Original Paper

Differences in soil organic matter and humus of sandy soil after application of biochar substrates and combination of biochar substrates with mineral fertilizers

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The effort to achieve the sustainable farming system in arable soil led to the intensive search for a new solution but an inspiration can also be found in the application of traditional methods of soil fertility improvement as it is shown in numerous examples in history. Recently many scientific teams have focused their attention on the evaluation of biochar effects on soil properties and crop yields. Since there are a lot of knowledge gaps, especially in explanations how biochar can affect soil organic matter (SOM) and humus substances, we aimed this study at the solution of these questions. Therefore, the objective of the experiment was to evaluate the impact of two biochar substrates (B1 – biochar blended with sheep manure, and B2 – biochar blended with sheep manure and the residue from the biogas station) at two rates (10 and 20 t ha^{-1}) applied alone or in combination with mineral fertilizers (Urea was applied in 2018, at rate 100 kg ha^{-1}, and Urea at rate 100 kg ha^{-1} + AMOFOS NP 12-52 at 100 kg ha^{-1} were applied in 2019) on the quantity and quality of SOM and humus of sandy soil (Arenosol, Dolná Streda, Slovakia). The results showed that application of the biochar substrates together with mineral fertilizers (MF) had more pronounced effect on the organic matter mineralization in the sandy soil which resulted in low accumulation of soil organic carbon (C_{org}) and labile carbon compared to biochar substrates treatments without MF. The share of humic substances in C_{org} significantly decreased by 16, 50, 16 and 24% in B1 at 10 t ha^{-1}, B1 at 20 t ha^{-1}, B2 at 10 t ha^{-1} and B2 at 20 t ha^{-1} treatments, respectively, compared to the control. A similar tendency was observed for biochar substrates treatments + MF, compared to MF control. The carbon content of humic substances (C_{HS}) was equal to 4.40 – 5.80 g kg^{-1} and the biochar substrates had statistically significant influence on C_{HS} content. On average, there was a smaller decrease of C_{HS} in B1 at rate 10 t ha^{-1} than at rate 20 t ha^{-1} and no effect of B2 compared to control. The carbon content of fulvic acid (C_{FA}) was 9% higher in B1 at 10 t ha^{-1}, and 20 t ha^{-1}, 47% higher in B2 at 10 t ha^{-1} and 17% higher in B2 at 20 t ha^{-1} compared to control. As a result of biochar substrates + MF application, the reduction in C_{FA} was observed. The results showed a decrease of C_{HA} : C_{FA} ratio with association to biochar substrates alone application compared to control on one hand, and a wider of C_{HA} : C_{FA} ratio in biochar substrates + MF treatments in comparison to MF control on the other hand. Humus stability was increased in biochar substrates alone treatments compared to control, on the other hand, compared to MF control, the application of biochar substrates + MF resulted in a lower humus stability.

Keywords: carbon sequestration, humus quality, Arenosol, biochar, *Effeco*

1 Introduction

In general, soil quality depends on quantity and quality of soil organic matter (SOM). SOM plays a key role in soil fertility and productivity through effects on physico-chemical and biological soil properties (Szombathová, 1999; Balashov and Buchkina, 2011; Gaida et al., 2013). SOM consists of different components, and each of them can contribute to different soil functions. Soil organic carbon is usually recognized as a quantitative characteristic of SOM and its content depends on soil type and texture, soil use and management etc. (Šimanský et al., 2008; Devine et al., 2014; Rabbi et al., 2014, Polláková et al., 2018). More stable part of SOM is humus. Humus represents a complicated and dynamic

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complex of organic components which are a result of decomposition and humification of organic substances in soils (Stevenson, 1994; Brady and Weill, 1999; Szombathová, 1999).

The agricultural land of the Slovak Republic covers 2, 432, 979 hectares and soil organic carbon content of arable land is typically unsatisfactory. In several most productive areas the soil organic carbon content has decreased to low levels (Kobza et al., 2017). The recently observed decrease in SOM is a result of intensifying soil degradation processes related to human activity such as incorrect and intensive soil management practices. Soil management plays a key role in changes of SOM and soil humus composition (Szombathová, 1999; Devine et al., 2014; Rabbi et al., 2014; Szombathová, 2010). Except for human activity, external factors such as temperature, soil moisture (Marschner and Kalbitz, 2003) and soil texture have significant impact on changes in SOM. For example, a higher accumulation of SOM is observed in loamy or clay soils than in sandy soil with coarse texture (Šimanský et al., 2009). Sandy soils occur in different environments but their use in agriculture is limited because of their low productivity. Typical features of sandy soils are high permeability, low water and nutrient storage capacity and low contents of SOM (Šimanský et al., 2019; Šimanský and Jonczak, 2020). If farmers wish to develop effective and ecological farming on any soil, they have to know the soil characteristics, the reasons of the soil low fertility and the pathways to eliminate the problems. In case of sandy soils, fertilizers are an important factor in the crop production intensification. Fertilization can increase crop yields, plant and root residues input into the soil system and eventually can result in an increase in the soil organic carbon content (Tian et al., 2015), but on the other hand, fertilizers can also decrease the C content compared to unfertilized soil (Shimizu et al., 2009) which can be reflected in some SOM parameters.

In the agro-ecosystems, the biggest primary source of organic substances is crop residues (Váchalová et al., 2016), which can compensate 50–60% of the loss in SOM. The remaining 40–50% has to be supplied into the soil in the form of manures, which is a big problem since the production of manures in Slovakia has a decreasing trend. So, a new solution has to be found. It turns out that application of biochar and biochar substrates or their combinations to soils could be a beneficial solution. Apparently, it is one of the new ways to improve soil quality and sustainability by increasing the soil carbon content and making significant changes in soil properties (Buchkina et al., 2017). Investing in biochar that is applied to soil is a long-term matter that may discourage farmers from using it. However, its beneficial effects in the soil persist for several years to decades, which in the next period may be of interest to the farmer compared to the application of, for example, composts, which are applied several times over the same time period (El-Naggar et al., 2019). However, technological conditions of pyrolysis and feedstock type have key impacts on biochar properties and its interaction with soils. Biochars in most cases do not provide high amounts of nutrients (Glaser and Birk, 2012) and therefore are applied to the soils together with composts, fertilizers or as biochar substrates (Trupiano et al., 2017; Fischer and Glaser, 2012; Šimanský et al., 2019a; Horák et al., 2020). The use of different biochar types has a great potential in stabilising humus content in soils (Gondek and Mierzwa-Hersztek, 2017), however, the effects of different biochar substrates and their combination with fertilizers at different rates are still insufficiently described in scientific literature. Therefore the aim of this study was:

a) to quantify the effects of different biochar substrates at different rates applied alone and in combination with mineral fertilizers on quantitative and qualitative parameters of SOM and humus in sandy soil;
b) to determine the linear relationships between soil organic carbon content and other SOM parameters of sandy soil with dependence on biochar substrates application.

2 Material and methods

The study was conducted in the south-western part of Slovakia near towns of Sereď at the Dolná Streda locality (Figure 1). The site is characterized by temperate climate, with an average annual temperature of 9–10 °C and average annual precipitation of 520–600 mm. The soil in an experimental field was classified according to the World Reference Base for Soil Resources (IUSS WRB, 2015) as sandy Haplic Arenosol (Arenic, Calcic). The main characteristics of the top layer of soil (0 – 20 cm) before the experiment establishment: sand – 81.9%, silt – 10.5%, clay – 7.64%, soil organic carbon – 9.70 g kg^{-1} soil, total nitrogen – 1.30 g kg^{-1} soil, available P – 175 mg kg^{-1} soil, available K – 165 mg kg^{-1} soil, and soil pH – 7.60.

The experiment was established in autumn of 2017. Before the experiment, the preceding crop was durum wheat in 2017. Sunflower and durum wheat were planted in 2018 and 2019, respectively. The area of one plot was 7.5 × 12 m (90 m^2) and the protective belt of 1 m was left between the individual plots. The experiment was set up by the method of long segments. In spring and autumn of 2018 and 2019, the soil samples (three replicates) were taken from:

1/ the trial with treatments consisting of: i. Control – no fertilization, ii. Biochar substrate 1 (B1) at rate of 10 t ha^{-1}, iii. Biochar substrate 1 (B1) at rate of 20 t ha^{-1},

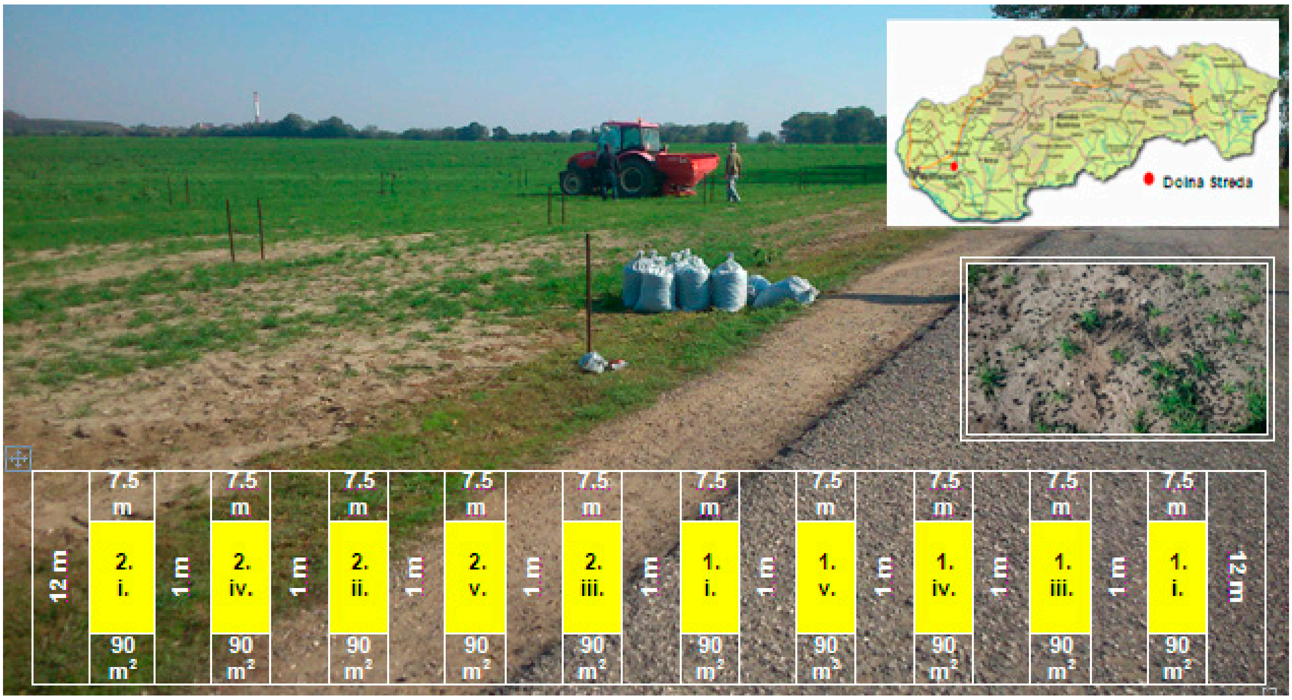

Figure 1 Location of the site and a schematic layout of the experiment
Trial 1: i. – control – no fertilization; ii. – biochar substrate 1 (B1) at rate of 10 t ha^{-1}; iii. – biochar substrate 1 (B1) at rate of 20 t ha^{-1}; iv. – biochar substrate 2 (B2) at rate of 10 t ha^{-1}; v. – biochar substrate 2 (B2) at rate of 20 t ha^{-1}
Trial 2: i. – fertilized control – mineral fertilization (MF); ii. – biochar substrate 1 (B1) at rate of 10 t ha^{-1} + MF; iii. – biochar substrate 1 (B1) at rate of 20 t ha^{-1} + MF; iv. – biochar substrate 2 (B2) at rate of 10 t ha^{-1} + MF; v. – biochar substrate 2 (B2) at rate of 20 t ha^{-1} + MF

iv. Biochar substrate 2 (B2) at rate of 10 t ha^{-1}, v. Biochar substrate 2 (B2) at rate of 20 t ha^{-1};

2/ from the trial with the following treatments: i. Fertilized Control – mineral fertilization (MF), ii. Biochar substrate 1 (B1) at rate of 10 t ha^{-1} + MF, iii. Biochar substrate 1 (B1) at rate of 20 t ha^{-1} + MF, iv. Biochar substrate 2 (B2) at rate of 10 t ha^{-1} + MF, v. Biochar substrate 2 (B2) at rate of 20 t ha^{-1} + MF.

Two types of biochar substrates were tested: B1 was the biochar mixed with the dried sheep manure in the proportion 1 : 1 (labelled *Effeco* 50 : 50) and B2 was the biochar mixed with the dried sheep manure and the residue from the biogas station (originally – cattle manure) in the proportion 1 : 1 : 1 (labled *Effeco* 33 : 33 : 33). Both products come under trade mark of Zdroje Zeme, a. g., company which develops biocarbon substrates. B1 contained 43% of total organic carbon, 1.2% total N, 0.49% P and 24.6% K, and its pH was 8.18. B2 contained 45.4% of total organic carbon, 1.3% of total N, 0.79% P and 15.5% K, and its pH was 8.44. The content of heavy metals in both biochar substrates did not exceed the limit rates, which are set by the regulation 577/2005. The regulation determines the manure types, composition, packaging and labelling of fertilizers, analytical methods, fertilizers testing, hazardous elements, their limit rates for the particular groups of fertilizers, tolerance and limit rates for organic fertilizers and according to the Act 220/2004 of protection and utilization of agricultural soil (in the Slovak Republic). Both biochar substrates are granulated into pellets with the size of about 2 × 1 × 1 cm. Mineral fertilizers in the field experiment were applied in the form of Urea (46% of N) at rate 100 kg ha^{-1} in 2018 and in the form of Urea at rate 100 kg ha^{-1} as well as AMOFOS NP 12-52 (12% of N and 52% of P_2O_5) at rate 100 kg ha^{-1} in 2019.

The following SOM and humus characteristics were determined in the soil samples: the soil organic carbon (C_{org}) content was determined by the wet combustion – oxidation of organic matter by a mixture of H_2SO_4 and $K_2Cr_2O_7$ with residue titration using Mohr's salt (Hrivňáková et al., 2011). The composition of humus fractions (humic acids – HA and fulvic acids – FA) was determined according to the Belchikova and Kononova procedure (Hrivňáková et al., 2011). The absorbance of humic substances and humic acids was measured at 465 and 650 nm to calculate the colour quotients $Q^{4/6}_{HS}$ and Q^{46}_{HA}. The labile carbon (C_L) was extracted from 1 g soil samples by shaking them in 50 mL of 0.005 M $KMnO_4$ for two hours. After centrifugation, the C_L content was determined by oxidation of organic matter by a mixture of 0.07 M H_2SO_4 and $K_2Cr_2O_7$ with residue titration using 0.05 M Mohr's salt (Łoginow et al., 1987).

The obtained data were analyzed using the statistic software Statgraphics Centurion XV.I programme

(Statpoint Technologies, Inc., USA). One-way ANOVA model was used for individual treatment comparisons at P <0.05, with separation of means by LSD multiple-range test. The linear analysis was used to determine the relationships between soil organic carbon and parameters of soil organic matter. Significant relationships were tested at P <0.05.

3 Results and discussion

3.1 Effects of biochar substrates and their combination with mineral fertilizers on SOM in sandy soil

The study has shown that application of the biochar substrates either through sole application or in combination with the mineral fertilizers increased the mean values of C_{org} in the soil. Higher content of C_{org} in the soil was measured at higher rates of both tested biochar substrates applied with or without the mineral fertilizers (Table 1). Biochar is a significant source of stable C (Fischer and Glaser, 2012; Šimanský et al., 2017) and its application into the soil can be linked with decreasing microbial activity, lower CO_2 production and fall in mineralization. Biochars in relation to their properties can provoke positive or negative priming effects (Cheng et al., 2016). In this study, the biochar substrates contained 43.0% (B1) and 45.4% (B2) of C and for this reason the increase of C_{org} in the sandy soil due to their applications is not surprising. Mineral fertilizers are considered to be a significant accelerator of some soil processes (Šimanský et al., 2016; Horák et al., 2017) and that was confirmed by our results. There was a statistically significant positive linear relationship between C_{org} and labile carbon (C_L) contents in the treatments with biochar substrates applied alone (Figure 1A). The soil C_L contents were significantly increased by B2 application compared to B1 and to the control (Table 1). The explanation could be the difference in C_L content in B1 and B2 substrates. There was no significant relationship between the C_{org} and C_L for the treatments where biochar substrates were combined with mineral fertilizers (Figure 1A). Biochar substrates together with mineral fertilizers had negative effect on the soil C_L contents compared to fertilized control (Table 1). This means that organic C_L substances with low stability from the biochar substances were very rapidly metabolized by micro-organisms, which resulted in lower C_L content in the sandy soil under the mentioned treatments. The biochar substrates applied together with mineral fertilizers were more intensively mineralised in the sandy soil and that resulted in low accumulation of C_{org} compared to the biochar substrates treatments without mineral fertilization (Table 1).

Biochar can play an important role in the formation of humic substances (HS) as reported by Jindo et al. (2016). The extraction of humus substances after the application of the biochar substrates into the soil was decreased (Šimanský et al., 2019a), because easy decomposable and instable sources from organic matter added to the soil were available and they were influenced by the extraction (Zaujec and Šimanský, 2006). The share of HS in C_{org} was significantly lower compared to control: 16, 50, 16 and 24% for B1 at 10 t ha^{-1}, B1 at 20 t ha^{-1}, B2 at 10 t ha^{-1} and B2 at 20 t ha^{-1} treatments, respectively. A similar picture was observed when the biochar substrates were applied with mineral fertilizers, compared to fertilized control (Table 1). A higher content of C_{org} resulted in lower HS share in C_{org}

Table 1 Soil organic matter parameters

Treatment	Biochar substrate dose (t ha^{-1})	Total mineral fertilizers in kg per ha during 2018–2019	C_{org}	C_L	HS	HA	FA
			g kg^{-1}	mg kg^{-1}	% share in C_{org}		
Control	–	–	9.03a	788a	57.5c	38.3b	19.3b
B1	10	–	10.0b	836a	48.4bc	32.2ab	16.2a
B1	20	–	10.6b	796a	29.0a	29.0a	15.5a
B2	10	–	12.0c	1,003b	48.5bc	28.8a	19.8b
B2	20	–	12.7c	947b	43.8b	28.5a	15.3a
MF	–	300	10.7a	1,085b	53.5c	31.7c	21.8c
B1 + MF	10	300	11.4a	883a	43.0b	28.4bc	14.6b
B1 + MF	20	300	13.1b	1,084b	44.6b	27.5b	17.2b
B2 + MF	10	300	13.3b	957a	43.9b	27.4b	14.9b
B2 + MF	20	300	13.4b	875a	34.6a	23.1a	11.5a

C_{org} – soil organic carbon, C_L – labile carbon, HS – share of humic substances in the content of C_{org}, HA – share of humic acids in the content of C_{org}, FA – share of fulvic acids in the content of C_{org}. Different letters between lines indicate statistically significant differences at P <0.05 – LSD multiple range test

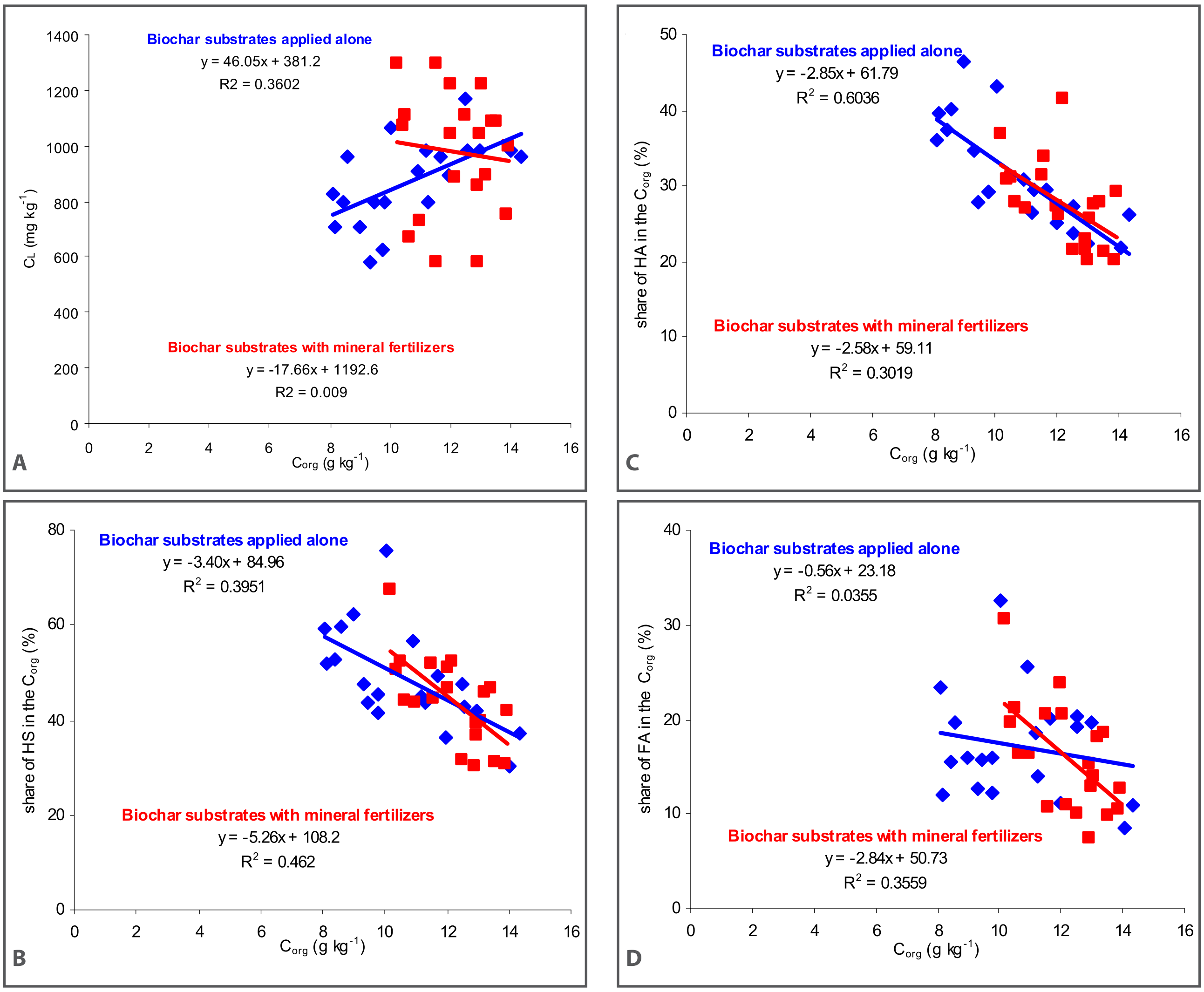

Figure 2 Linear relationship between soil organic carbon content and A) labile carbon content, B) share of humic substances in the soil organic carbon, C) share of humic acids in the soil organic carbon, and D) share of fulvic acids in the soil organic carbon

under biochar substrates alone and biochar substrates in combination with mineral fertilizers treatments; however this relationship (linear) was more significant in biochar substrates with mineral fertilizers treatments (Figure 1B). Statistically significant negative linear relationships between C_{org} and share of humic acid (HA) in C_{org} were determined for both biochar substrates alone and the biochar substrates with mineral fertilizers (Figure 1C) with the same tendency as in the case of HS. The share of HA in of C_{org}, irrespective of the biochar substrate, their combination with mineral fertilizers, or rates did not exceed the value of 40%. The level of 40% indicates a very high level of SOM humification (Grishina et al., 1986). A significantly higher decrease of HA in the C_{org} was noted in the treatments with the addition of biochar substrates alone and rather at rate of 20 t ha^{-1} than 10 t ha^{-1}. There was no any linear relationship between the C_{org} and share of fulvic acids (FA) in the soil organic carbon (Figure 1D). On the other hand, a negative linear trend in biochar with mineral fertilizers treatments (Figure 1D) was still visible. This means that part of FAs as the most reactive humus fraction with lower chemical stability from the biochar substances were very rapidly metabolized by micro-organisms, which resulted in decline in FA. On the contrary, after application of biochar substances alone there was a possibility of FAs formation from SOM which were not metabolized by micro-organisms (Jiang et al., 2016).

3.2 Effects of biochar substrates and their combination with mineral fertilizers on humus in sandy soil

According to Li et al. (2015), biochar is very beneficial for the formation of humus in the soil because biochar increases contents of humic acids, fulvic acids, and humins which have a very positive effect on the stability

Table 2 Humus parameters

Treatment	Biochar substrate rate (t ha^{-1})	Total mineral fertilizers rate during 2018–2019 (kg ha^{-1})	C_{HS}	C_{HA}	C_{FA}	$C_{HA} : C_{FA}$	$Q^{4/6}_{HS}$	$Q^{4/6}_{HA}$
			g kg^{-1}					
Control	–	–	5.20b	3.43a	1.52a	2.24b	6.470b	4.672b
B1	10	–	4.82ab	3.17a	1.65ab	2.08b	5.393a	4.138a
B1	20	–	4.72a	3.07a	1.65ab	1.94ab	5.531ab	4.339ab
B2	10	–	5.58b	3.33a	2.24c	1.64a	5.102a	4.174a
B2	20	–	5.06b	3.28a	1.78b	1.96ab	4.944a	4.118a
MF	–	300	5.70b	3.38b	2.32b	1.50a	4.627a	3.875a
B1 + MF	10	300	4.40aa	2.87a	1.53a	2.01ab	5.655b	4.355ab
B1 + MF	20	300	5.80b	3.59b	2.21b	1.68a	5.021ab	4.010a
B2 + MF	10	300	5.13b	3.34b	1.80ab	2.13b	6.121bc	4.371ab
B2 + MF	20	300	4.65a	3.10ab	1.55a	2.18b	6.020bc	4.557b

C_{HS} – humic substances carbon, C_{HA} – humic acids carbon, C_{FA} – fulvic acids carbon, $C_{HA} : C_{FA}$ – humic acids carbon to fulvic acids carbon ratio, $Q^{4/6}_{HS}$ – colour quotient of humic substances, $Q^{4/6}_{HA}$ – colour quotient of humic acids. Different letters between lines (different treatments – means) indicate statistically significant differences at $P < 0.05$ – LSD multiple range test

of the organic carbon pool in the soil. Humus parameters of the studied sandy soil are presented in Table 2. The content of humic substances carbon (C_{HS}) was changing from 4.40 to 5.80 g kg^{-1} and the biochar substrates had statistically significant influence on C_{HS}. On average, there was a smaller decrease of C_{HS} in B1 at rate 10 t ha^{-1} than at rate 20 t ha^{-1} and there was no effect of B2 application compared to control. Biochar substances and application rates had an effect on C_{HS} when applied with mineral fertilizers. B1 at rate 20 t ha^{-1} + MF and B2 at rate 10 t ha^{-1} + MF had no effect on C_{HS} compared to MF treatment but applications of B1 at rate 10 t ha^{-1} + MF and B2 at rate 20 t ha^{-1} + MF significantly decreased C_{HS} by 23 and 18%, respectively, compared to MF treatment.

The application of both biochar substrates alone did not have statistically significant influence on the content of humic acids carbon (C_{HA}) compared to control. Added biochar substrate B1 at rate 10 t ha^{-1} with mineral fertilizers significantly decreased C_{HA} content compared to MF treatment. Different results were received for the C_{FA} contents. The C_{FA} content was 9% higher in B1 treatment at 10 t ha^{-1} and 20 t ha^{-1}, 47% higher in B2 treatment at 10 t ha^{-1} and 17% higher in B2 treatment at 20 t ha^{-1} compared to control. As a result of biochar substrates combination with MF, the reduction in C_{FA} was observed (Table 2). The applications of B1 at 10 t ha^{-1} + MF, B1 at 20 t ha^{-1} + MF, B2 at 10 t ha^{-1} + MF and B2 at rate 20 t ha^{-1} + MF resulted in the reduction of C_{FA} content by 34, 5, 22 and 33%, respectively, compared to the MF treatment. Mineral fertilizers can act as accelerators (Šimanský et al., 2016; Horák et al., 2017) and support the reduction of C_{FA} as the most reactive humus fraction with lower chemical stability.

The use of different types of biochars has a great potential for the formation (Li et al., 2015) and stabilisation of humus substances of soils (Gondek and Mierzwa-Hersztek, 2017), which was confirmed in our research only partly (Table 2). The humus quality based on $C_{HA} : C_{FA}$ for the studied soil was above 1 which indicates favourable humus quality (Szombathová, 2010). Our results showed a decrease of $C_{HA} : C_{FA}$ ratio under biochar substrates alone treatment compared to control on one hand, and a wider $C_{HA} : C_{FA}$ ratio in biochar substrates + MF treatments in comparison to MF treatment, on the other. Similar results were published by Mierzwa-Herszek et al. (2018). In their study, the values of the $C_{HA} : C_{FA}$ ratio did not exceed 1, which indicated a low degree of humification of organic matter in the soil of each treatment, however, application of biochar alone in their cases decreased of $C_{HA} : C_{FA}$ ration on one hand, and added biochar together with wheat straw increased of $C_{HA} : C_{FA}$ ratio. In our experiment the degree of humification was decreased as a result of biochar substrates application alone or in combination with MF – more intensive in the treatments biochar substrates + MF (Table 1). In our case increase/decrease in HS can be linked with mineralization rather than humification. Above-mentioned trend was also confirmed by the values of colour quotients of humic substances as well as humic acids. Humus stability was higher in biochar substrates alone treatments compared to control, but on the other hand, the application of biochar substrates + MF resulted in lower humus stability compared to MF control. After application of biochar, which is a source of stabile C_{org}, a lower level of microbial activity resulted in C sequestration (Šimanský et al., 2017) on one hand, while mineral fertilizer application can be linked with intensive decomposition of SOM and biochar carbon in the soil (Whitman et al., 2015) on the other.

4 Conclusions

Our results confirmed that significant changes in SOM and humus of the studied sandy soil happen after the biochar substrates application. The biochar substrates application led to an increase in soil organic carbon content on one hand and to a decrease in the share of humic substances in soil organic matter on the other. Above-mentioned changes in SOM are reflected in the humus parameters. In this study a significant role was played by biochar substrate type, rate and especially combination with mineral fertilizers.

The obtained results have shown that in the sandy soil under biochar substrates alone treatments a higher content of soil organic carbon was related to an increase in labile carbon content. The application of biochar substrates alone or in combination with mineral fertilizers resulted in lower share of humic substances, including humic acids, in soil organic carbon. A higher content of soil organic carbon was observed together with the increasing share of fulvic acids in the soil organic carbon under biochar substrates applied together with mineral fertilizers treatments.

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