and oxygen noted in biochar (WSB) were smaller and carbon and nitrogen contents were greater than those discovered in thermally unconverted straw (WS). During the analysis of biochar physical parameters, we discovered that the specific surface area as well as pore volume and diameter increased as a result of thermal conversion.

The soil used in the studies was sandy and acidic and contained 6.43 g·kg^{-1} DM of total carbon and 0.54 g·kg^{-1} DM of total nitrogen. The total contents of trace elements were typical for uncontaminated soils (Table 2).

Regardless of the type of organic material and its dose, the content of organic carbon in soil on the day of setting up the experiment (0 day) increased significantly compared to control treatments without the addition of organic materials and mineral fertilisers (C) and the treatment with mineral salts (MF) (Table 3). After 254 days of incubation of soil with organic materials, a similar relationship was noted, except for treatments in which 0.2% of thermally unconverted straw and 0.2% of biochar were applied (Table 3). Lower organic carbon content was determined after 254 days of incubation in the soil on all objects except treatments into which 1% and 2% doses of biochar were introduced.

After 254 days of incubation, C_{ext} soil content significantly increased in treatments with 0.5%, 1%, and 2% additions of WS and 2% addition of WSB compared to the content determined in the soil of C and MC treatments (Table 3). However, it should be noted that the C_{ext} contents determined in treatments with WS were much lower after 254 days than on the day of starting the experiment (0 day). The mean share of C_{ext} in the total C_{org} content after 245 days of

TABLE 2. Selected properties of soil used for the incubation experiment

Determination	Unit	Value
Sand 1.0–0.1	mm	85
Silt 0.1–0.02		9
Clay <0.02		6
pH H_2O	–	5.67±0.05
EC	$\mu S \cdot cm^{-1}$	32.2±4.35
$C_{tot.}$	g·kg^{-1} DM	6.43±0.08
$N_{tot.}$		0.54±0.01
C:N	–	11.9
$Cd_{tot.}$	mg·kg^{-1} DM	0.19±0.01
$Cu_{tot.}$		1.85±0.06
$Pb_{tot.}$		37.9±1.53
$Zn_{tot.}$		16.5±1.07

±standard deviation, n=3.

TABLE 3. The content of soil humus compounds on the start day the experiment (0 day) and after 254 days of the experiment

Treatment	C_{org}	C_{ext}	CHA	CFA	CNH
	g·kg⁻¹ DM	g·kg⁻¹ DM (% share in C_{org})			
On the start day the experiment (0 day)					
C	5.09a	2.37b (46.6)	0.49b (9.6)	1.88b (37.0)	2.72a (53.4)
MF	5.54a	2.72bc (49.0)	0.32a (5.8)	2.39c (43.2)	2.82a (51.0)
WS 0.2%	8.15d	2.94bc (36.2)	0.55bc (6.8)	2.39c (29.4)	5.20c (63.8)
WS 0.5%	7.53cd	5.18e (68.9)	0.63cd (8.3)	4.56e (60.6)	2.34a (31.1)
WS 1%	10.76e	5.34e (52.9)	0.65de (6.4)	4.69e (46.4)	5.42c (49.8)
WS 2%	12.60f	5.45e (43.1)	0.72e (5.7)	4.73e (37.4)	7.19d (56.9)
WSB 0.2%	6.42bc	1.65a (26.0)	0.47b (7.3)	1.18a (18.4)	4.77c (74.3)
WSB 0.5%	7.96d	2.75bc (34.7)	0.49b (6.2)	2.26b (28.5)	5.21c (65.3)
WSB 1%	10.90e	2.83bc (26.1)	0.51b (4.7)	2.32c (21.3)	8.05d (74.0)
WSB 2%	13.30f	2.87bc (21.6)	0.53b (4.0)	2.34c (17.6)	10.40e (78.4)
Mean	7.93a	3.11a	0.54a	2.55a	4.81a
After 254 days of the experiment					
C	5.91ab	2.74bc (46.9)	0.45a (7.6)	2.29b (39.2)	3.16ab (53.2)
MF	5.04a	2.92bc (57.9)	0.46ab (9.1)	2.46bc (48.8)	2.13a (42.1)
WS 0.2%	5.45a	2.96bc (54.2)	0.52bc (9.5)	2.44bc (44.8)	2.49a (45.7)
WS 0.5%	6.63abc	3.18c (47.9)	0.63de (9.5)	2.55cd (38.4)	3.44bc (52.1)
WS 1%	7.27bc	3.80d (52.5)	0.65d (8.9)	3.15d (43.6)	3.46bc (47.5)
WS 2%	8.93d	3.89d (43.8)	0.78e (8.8)	3.11d (35.0)	5.04c (56.2)
WSB 0.2%	5.91ab	2.39a (40.6)	0.55c (9.3)	1.84a (31.2)	3.52bc (59.5)
WSB 0.5%	7.72cd	2.92bc (37.8)	0.52bc (6.7)	2.40c (31.1)	4.80c (62.2)
WSB 1%	11.3e	3.05bc (27.2)	0.55c (4.9)	2.50cd (22.3)	8.20d (72.8)
WSB 2%	15.2f	3.32c (22.0)	0.52bc (3.4)	2.80cd (18.6)	11.89f (78.0)
Mean	8.82b	3.41b	0.56b	2.87b	5.41b

Each value represents the mean of three replicates. The different letters within a column indicate a significant difference at $\alpha \leq 0.05$ according to Duncan's multiple range tests – two-factor analysis: treatment × analysis date.

incubation decreased (about 1%) on WS and increased (about 4%) on WSB (Table 3).

The CHA content in soil was comparable in both cases (on 0 day and after 254 days) in treatments with organic materials, regardless of the dose (Table 3). The share of humic acid carbon (CHA) in the content of C_{org}, irrespective of the material type, dose, and analysis date, did not exceed 10% (Table 3). Irrespective of the date, the analysis of the effect of the material dose revealed the generally decreasing share of this humus fraction in C_{org}. After 254 days of incubation, there was an increase in the share of CHA in the organic carbon content after adding both WS and WSB (apart from the 2% WSB dose). A similar tendency was observed for fulvic acids (CFA), in which the share of C_{org} was much higher (Table 3).

Both on the day of setting up the experiment (0 day) and after 254 days of incubation, a significantly higher content and share of non-hydrolysing carbon (CNH) in the C_{org} content was noted in the soil of treatments with the addition of biochar compared to treatments with thermally unconverted straw (Table 3). In general, irrespective of the type of organic fertilisation, 1% and 2% doses had a more favourable effect on the share of this humus fraction in the C_{org} content. It should also be noted that a very strong significant relationship was demonstrated between C_{org} and CNH (Figure).

The values of CHA:CFA ratio, regardless of the type of organic material, dose, and date (0 and 254 days) were relatively low (0.19–0.40) (Table 4). After 254 days, the parameter values in treatments with WS were higher than on the day of setting up the experiment (0 day). Comparable or smaller values of the CHA:CFA ratio were discovered for WSB. In general, the amount of material added did not have a major effect on the parameter value.

The values of the A2/6 quotient were generally comparable on the day of setting up the experiment (0 day) and after 254 days of incubation. It was

TABLE 4. The ratio of humic to fulvic acids and the optical properties of humic acid extracts on the start day (0 day) and after 254 days of the experiment (254)

Treatment	CHA:CFA		A2/6		A2/4		A4/6	
	0	254	0	254	0	254	0	254
C	0.26ef	0.20bc	5.19bc	5.38bc	1.20d	1.13cd	4.60bc	4.75c
MF	0.14a	0.19ab	5.28bc	4.75a	1.18d	1.10bc	4.48bc	4.30ab
WS 0.2%	0.23cd	0.22cd	5.56c	5.20abc	1.22d	1.09bc	4.54bc	4.78c
WS 0.5%	0.14a	0.25de	5.23bc	5.43c	1.17c	1.10bc	4.48bc	4.95d
WS 1%	0.14a	0.21bc	4.11a	5.07abc	1.08b	1.00b	3.81a	5.05e
WS 2%	0.15ab	0.25de	4.63abc	4.86abc	1.07b	0.93a	4.34ab	5.23d
WSB 0.2%	0.40g	0.31f	5.15bc	5.11abc	1.18cd	1.07bc	4.38ab	4.79c
WSB 0.5%	0.22cde	0.22cd	4.72abc	5.04abc	1.10bc	1.07bc	4.28ab	4.71c
WSB 1%	0.22cde	0.22cd	4.96abc	4.93abc	1.09bc	1.00b	4.55a	4.93d
WSB 2%	0.23cde	0.19ab	4.41ab	4.80ab	1.01a	0.97ab	4.37ab	4.97d
Mean	0.21a	0.23a	4.92a	5.06a	1.05a	1.13b	4.43a	4.85b

Each value represents the mean of three replicates. The different letters within a column indicate a significant difference at $\alpha \leq 0.05$ according to Duncan's multiple range tests – two–factor analysis: treatment × analysis date.

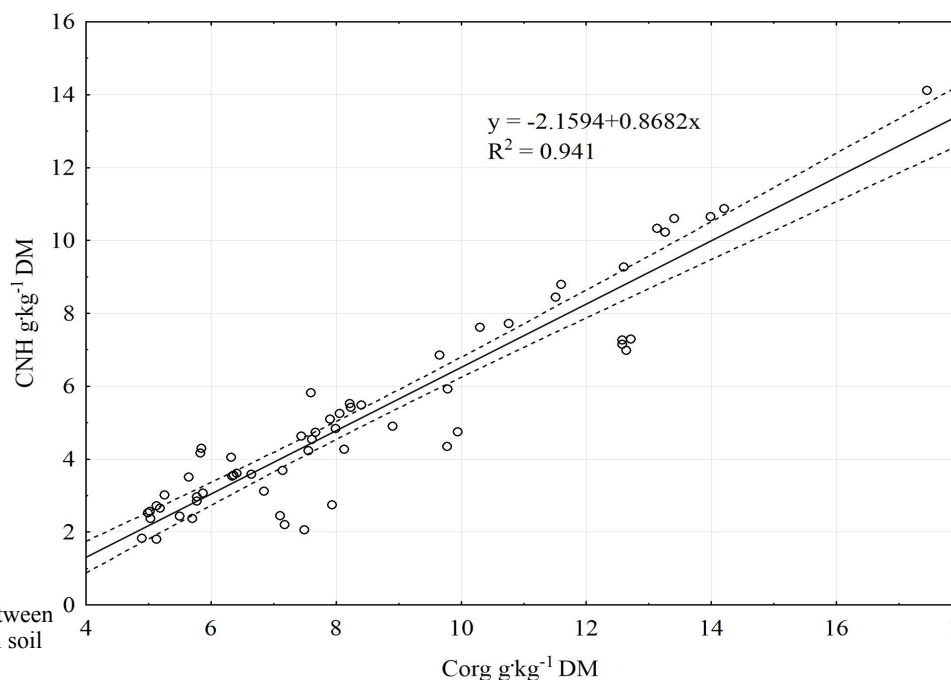$$y = -2.1594 + 0.8682x$$
$$R^2 = 0.941$$

FIGURE. The relationship between the content of C_{org} and CNH in soil

found that the larger addition of both WS and WSB resulted in lower values of A2/6. Similar relationships were determined for the A2/4 quotient value. Reverse relations were found for the A4/6 quotient (Table 4). Especially after 254 days of incubation, this parameter value increased with the increased additions of WS and WSB.

DISCUSSION

The pH values of WSB used in our study (Table 1) was lower than the pH values of different biochars used in the study of Jindo et al. (2014). This was determined by the temperature of biomass thermal conversion. According to Novak et al. (2009), pH changes in the pyrolysed material occurred probably as a consequence of the relative concentration of non-pyrolysed inorganic elements already present in original feedstocks. In our study, in the case of WSB, higher pH values can be attributed to a very stable organic carbon content in this material, which gives more surface functional groups and a large area thanks to micropores and aromatic ring structures (Jien and Wang 2013, Chintala et al. 2014, Gai et al. 2014, Obia et al. 2015, Gondek and Mierzwa-Hersztek 2016). Jindo et al. (2014) argued that the temperature used

in the process of thermal conversion of organic material greatly effects losses of different elements. These authors indicated that the temperature increase during pyrolysis results in a greater loss of H and O compared to C. In the present study, the content of both H and O in WSB was lower than their content determined in WS. As indicated by Cheah et al. (2014), the total sulphur content in biochar is mostly affected by the type of feedstock. However, it should be remembered that this element content will decrease during the thermal conversion of biomass (Al-Wabel et al. 2013), which was also demonstrated in the present study. Additionally, as stated by Yu et al. (2007) and Knudsen et al. (2004), the large variation in N and S content in biochars results from the retention of N in heterocyclic compounds, such as pyridones or pyrrols and the complexation of inorganic S forms by biochar. The porosity and specific surface area of biochar are important physical properties taking into account the effect of this type of material on soil properties. Jindo et al. (2014) obtained higher values of S_{BET} compared to these determined in the present study. However, it should be noted that these authors analysed biochars produced from different materials and at higher temperatures of the pyrolysis process.

Soil degradation and nutrient depletion are a global concern. Soil restoration techniques aimed at increasing soil organic matter (SOM) and stability of soil carbon (C) are required to enhance productivity and minimise risks of both soil degradation and environmental pollution (Mukherjee et al. 2014). Human interference in the soil environment, i.e. introduction of different organic materials to soil, significantly influences the properties and process of humic substance humification. When it comes to biochar, the wide variety of substrates and a series of processes taking place in the first stage of humification make it difficult to determine the humic substance structures. It is a common concept that bridged aromatic rings, which contain varied functional groups, are the structural basis of the humic acid molecule. Functional groups are believed to have critical effect on chemical properties of humic acids. Scientific literature provides some examples that the type and content of functional groups in humic acid molecules condition a number of humic acid properties, such as hydrophilicity, acidic character, ionic exchange capacity, as well as formation of mineral-organic bonds. Unfortunately, due to its polymolecular and polydispersive structure, humic substances undergo constant biochemical and chemical changes in soil, making it difficult to unambiguously determine the structure of their molecules. Therefore, comprehensive studies on an organic soil substance after the application of biochar are important not only

from a cognitive point of view, but also for the fertility assessment and degree of soil degradation.

In the present study, the increase in the content of organic C after 254 days of incubation and applying higher doses of biochar (1% and 2%) (Table 3) was comparable to those treatments with the same doses of unconverted straw applied. As stated by Hernandez-Sariano et al. (2016), the application of biochar to soil may cause accumulation of C, which is a result of a spatial reorganisation of C in soil particles, as well as a decrease in its respiration activity. According to Jiang et al. (2016), causes of higher C content in soil after application of biochar compared to thermally unconverted materials should be sought in biochar resistance to microbial decomposition and the content of nitrogen and low molecular weight C compounds in soil. This observation is partly confirmed by our results, as we demonstrated an increase in C_{ext} content, but, at the same time, lower share of C_{ext} in C_{org} content in the soil of treatments with 1% and 2% additions of WSB compared to the same doses of WS (Table 3). In addition, Jiang et al. (2016) showed that the addition of biochar to soil promoted the formation of dissolved organic carbon (DOC) derived from soil organic carbon (SOC) however, DOC accumulated in soil and was not mineralised to CO_2. Also Lu et al. (2014) demonstrated the significant effect of biochar, even with an addition of N, on reducing CO_2 emissions from native SOC by 64.9–68.8%. This indicates that biochar inhibited the decomposition of native SOC and the stimulation effect of inorganic N on native SOC degradation. The authors also indicated a reduction in the soluble carbon (DOC) content in the soil, primarily due to the sorption of DOC by biochar.

Literature data indicate that humic substances are composed of humic and fulvic acids as well as humins which have different properties. However, they are humic acids that have a high sorption capacity and are believed to be the most important components of soil humus (Tahir et al. 2011, Motojima et al. 2012, Mukherjee et al. 2014, Hernandez-Sariano et al. 2016). Humic substances are recognised by most soil scientists and agronomists as the most important components of healthy fertile soil. According to Mukherjee et al. (2014), the increase in the CHA content in soil improves soil buffering properties, micronutrient chelation by increasing acidic ligands (-COOH or Ph–OH), as well as improving soil available water capacity (AWC). The latest data (Lehmann 2007) indicate significant progress in understanding the properties of biochar and its impact on soil and plants. However, the biochar's effect on the process of creating humic substances,

which are extremely important in the context of improving productivity, health, and functional properties of degraded soils, remains not fully described. Biochar in soil undergoes multidirectional and varied changes that lead to significant amendments in its structure, including π–π electron donor-acceptor – type interaction between sorbed SOM and functional groups, surface hydrophilic or hydrophobic interactions, and complexation by multidented organic acids with metal ions in soil (Mukherjee et al. 2014). The knowledge of surface oxidation of biochar by means of chemisorption during its aging in the environment at varied access of air and water, which can significantly affect the elemental composition of humic substances, acquires importance for a full understanding of these processes.

In our study, after 254 days of incubation, the addition of WSB to soil increased the content of humic acid carbon (CHA, CFA) with the simultaneous decrease of its share in the organic carbon content compared to C, MF treatments as well as treatments amended with WS, especially at higher doses (1% and 2%). It was also found that the content of CFA (being the most reactive humus fraction with lower chemical stability) in the soil of all experimental treatments was higher than the content of CHA, and increased relative to higher doses of WS and WSB (Table 3). Considering the significant role of humus acids (humic acids, in particular) in soil fertility, the increase in the content of CHA and CFA in these treatments can be regarded as a very beneficial phenomenon. On the other hand, higher CFA content may contribute to faster leaching of heavy metals and alkaline cations from soil compared to the situation when the share of CHA predominates. This is due to the fact that fulvic acids are very easily soluble (like most of their salts), and this determines their mobility and ability to penetrate deep into the soil profile. Additionally, these acids dissociate in solutions much more strongly than humic acids and show a strongly acidic pH.

According to Li et al. (2015), biochar is very beneficial for the formation of humus in the soil. The authors demonstrated increased contents of humic acids, fulvic acids, and humins after the application of biochar, which was very positive for the stability of the organic carbon pool in the soil. In the studies of Li et al. (2015), after 210 days of incubation, the CHA content increased by 9.8% and reached 13.6%, and the CFA content by 17.2% compared to the control. In our study (Table 3), the content of CNH and the share of this humus fraction in the C_{org} content significantly increased, which indicates a greater stabilisation of humus compounds and, at the

same time, the probable smaller emission of CO_2 (Li et al. 2015). The biochar stability in the soil environment, although modified by the type of biomass and technology used to produce this material, as well as environmental factors, including soil moisture, is conditioned mainly by the strong carbon bond in the material structure and the degree of aromatic carbon condensation (Schmidt et al. 2011, Singh and Cowie 2014, Qayyum et al. 2014). Some previous studies also suggest that biochar can stabilise native organic carbon in soil by creating organic-mineral connections, as well as by the sorption of DOC on the biochar surface and in pore spaces due to the exceptionally large specific surface area (Keith et al. 2011, Zimmerman et al. 2011, Singh and Cowie 2014). Ngo et al. (2014) also argued that the application of biochar to soil increased the content of not readily mineralised lignin compounds.

In addition to the quantitative and qualitative composition of humus compounds in soil fertilised with biochar, of great importance is also the CHA:CFA ratio, which informs about the internal structure and quality of soil humus substances. It is assumed that the higher the relationship values, the more fertile the soil. In the present study, the values of the CHA:CFA ratio did not exceed 1.0 (Table 4), which indicated a low degree of humification of organic matter in the soil of each treatment.

Changes in the optical density of humic acid solutions expressed by absorbance at the wavelength of 280, 464, and 664 nm indicated that humic acids in the soil of treatments amended with biochar, especially at 1% and 2% doses, were characterised by lower A2/6 and A2/4 ratios (Table 4). This indicates a higher molecular weight and a higher condensation level of CHA aromatic structures. In addition, the values of the A2/4 ratio, which expresses the ratio of lignin-like substances to the content of substances in the initial stage of decomposition, showed that higher addition of organic material increased the content of substances resistant to humification (Gondek and Mierzwa 2014). The optical properties of humic acids can be changed not only due to humification and mineralisation processes taking place in the soil, but also the type of external organic matter introduced. According to Baglieri et al. (2007), the optical density of humic substances depends on the ratio of carbon in the aromatic nucleus to carbon in the side radicals. The author stated that the chemically 'younger' humic acids are characterised by lower optical density compared to 'more mature' acids. This is due to the large condensation of the aromatic nucleus in 'mature' humic acids – while in 'younger' acids – due to the greater number of side

chains. In the research, Adani et al. (2007) discovered that the introduction of external organic matter into the soil can significantly affect the properties of soil humus compounds.

CONCLUSIONS

1. Higher doses of biochar resulted in a higher in C_{org} contents in soil.
2. The share of C_{ext} in C_{org} in the soil with biochar was smaller than in the soil with straw
3. The addition of biochar to the soil decreased the share of humic acid carbon (CHA, CFA) in the C_{org} content.
4. The content of non–hydrolysing carbon significantly increased in the soil with biochar.
5. Humic acids in the soil with biochar had a higher molecular weight and a higher aromatic structure condensation level.

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Przemiany biowęgla w glebie na podstawie ilościowych i jakościowych parametrów związków humusowych

Streszczenie: Ze względu na niepodważalne znaczenie próchnicy glebowej w wielu procesach biochemicznych, a także jej rosnący deficyt szczególnie w glebach lekkich, stale poszukuje się alternatywnych źródeł tego składnika. Biorąc pod uwagę stabilność fizyczną, chemiczną i biologiczną, takim źródłem może być stały produkt termicznej konwersji biomasy zwany biowęglem. Celem niniejszej pracy była ocena wpływu dodatku słomy pszennej (*Triticum aestivum* L.) i wyprodukowanego z niej biowęgla w dawkach 0,2%, 0,5%, 1% i 2% na parametry ilościowe i jakościowe próchnicy. Stwierdzono, że niezależnie od terminu analizy po zastosowaniu 1% i 2% biowęgla do gleby, w porównaniu do takich samych dawek słomy nieprzekształconej termicznie, zawartość C_{org} w glebie istotnie się zwiększyła. Wykazano znacznie mniejszy udział C_{ext} w zawartości C_{org} w glebie obiektów, w których zastosowano biowęgiel w dawkach 1% i 2% w porównaniu do równoważnych dawek słomy pszennej. Dodatek biowęgla do gleby w porównaniu do obiektów bez dodatków organicznych (C, MF) oraz do obiektów ze słomą pszenną, zwłaszcza w większych dawkach (1% i 2%), po 254 dniach inkubacji zmniejszył udział węgla kwasów huminowych (CHA, CFA) w zawartości węgla organicznego. Niezależnie od terminu istotnie zwiększyła się także zawartość węgla niehydrolizującego w glebie obiektów, w których zastosowano dodatek 1% i 2% biowęgla. Wskazuje to na większą stabilizację związków próchniczych, a zarazem mniejszą emisję CO_2. Kwasy huminowe w glebie obiektów, w których zastosowano biowęgiel, zwłaszcza w dawkach 1% i 2%, charakteryzowały mniejsze wartości współczynników A2/6 i A2/4, co wskazywało na większą masę cząsteczkową i większy stopień skondensowania ich struktur aromatycznych.

Słowa kluczowe: biowęgiel, węgiel kwasów humusowych, gleba, słoma pszenna, węgiel organiczny