Acta fytotechn zootechn, 27, 2024(2): 172–178
http://www.acta.fapz.uniag.sk

Original Paper

Soil Organic Carbon Sequestration in Different Size-Fractions of Water-Stable Aggregates in Haplic Luvisol after Organic Amendment

Vladimír Šimanský*, Martin Juriga
Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources,
Institute of Agronomic Sciences, Slovakia

Article Details: Received: 2024-04-17 | Accepted: 2024-06-03 | Available online: 2024-06-30

https://doi.org/10.15414/afz.2024.27.02.172-178

Contents of different size-fractions of water-stable aggregates (WSA), soil organic carbon content (SOC) in soil bulk, and in different sizes of WSA and their contributions to C sequestration were determined under different rates of biochar application (B0: no biochar, B10: 10 t.ha^{-1}, and B20: 20 t ha10 t.ha^{-1}) at the experimental station of the Slovak University of Agriculture in Nitra (SUA), Slovakia. The results showed that only the application of 20 t of biochar ha^{-1} significantly reduced the content of water-stable micro-aggregates (WSAmi) compared to B0. In B10 treatment, SOC in soil bulk significantly decreased by 7%, but in case of B20, it increased by 14% compared to B0. In B20, the higher SOC content in soil bulk was also reflected in higher SOC concentration in size-fractions of WSA than in B0 and B10 treatments. However, a higher share of SOC in WSA from the total SOC in the soil bulk was found in the B10 when comparted to B0 and B20 treatments. Contents of SOC in WSAma >3 mm as well as 0.25–0.5 mm were polynomial increased with increasing of contents WSAma >3 mm and 0.25–0.5 mm, respectively in B0 and B10, and opposite SOC in WSAma 3-0.5 mm and WSAmi decreased with decreasing of contents of WSAma 3–0.5 mm and WSAmi, respectively along in all treatments. These results indicated that the rate of biochar is crucial for C sequestration in soil bulk and in WSA. WSAma have a great potential for C sequestration in soils after organic amendments.

Keywords: aggregation, organic carbon, fertilization, soil structure

1 Introduction

Carbon sequestration is a significant method for reducing climate change and enhancing soil fertility in agriculture (Shivangi et al., 2024). Many people are becoming increasingly concerned about climate change, and researchers have been studying soils as an area to store CO_2 (Horák and Šimanský, 2027; Kumar et al., 2020; Shivangi et al., 2024). Carbon sequestration can be realized also through incorporation of organic amendments to the soil (Šimanský et al., 2019; Kumar et al., 2020; Šrank and Šimanský, 2020; Shivangi et al., 2024). It is important, however, in addition to increasing the organic carbon content in the soil, that the mitigation of CO_2 release from the mineralization of native soil organic matter and organic amendments applied to the soil must be ensured (Shivangi et al., 2024). From this point of view, it is essential to pay attention to the properties of organic materials that are applied to the soil and the mechanisms of C stabilization in the soil. Several recent studies show that also biochar has such potential, i.e. it can stabilize C in the soil (Šimanský et al., 2019; Kumar et al., 2020; Šrank and Šimanský, 2020). Biochar is one of several products of organic matter pyrolysis (IBI, 2013) and, in addition to stabilizing C, it has the potential to improve soil properties. Biochar improves the structure of the soil and boosts its capacity to hold water and nutrients, which fosters plant growth (Aydin et al., 2020; Balashov et al., 2022; Kotuš et al., 2022). Through photosynthesis, this additional plant biomass adds to the carbon sequestration process.

It is known that after the application of biochar in the soil, various situations arise in connection with the production of CO_2 – priming effects (Yin et al., 2022). This process is influenced by several factors, such as the properties of biochar, and different soil and climate conditions (Ganesan et al., 2024). The cases of monitoring

---

***Corresponding Author:** Vladimír Šimanský, Slovak University of Agriculture, Faculty of Agrobiology and Food Resources, Institute of Agronomic Sciences, 949 76 Nitra, Slovakia
vladimir.simansky@uniag.sk https://orcid.org/ 0000-0003-3271-6858

changes in soil properties after the application of biochar have grown significantly in recent times (Lehmann and Joseph, 2015). In Slovakia, this topic is relatively new and, according to our knowledge, so far, the only continuously running experiment is localized at the research base of SUA in Nitra. So far, the results published from this experiment point to biochar potential to be an effective tool for reduction greenhouse gases, including CO_2, into the atmosphere in soil and climate conditions of Slovakia (Kotuš et al., 2022). As part of this study, we are trying to investigate the effect of biochar applied at different rates on the sequestration of C in the soil, but also in WSA, because WSA affects of physical stabilization of organic C in the soil (Šimanský et al., 2024). In addition, from the theoretical and practical viewpoints, several important problems related to soil carbon sequestration can be formulated (Semenov et al., 2008). However, through this study we primarily wanted to provide an answer to the question: What is the contribution of biochar to C sequestration into WSA from content of C in soil bulk?

2 Material and methods

2.1 Study area

The experimental field (latitude 48° 19′ 23.41″; longitude 18° 09′ 0.7″) is located on the Žitavská Upland east of Nitra city, Slovakia. The region has a warm lowland climate, warm summers, and brief, dry winters, with an average annual air temperature of 10.7 °C and mean annual precipitation of 559 mm. The soil (Haplic Luvisol) mainly developed from an Young Neogene deposits includes diverse clays, loams, and sand gravels overlaid with loess in the Pleistocene epoch. In A-horizon, the soil before the experiment establishment contained: clay 249 g.kg^{-1}, silt 599 g.kg^{-1}, sand 152 g.kg^{-1}, soil organic carbon 9.13 g.kg^{-1}, CEC 142 mmol$_{(+)}$ kg^{-1}, base saturation 85%, and soil pH$_{KCl}$ was 5.7.

2.2 Experimental design

The study employed a randomized block design (Figure 1). The experimental design involved the application of two different amounts of biochar to the plots: no biochar (B0: 0 t.ha^{-1}); low biochar (B10: 10 t.ha^{-1}), and high biochar (B20: 20 t.ha^{-1}). Each treatment had three replicates. The field experiment followed an annual crop rotation sequence: spring barley (*Hordeum vulgare* L.), maize (*Zea mays* L.), spring wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), spring barley (*Hordeum vulgare* L.), maize (*Zea mays* L.), pea (Pisum sativum L.), winter wheat (*Triticum aestivum* L.), and maize (*Zea mays* L.) in 2014, 2015, 2016, 2017, 2018, 2019, 2020, 2021 and 2022, respectively. The application of biochar as an organic amendment occurred in 2014. Biochar was applied manually to the soil surface using rakes in all relevant plots and then incorporated into the 0–10 cm soil layer.

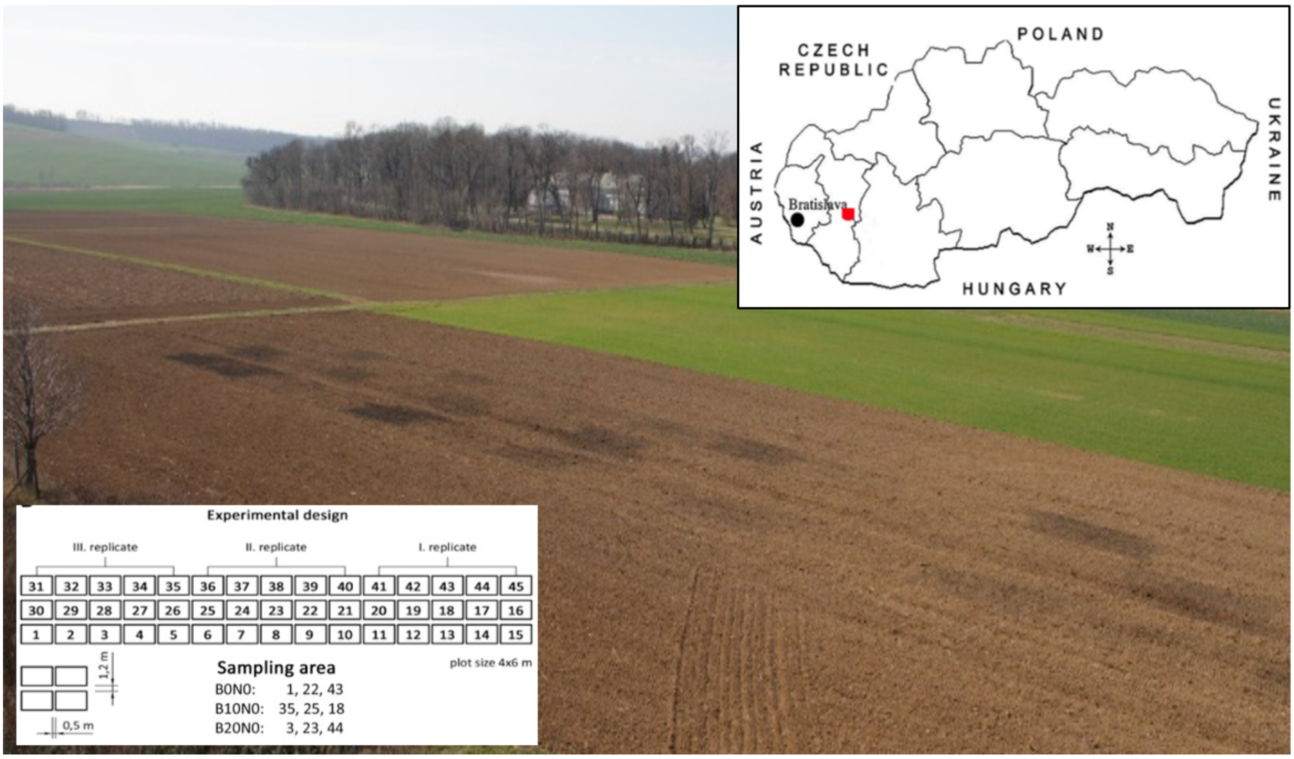

Figure 1 Location of the study area and treatments (B0: no biochar, B10: 10 t.ha^{-1}, and B20: 20 t.ha^{-1})

Subsequent applications of biochar were not carried out. The remaining practices conformed with the respective established site management.

2.3 *Biochar properties*

The biochar utilized in the experiment was derived from a mixture of paper fibre sludge and grain husks (1 : 1 w/w). The mix was pyrolyzed at a temperature of 550 °C for 30 min in a Pyreg reactor (Pyreg GmbH, Dörth, Germany), and the resulting product had the following basic properties: total organic C content – 531 g.kg^{-1}, total N content – 14 g.kg^{-1}, total Ca content – 57 g.kg^{-1}, total Mg content – 3.9 g.kg^{-1}, total K content – 15 g.kg^{-1}, total Na content – 0.77 g.kg^{-1}. It had a specific surface area of 21.7 m^2.g^{-1}, an ash content of 38.3%, pH of 8.8, and particle size ranging from 1 to 5 mm.

2.4 *Soil sampling and analysis*

Sampling occurred in spring 2022. Soil samples were collected from A-horizon of Haplic Luvisol across all treatments. In each individual treatment repetition (n = 3), three random sub-areas were chosen for collection of soil samples from each plot. Soil samples for determining the distribution water-stable (WSA) aggregates size-fractions were taken with a spade to preserve the natural lines of soil aggregates. Soil samples for the determination of soil organic carbon content (SOC) were taken from the same areas. A set of 3 samples from each replicate was mixed into the average soil sample. The determination of individual fractions of water-stable aggregates was done by wet sieving Baksheev method (Hrivňáková et al., 2011). The determined size-fractions of water-stable aggregates (WSAs) were as follows: >5, 3–0.5, 0.5–0.25 mm (macro-aggregates), and <0.25 mm (micro-aggregates). The soil samples for determination of SOC were air-dried for a few days at lab temperature. Visible stones and plant roots were hand-removed, and all samples were sieved through a 0.25 mm. The content of SOC in each set of WSA size-fractions as well as in soil bulk was measured via the potassium dichromate-sulfuric acid dilution heat method (Hrivňáková et al., 2011).

2.5 *Statistical analysis*

Effects of biochar treatments on content of WSA, SOC in WSA and soil bulk were compared through one-way ANOVA analysis of variance in the software package Statgraphics Centurion XV.I (Statpoint Technologies, Inc., USA) with means being separated using the Tukey's test. P <0.05 was the threshold for significance. The quantitative (linear, polynomial, exponential, logarithmic, and power) relationships between percentage share of SOC in the size-fraction of WSA from SOC in the soil bulk and WSA content were identified. Then the equation and correlation coefficient (R^2) from the regression trend line were obtained.

3 Results and discussion

3.1 *Effect of biochar rates on aggregation and soil organic carbon*

Soil management alters aggregation (Bronick and Lal, 2005) through practices such as biochar application, but with varying effects as reported by several authors (Šimanský et al., 2019; Yang et al., 2024). In this study, there was no significant difference in WSAma content as a result of biochar application; however, WSAma >3 mm and 3–0.5

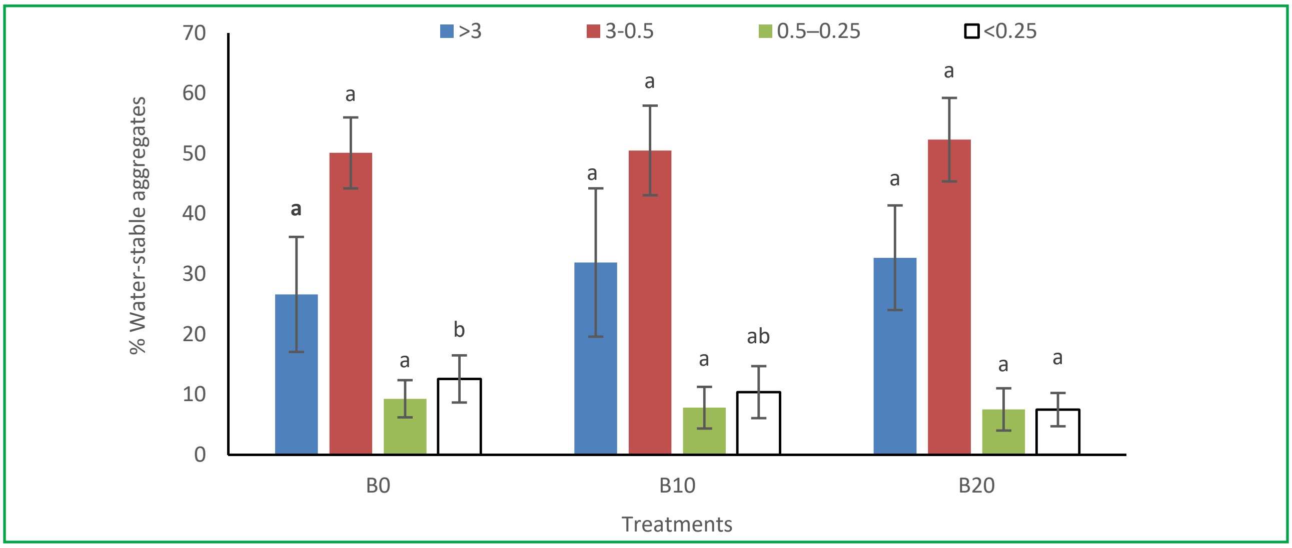

Figure 2 Content of size-fractions of water-stable aggregates (average and ±standard deviation). Different letters between columns at the same color indicate that treatment means are significantly different at p <0.05 according to the Tukey test

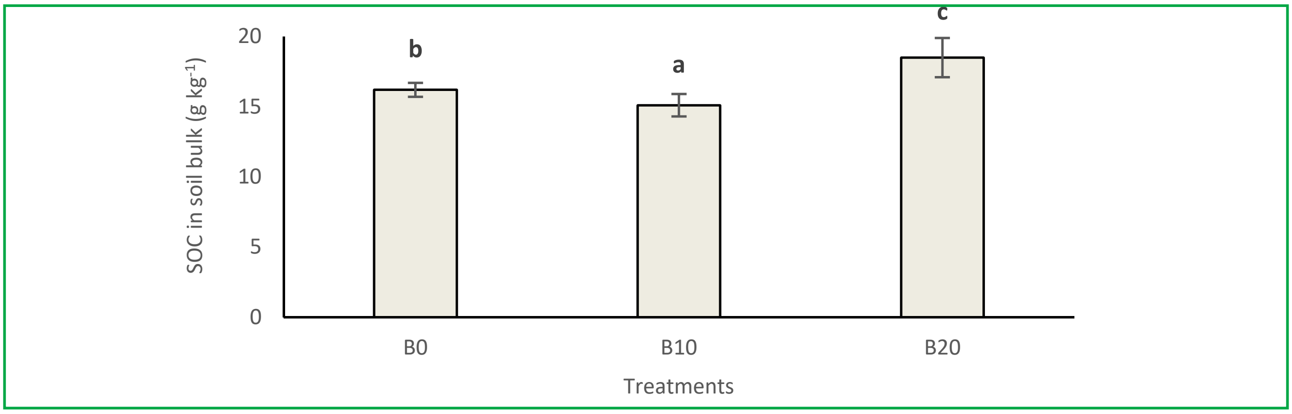

Figure 3 Soil organic carbon in soil bulk (average and ±standard deviation). Different letters between columns at the same color indicate that treatment means are significantly different at *p* <0.05 according to the Tukey test

mm contents were higher (not significantly) in the B10 and B20 treatments compared to the B0 treatment (Figure 2). The effect of different rates of biochar was statistically significant primarily in the changes in WSAmi content. In the B10 and B20 treatments, the content of WSAmi was lower by 17% and 40%, respectively compared to the control (B0). These findings suggest that a higher dose of biochar significantly reduces the content of WSAmi and promotes aggregation, particularly of larger WSAma. This means that the rate of biochar application is a key factor influencing soil aggregates. Improvements in soil aggregation can be attributed to microbial activity resulting from the formation of macro- and micro-aggregates due to the production of mucilage and hyphae at the interface between biochar and soil particles (Jien and Wang 2013; Juriga and Šimanský, 2018).

The results of this study suggest that soil aggregation and SOC sequestration both increased with the application of higher rates of biochar. In all treatments, higher SOC content was observed in soil bulk compared to WSA (Figures 3 and 4). When comparing the control (B0) to the application of a lower rate of biochar (B10), there was a significant decrease of 1.1 g.kg^{-1} in SOC content in soil bulk, while an application of 20 t of biochar ha^{-1} significantly increased the SOC by 2.3 g.kg^{-1}. These results can be attributed to priming effects following the application of biochar, as described by Yin et al., 2022. The content of SOC in individual WSA size-fractions ranged from 68 to 99% of SOC in the soil bulk. Its share depended on the size-fraction of WSA, and the different rates of biochar applied to the soil. SOC content in WSAma size-fractions 0.25–0.5 mm, 0.5–3 mm, >3 mm, and WSAmi, represented 7–16, 7–14, 5–9 and 3–10% of the SOC content in the soil bulk, respectively, in B10 compared to B0 treatment. The opposite situation was in the B20 treatments compared to the control. There, the percentage content of SOC in WSA from the content of SOC in the soil bulk was considerably smaller and varied in a range from -4 to 1%, -7 to 4%, -9 to 2% and -2 to -1% in WSAmi, in WSAma 0.25–0.5 mm, WSAma 0.5–3 mm and in WSA > 3 mm, respectively. This means that SOC was more efficiently incorporated into WSA in the case of B10 than in B20. However, in all treatments, SOC content was higher in WSAma than WSAmi, and the biochar application rate played a significant role in its increase (Figure 4). The higher SOC content in WSA resulted from higher rate of biochar applied to the soil. In the B20 treatment, SOC content in WSAma >3 mm, 3–0.5 mm, 0.5–0.25 mm and WSAmi was greater by 13, 12, 14 and 13%, respectively, compared to B0 (Table 1). As reported Dungait et al. (2012) a higher SOC contents in WSAma resulted from biochar supplement and reduced rate of SOC decomposition in WSAmi by spatial inaccessibility.

Table 1 Contents of SOC in individual size-fractions of WSA

Treatments	Individual size-fractions of WSA (mm)			
	> 3	3-0.5	0.5–0.25	<0.25
B0	13.8 ±0.37^{a}	13.7 ±0.35^{a}	13.2 ±0.46^{a}	11.8 ±044^{a}
B10	13.9 ±0.65^{a}	13.3 ±0.32^{a}	13.5 ±0.69^{a}	12.0 ±0.078^{a}
B20	15.7 ±0.57^{b}	15.4 ±0.74^{b}	15.1 ±0.96^{b}	13.3 ±0.65^{b}

different letters between lines indicate that treatment means are significantly different at *p* <0.05 according to the Tukey test

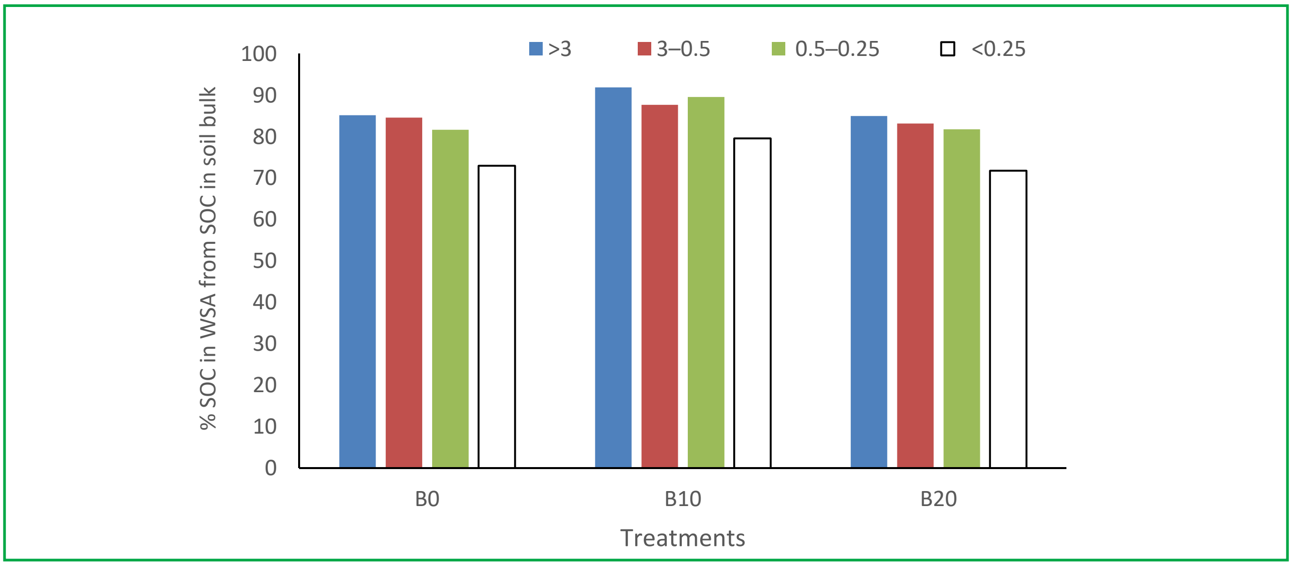

Figure 4 Percentage share of SOC in the size-fractions of WSA from SOC in the soil bulk (average)

Table 2 The quantitative relationships between individual size fractions of WSA and the percentage share of SOC in the size fractions of WSA from SOC in soil bulk

	>3 mm	R^2	3–0.5 mm	R^2	0.5–0.25 mm	R^2	<0.25 mm	R^2
Linear								
B0	$y = -0.05x + 86.7$	0.052	$y = 0.01x + 84.0$	0.001	$y = 0.05x + 81.3$	0.003	$y = -0.39x + 77.9$	0.319
B10	$y = -0.02x + 92.7$	0.005	$y = 0.10x + 82.5$	0.126	$y = 0.96x + 82.1$	**0.530**	$y = -0.66x + 86.4$	0.308
B20	$y = -0.07x + 87.3$	0.037	$y = 0.14x + 76.1$	0.056	$y = 0.36x + 79.1$	0.060	$y = -0.92x + 78.7$	**0.533**
Exponential								
B0	$y = 86.54e^{-6E-04x}$	0.052	$y = 83.89e^{0.0002x}$	0.001	$y = 81.29e^{0.0005x}$	0.003	$y = 78.11e^{-0.005x}$	0.313
B10	$y = 92.47e^{-2E-04x}$	0.005	$y = 82.72e^{0.0012x}$	0.126	$y = 82.48e^{0.0105x}$	**0.541**	$y = 86.83e^{-0.009x}$	0.303
B20	$y = 87.29e^{-8E-04x}$	0.037	$y = 76.15e^{0.0017x}$	0.054	$y = 78.68e^{0.0049x}$	0.058	$y = 79.06e^{-0.013x}$	**0.529**
Logarithmic								
B0	$y = -1.88\ln(x) + 91.3$	0.071	$y = 1.27\ln(x) + 79.6$	0.005	$y = 0.10\ln(x) + 81.5$	0.000	$y = -3.32\ln(x) + 81.2$	0.203
B10	$y = -1.70\ln(x) + 97.7$	0.018	$y = 4.99\ln(x) + 68.2$	0.122	$y = 5.12\ln(x) + 79.7$	0.387	$y = -4.66\ln(x) + 89.9$	0.241
B20	$y = -1.05\ln(x) + 88.6$	0.010	$y = 8.45\ln(x) + 49.9$	0.082	$y = 3.63\ln(x) + 74.9$	0.136	$y = -6.72\ln(x) + 84.9$	**0.480**
Polynomial								
B0	$y = 0.01x^2 – 0.78x + 96.8$	0.150	$y = -0.05x^2 + 5.05x - 34.1$	**0.545**	$y = 0.08x^2 – 1.62x + 88.7$	0.079	$y = -0.09x^2 + 1.69x + 66.8$	**0.597**
B10	$y = 0.02x^2 – 1.30x + 114.2$	0.195	$y = -0.00x^2 + 0.16x + 81.1$	0.127	$y = 0.27x^2 – 2.87x + 93.2$	**0.822**	$y = -0.10x^2 + 1.18x + 79.5$	**0.414**
B20	$y = -0.04x^2 + 2.46x + 51.0$	**0.667**	$y = -0.07x^2 + 6.95x - 97.2$	**0.665**	$y = -0.48x^2 + 7.58x + 57.2$	**0.536**	$y = -0.07x^2 + 0.30x + 74.1$	**0.552**
Power								
B0	$y = 91.2x^{-0.021}$	0.071	$y = 79.4x^{0.0161}$	0.005	$y = 81.6x^{0.0005}$	0.000	$y = 81.8x^{-0.046}$	0.197
B10	$y = 97.4x^{-0.017}$	0.019	$y = 70.4x^{0.0563}$	0.123	$y = 80.3x^{0.056}$	0.395	$y = 90.9x^{-0.061}$	0.237
B20	$y = 88.8x^{-0.013}$	0.010	$y = 55.2x^{0.1037}$	0.079	$y = 74.6x^{0.0478}$	0.131	$y = 86.4x^{-0.095}$	**0.473**

x – mass proportion of size-fractions of WSA, y – percentage of SOC in size-fractions of WSA from SOC bulk

3.2 *Relationships between soil aggregation and SOC sequestration*

C sequestration is dependent on several factors such as soil texture, carbonate content, biological activity, soil management practices (Šimanský, 2015; Yang et al., 2021; Batool et al., 2024), etc. Soil aggregation is one of the crucial physical mechanisms of SOC stabilization in soil (Bronick and Lal, 2005), which is also confirmed by the findings in this study. The relationships between individual size-fractions of WSA and the percentage share of SOC in the size-fractions of WSA from SOC in soil bulk were expressed through a second-order polynomial relationship, which was found to be the most reasonable compared to linear, exponential, logarithmic, and power relationships, as shown in Table 2.

Positive or negative correlations between WSA and the percentage share of SOC in the size-fractions of WSA from SOC in the soil bulk depended on treatments. Positive correlations were found between WSAma 3–0.5 mm and 0.5–0.25 mm and SOC content in WSAma 3–0.5 mm and 0.5–0.25 mm, whereas negative correlations were observed between WSAma >3 mm and WSAmi and SOC in WSAma >3 mm and <0.25 mm in B0 and B10 treatments. Therefore, the positive second-order polynomial indicated a potential for SOC sequestration in WSAma 0.25–0.5 mm only in the B10 treatment. No significant potential for SOC sequestration into WSA was found in the other size-fractions of WSA in the B10 treatment, as well as in all WSAs in B0 and B20 treatments. These findings suggest different effectiveness of biochar application rates on SOC sequestration into WSA.

4 Conclusions

In general soil aggregation and SOC sequestration in soil bulk and individual size-fractions of WSA depended on rates of biochar. SOC in the soil bulk significantly increased after the application of biochar at the higher application dose. SOC in aggregates represented a range of 66–99% of its content in the soil bulk. Overall, more SOC occurred in WSAma >3 mm than in other WSA size-fractions in all treatments. A dose of 10 t of biochar ha^{-1} had a greater effect on SOC addition in aggregates than its content in soil bulk, but it had no effect on soil aggregation. In general, the formation of aggregates was more pronounced at a higher application rate of biochar (20 t.ha^{-1}).

Acknowledgments

This study was supported by the Grant Agency of the Slovak University of Agriculture in Nitra project, Faculty of Agrobiology and Food Resources, no. GA FAFR 7/2023 Biochar as an improver of humic substances in agricultural soil. Further, this publication is the result of the implementation of the projects by the Slovak Research and Development Agency under the contract No. APVV-21-0089.

References

Aydin, E. et al. (2020). Potential of biochar to alternate soil properties and crop yields 3 and 4 years after the application. *Agronomy*, 10, 889. https://doi.org/10.3390/agronomy10060889

Balashov, E. et al. (2022). Content of adsorbed film water and density of oxygen-containing functional groups on surface of ageing biochar in sandy spodosol. *Acta Horticulturae et Regiotecturae*, 25(2), 115–120. https://doi.org/10.2478/ahr-2022-0015

Batool, M. et al. (2024). Soil inorganic carbon formation and the sequestration of secondary carbonates in global carbon pools: A review. *Soil System*, 8, 15. https://doi.org/10.3390/soilsystems8010015

Bronick, C.J. & Lal, R. (2005). Soil structure and management: a review. *Geoderma*, 124, 3–22. https://doi.org/10.1016/j.geoderma.2004.03.005

Dungait, J.A.J. et al. (2012). Soil organic matter turnover is governed by accessibility not recalcitrance. *Global Change Biology*, 18, 1781–1796. https://doi.org/10.1111/j.1365-2486.2012.02665.x

Ganesan, S.P. (2024). Exploring implication of variation in biochar production on geotechnical properties of soil. *Biomass Conversion and Biorefinery*, 14, 5791–5801. https://doi.org/10.1007/s13399-020-00847-2

Hrivňaková, K. et al. (2011). *The uniform methods of soil analysis*. Bratislava: VÚPOP. In Slovak.

Horák, J., & Šimanský, V. (2017). Effect of biochar on soil CO_2 production. *Acta Fytotechnica et Zootechnica*, 20(4), 72–77. http://dx.doi.org/10.15414/afz.2017.20.04.72–77

IBI (2013). Standarized product definition and product testing guidelines for biochar that i sused in soil, IBI-STD-0.1-1, International Biochar Initiative.

Jien, S.H., & Wang, CH.S. (2013). Effects of biochar on soil properties and erosial potencial in a higly weathered soil. *Catena*, 110, 225–233. https://doi.org/10.1016/j.catena.2013.06.021

Juriga, M. & Šimanský, V. (2018). Effect of biochar on soil structure – review. *Acta Fytotechnica et Zootechnica*, 21(1), 11–19. https://doi.org/10.15414/afz.2018.21.01.11-19

Kotuš, T. et al. (2022). Effect of biochar amendment and nitrogen fertilization on soil CO_2 emission during spring period. *Acta Horticulturae et Regiotecturae*, 25(2), 121–128. https://doi.org/10.2478/ahr-2022-0016

Kumar, S.S. et al. (2020). Mitigation of climate change through approached agriculture-soil carbon sequestration (a review). *Current Journal of Applied Science and Technology*, 39(33), 47–64. https://doi.org/ 10.9734/cjast/2020/v39i3331017

Lehmann, J. & Joseph, S. (2015). Biochar for environmental management (2nd ed.). London, New York: Routledge, Taylor and Francis Group.

Semenov, V. M. (2008). Mineralization of organic matter and the carbon sequestration capacity of zonal soils. *Eurasian Soil Science*, 41, 717–730. https://doi.org/10.1134/S1064229308070065

Shivangi, et al. (2024). Carbon sequestration through organic amendments, clay mineralogy and agronomic practices: A review. *Egyptian Journal of Soil Science*, 64(2), 581–598. https://doi.org/10.21608/EJSS.2024.260719.1707

Šimanský, V. (2015). Fertilization and carbon sequestration. *Acta Fytotechnica et Zootechnica*, 18(3), 56–62.

Šimanský, V. et al. (2019). Differences in soil properties and crop yields after application of biochar blended with farmyard manure in sandy and loamy soils. *Acta Fytotechnica et Zootechnica*, 22(1), 21–25.
https://doi.org/10.15414/afz.2019.22.01.21-25

Šimanský, V. et al. (2024). A field investigation on the soil management practices in a productive vineyard considering C sequestration and water resistance of soil structure. *Biologia*, https://doi.org/10.1007/s11756-024-01676-8

Šrank, D., & Šimanský, V. (2020). Differences in soil organic matter and humus of sandy soil after application of biochar substrates and combination of biochar substrates with mineral fertilizers. *Acta Fytotechnica et Zootechnica*, 23(3), 117–124. https://doi.org/10.15414/afz.2020.23.03.117-124

Yang, P. et al. (2021). Factors affecting soil organic carbon content between natural and reclaimed sites in Rudong coast, Jiangsu province, China. *Journal of Marine Science and Engineering*, 9(12),1453. https://doi.org/10.3390/jmse9121453

Yang, W.J. et al. (2024). Biochar application influences the stability of soil aggregates and wheat yields. *Plant Soil and Environment*, 70, 125–141.
https://doi.org/10.17221/199/2023-PSE

Yin, J. et al. (2022). Evaluation of long-term carbon sequestration of biochar in soil with biogeochemical field model. *Science Total Environment*, 822, 153576.
https://doi.org/10.1016/j.scitotenv.2022.153576