J. Hydrol. Hydromech., 66, 2018, 4, 429–436
DOI: 10.2478/johh-2018-0033

Response of soil organic carbon and water-stable aggregates to different biochar treatments including nitrogen fertilization

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Abstract: Recent studies show that biochar improves physical properties of soils and contributes to the carbon sequestration. In contrast to most other studies on biochar, the present study comprise a long-term field experiment with a special focus on the simultaneous impact of N-fertilizer to soil structure parameters and content of soil organic carbon (SOC) since SOC has been linked to improved aggregate stability. However, the question remains: how does the content of water-stable aggregates change with the content of organic matter? In this paper we investigate the effects of biochar alone and in a combination with N-fertilizer (i) on the content of water-stable macro- (WSA_{ma}) and micro-aggregates (WSA_{mi}) as well as soil structure parameters; and (ii) on the contents of SOC and labile carbon (C_L) in water-stable aggregates (WSA).

A field experiment was conducted with different biochar application rates: B0 control (0 t ha^{-1}), B10 (10 t ha^{-1}) and B20 (20 t ha^{-1}) and 0 (no N), 1st and 2nd level of nitrogen fertilization. The doses of level 1 were calculated on required average crop production using the balance method. The level 2 included an application of additional 100% of N in 2014 and additional 50% of N in the years 2015–2016 on silty loam Haplic Luvisol at the study site located at Dolná Malanta (Slovakia). The effects were investigated after the growing season of spring barley, maize and spring wheat in 2014, 2015 and 2016, respectively.

The results indicate that the B10N0 treatment significantly decreased the structure vulnerability by 25% compared to B0N0. Overall, the lower level of N combined with lower doses of biochar and the higher level of N showed positive effects on the average contents of higher classes of WSA_{ma} and other soil structure parameters. The content of SOC in WSA in all size classes and the content of C_L in WSA_{ma} 3–1 mm significantly increased after applying 20 t ha^{-1} of biochar compared to B0N0. In the case of the B20N1 treatment, the content of SOC in WSA_{ma} within the size classes >5 mm (8%), 5–3 mm (19%), 3–2 mm (12%), 2–1 mm (16%), 1–0.5 mm (14%), 0.5–0.25 mm (9%) and WSA_{mi} (12%) was higher than in B0N1. We also observed a considerably higher content of SOC in WSA_{ma} 5–0.5 mm and WSA_{mi} with the B10N1 treatment as compared to B0N1. Doses of 20 t biochar ha^{-1} combined with second level of N fertilization had significant effect on the increase of WSA_{ma} and WSA_{mi} compared to the B0N2 treatment. A significant increase of C_L in WSA was determined for size classes of 2–0.25 mm and WSA_{mi} in the B20N2 treatment. Our findings showed that biochar might have beneficial effects on soil structure parameters, SOC, C_L in WSA and carbon sequestration, depending on the applied amounts of biochar and nitrogen.

Keywords: Soil structure; Soil organic carbon; Labile carbon; Aggregate stability; Biochar; N fertilizer.

INTRODUCTION

Refining our understanding of how soil structure develops is important because soil structure has a major influence on plant growth (Millar et al., 1962). SOC is one of the most important factors affecting soil structure (Kodešová et al., 2015; Saha et al., 2011; Šimanský and Jonczak, 2016), since SOC acts as a significant binding agent for soil particles (Bronic and Lal, 2005). Organic manure is one of the most important secondary sources of SOC in arable soils. A decline in livestock population in Slovakia over the last years has been reported, which leads to a reduced organic fertilizer production. At present, a deficit of organic matter on agricultural soils between 30 up to 50% is observed. The annual production of organic fertilizers is now around 10 mil. t y^{-1}, which is less than 5 t ha^{-1} of agricultural soil (Green Report, 2014). With respect to sustainable land management, it is essential to look for new resources in order to achieve a sufficient balance of organic substances. The application of biochar could serve as a plausible and innovative solution to this problem. Biochar is a stable source of organic carbon (Fischer and Glaser, 2012). Recent results showed that biochar incorporated to soils increases organic carbon content what contributed to its sequestration (Agegnehu et al., 2016; Cross et al., 2016). Over the last decade, biochar research focused on the agricultural sector also due to its positive effects on improvement of soil physical properties such as retention water capacity, total porosity, soil structure and soil water content (Atkinson et al., 2010; Barrow, 2012; Jones et al., 2010; Obia et al., 2016; Vitkova et al., 2017). Biochar particles associate with soil particles resulting in stable soil aggregates with favourable structure (Brodowski et al., 2006; Jien and Wang, 2013). Potential benefits of biochar applications needs to be carefully discussed with farmers, particularly when compared in relation to soil organic matter. Application of farmyard manure, compost or crop residues are classical methods used to improve soil fertility. Although biochar addition might be accompanied with an elevated content of organic matter, improved structure of soil and soil fertility, the residual effects might be very different (Cross et al., 2016).

The application of biochar to the soil could increase the immobilization of macro- (especially nitrogen) and micronutrients and decrease their uptake by plants (Šimanský et al., 2018). The effects of biochar on soil properties largely depends on biochars' properties, which vary widely between different biochars, mainly due to variations in feedstock materials (Butnan et al., 2015) and also pyrolysis conditions (Wang et al., 2013). The different effects might be a result of differences in the biochar reactivity (i.e., amount of reactive functional groups) that strongly depends on production conditions and feedstock (Keluweit et al., 2010). Combining biochar with a nitrogen fertilizer appears to be a promising practice for sustainable agriculture. Since the interactions between biochar, mineral fertilizer and soil are complex processes, additional research is needed.

In this context, we hypothesised that: (i) a higher application rate of biochar combined with N-fertilizer will improve soil structure since nitrogen may intensify the mineralization processes of biochar and enhance the association of biochar particles with soil particles, (ii) higher doses of biochar are responsible for more water-stable macro-aggregates compared to micro-aggregates and for a higher content of SOC inside of individual size classes of the water-stable aggregates. Since labile carbon is a very sensitive soil parameter (Szombathová, 1999), we suppose that (iii) adding biochar combined with N fertilizer will lead to intensive changes in labile carbon content.

The objective of this study were to quantify the effects of biochar and biochar in combination with N fertilizer (i) on the content of water-stable aggregates and soil structure parameters, and (ii) on the content of soil organic and labile carbon in water-stable aggregates.

MATERIAL AND METHODS

Description of study site

The field experiment was conducted at the experimental site of the Slovak University of Agriculture in Nitra, Dolná Malanta (48^{o}19′00″ N; 18^{o}09′00″ E). The site has a temperate climate, with a mean annual air temperature of 9.8°C, and with mean maximum and minimum temperatures of 17°C (July) and –3°C (January). The mean annual precipitation at this site is 540 mm (150–200 mm between June and August). The parent material consists of little previous rocks with high quantities of fine particles. Young Neogene deposits consist of various clays, loams, sand gravels on which loess were deposited in the Pleistocene Epoch. The soil is classified as Haplic Luvisol according to the Soil Taxonomy (IUSS WRB, 2014) with 9.13 g kg^{-1} of soil organic carbon, 5.71 pH and a silty loam texture (content of sand 15.2%, silt 59.9% and clay 24.9%).

Experimental design and field management

Prior to the experiment, the soil at the experimental site were cultivated for over 100 years using conventional agriculture techniques. Our experiment started in March 2014. The experimental field is shown in Fig. 1a. Fig. 1b shows a schematic layout of the experimental design. The investigated treatments are presented in Table 1. The study was carried out on 27 plots. Each plot had an area of 24 m^2 (4 m x 6 m). Three groups consisting of nine plots was arranged in a row and treated as replications. The spacing between the neighbouring replications was 0.5 m. The field was ploughed, harrowed and biochar was evenly applied to the soil surface and immediately incorporated into the 0–10 cm soil layer combined with or without N fertilizer.

Table 1. The investigated treatments.

Treatment	Description
B0N0	no biochar, no N fertilization
B10N0	biochar at rate of 10 t ha^{-1}
B20N0	biochar at rate of 20 t ha^{-1}
B0N1	no biochar combined with first level of N fertilization: dose of N were 40, 160 and 100 kg N ha^{-1} in 2014, 2015 and 2016, respectively.
B10N1	biochar at rate of 10 t ha^{-1} with N: dose of N were 40, 160 and 100 kg N ha^{-1} in 2014, 2015 and 2016, respectively.
B20N1	biochar at rate of 20 t ha^{-1} with N: dose of N were 40, 160 and 100 kg N ha^{-1} in 2014, 2015 and 2016, respectively.
B0N2	no biochar combined with second level of N fertilization: dose of N were 80, 240 and 150 kg N ha^{-1} in 2014, 2015 and 2016, respectively.
B10N2	biochar at rate of 10 t ha^{-1} with N: dose of N were 80, 240 and 150 kg N ha^{-1} in 2014, 2015 and 2016, respectively.
B20N2	biochar at rate of 20 t ha^{-1} with N: dose of N were 80, 240 and 150 kg N ha^{-1} in 2014, 2015 and 2016, respectively.

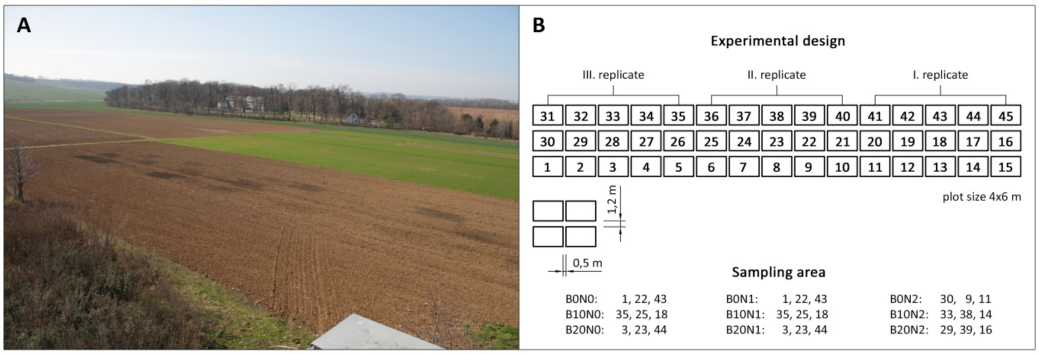

Fig. 1. Schematic layout of the experimental field.

A standard N fertilizer (Calc-Ammonium nitrate with dolomite, LAD 27) was used in this experiment. The biochar used in this study was provided from commercial producer (Sonnenerde, Austria). The biochar was produced from paper fiber sludge and grain husks (1:1 w/w). As declared by the manufacturer, the biochar was produced at a pyrolysis temperature of 550°C applied for 30 minutes in a Pyreg reactor. The pyrolysis product (biochar) has particle sizes between 1 to 5 mm. On average, the product contains 57 g kg^{-1} of Ca, 3.9 g kg^{-1} of Mg, 15 g kg^{-1} of K and 0.77 g kg^{-1} of Na. The total C content of the biochar is 53.1%, while the total N content is 1.4%, with the C:N ratio of 37.9. The specific surface area (SSA) is 21.7 m^2 g^{-1} and the content of ash is 38.3%. On average, the pH of the biochar is 8.8. The field experiment had the following annual crop rotation starting with spring barley (*Hordeum vulgare* L.) in 2014, followed by maize (*Zea mays* L.) in 2015 and spring wheat (*Triticum aestivum* L.) in 2016.

Sampling and measurements

Soil samples (depths 0–20 cm) were collected from all treatments. Sampling of soil was conducted monthly to cover the whole growing season of spring barley (from 17 April to 13 July in 2014), maize (from 15 April to 28 September in 2015) with sampling extended to 2016 to cover the whole spring growing season of wheat (sampling dates: on 20 April, 17 May, 22 June, and 18 July). The sampling campaign was conducted one, two, three and four months after biochar application in 2014. In 2015, sampling was conducted 13, 14, 15, 16, 17 and 18 months after biochar application. In 2016, sampling was conducted 26, 27, 28 and 29 months after the biochar was applied.

The soil samples were carefully taken using a spade to avoid disruption of the soil aggregates. The samples were mixed to produce an average representative sample from each plot. Roots and large pieces of crop residues were removed. Large clods were gently disrupted along natural fracture lines in the laboratory, and air-dried at the laboratory temperature. The dry soil samples were sieved (dry sieving) to the following seven size fractions: >7, 7–5, 5–3, 3–2, 2–1, 1–0.5, 0.5–0.25 mm. The percentage of water-stable aggregates (*WSA*) was determined by the Baksheev method (Vadjunina and Korchagina, 1986). The analysis started with a 30g sample of aggregates. The aggregates were first rinsed with distilled water. After two hours, each sample was transferred to the top sieve (>5 mm) with a cylindrical container (Baksheev device) filled with distilled water. Six sieves with mesh sizes of 5, 3, 2, 1, 0.5 and 0.25 mm were used. The cylinder was hermetically sealed and the samples were sieved for 12 minutes (soaked in water). The obtained fractions of water-stable aggregates were transferred from the individual sieves to a filter paper and dried in an oven at 45 °C. The residual material was quantified on each sieve except for micro-aggregates, i.e., water-stable aggregates < 0.25 mm (WSA_{mi}), for which the content was calculated as the difference between the total weight of the soil sample and the sum of the macro-aggregates (WSA_{ma}). The soil organic carbon (Dziadowiec and Gonet, 1999) and the labile carbon (Loginow et al., 1987) were analyzed for all fraction sizes of the WSA. Briefly, the content of soil organic carbon (SOC) in the water-stable aggregates (WSA) was determined using the wet combustion method - oxidizing organic matter in a mixture of 0.07 M H_2SO_4 and $K_2Cr_2O_7$ with titration using 0.01 M Mohr's salt. The labile carbon content (C_L) in the WSA was extracted from the samples containing 1 g of individual particle-size fractions of WSA by shaking in 50 mL of 0.005 M $KMnO_4$ for 2 hours. After centrifugation, the C_L was determined by oxidation of 0.07 M H_2SO_4 and $K_2Cr_2O_7$ with titration using 0.05 M Mohr's salt.

The dried and wet-sieved samples were used to calculate the mean dry and wet weight diameters (MWD_d and MWD_w) of water-stable aggregates, vulnerability coefficient (K_v) by Valla et al. (2000) as well as the stability index of water-stable aggregates (*Sw*) by Henin (Lal and Shukla, 2004). The above-mentioned soil structure parameters (MWD_d, MWD_w, K_v and *Sw*) were calculated according to the following equations (1–4):

$$Sw = \frac{WSA - 0.09sand}{silt + clay} \tag{1}$$

where: *Sw* is the index of aggregate stability and *WSA* is the content of water-stable aggregates (%).

$$MWD_d = \sum_{i=1}^{n} x_i w_i \tag{2}$$

where: MWD_d is the mean weight diameter of aggregates for dry sieving (mm), x_i is the mean diameter of each size fraction (mm) and w_i is the portion of the total sample weight within the corresponding size fraction, and *n* is the number of size fractions.

$$MWD_w = \sum_{i=1}^{n} x_i WSA \tag{3}$$

where: MWD_w is mean weight diameter of water stable aggregates (mm), x_i is mean diameter of each size fraction (mm), and *WSA* is portion of the total sample weight within the corresponding size fraction, and *n* is the number of size fractions.

$$K_v = \frac{MWD_d}{MWD_w} \tag{4}$$

where: K_v is the vulnerability coefficient, MWD_d is the mean weight diameter of aggregates for dry sieving (mm), and MWD_w is the mean weight diameter of water stable aggregates (mm).

The Philips scanning electron microscope (SEM) with energy dispersive X-ray microanalysis (EDX) was used to characterize the biochar in the analyzed soil aggregates. The measurements were performed at VŠB – Technical University of Ostrava (Nanotechnology Centre), Czech Republic. SEM images were recorded using secondary electrons in the mode of back scattering electrons at an operating voltage of 20 kV.

The mineral components in the soil aggregates were identified by X-ray powder diffraction (XRD) analysis on the diffractometer PW1710 (Philips, The Netherlands) under the following conditions: Bragg-Brentano geometry (Theta-2Theta), Cu anticathode (λa_1 = 1.54060 Å), beam current 40 mÅ, and accelerating voltage of 40 kV. The step size was 0.01°2 Θ, the settled step time 1 s per one step at measurement range from 4 to 65° 2Θ. X-ray powder diffraction analysis was conducted at the Slovak Academy of Sciences.

Statistics

The data were analyzed by ANOVA tests implemented in the software package Statgraphics Centurion XV.I (Statpoint Technologies, Inc., USA). Comparisons between samples were conducted using least significant differences (LSD) at the probability level of $P = 0.05$.

RESULTS AND DISCUSSION

Content of water-stable aggregates and soil structure parameters

The average parameters of soil structure such as: MWD_w, K_v, Sw, WSA_{ma} and WSA_{mi} as a result of biochar amendment are shown in Table 2. The results of Atkinson et al. (2010) and Barrow (2012) show that biochar has positive effects on soil structure. In our case, the effects of biochar and biochar combined with N fertilization on soil structure parameters were different. The one-way ANOVA test did not show any significant differences between the biochar treatments without N in terms of WSA_{ma}, WSA_{mi}, Sw and both values of MWD. Our results suggest that biochar without N fertilization did not enhance the soil structure parameters except the K_v values. When no nitrogen was applied, a significant effect on K_v was observed in the treatment with 10 t biochar ha^{-1}, and no significant effect was determined after application 20 t biochar ha^{-1} compared to B0N0. The lower soil structure vulnerability can be explained also by the lower dose of biochar, an effect already observed after one year after the incorporation of biochar to the soil (Šimanský et al., 2016). High levels of labile C fraction in biochar explain this effect (Cross et al., 2016). Adding less biochar with no N fertilizers may thus be more beneficial for soil aggregation that higher doses of biochar. On the other hand, biochar with both levels of N fertilization improved the soil structure (Table 2). The average contents of WSA_{ma} in the B0N1, B10N1, B20N1, B0N2, B10N2 and B20N2 treatments were 69.9, 75.0 and 77.5% or 67.3, 73.1 and 78.7%, respectively. These figures indicates a significant increase in the WSA_{ma} contents due to higher doses of biochar in combination with N fertilization. The same trends (significant increase/decrease) were observed in the case of Sw, K_v, MWD_w and contents of WSA_{mi}. Combinations 10 t biochar ha^{-1} with the first level of N fertilization (N1) and biochar at a rate of 20 t ha^{-1} with a second level of N fertilization (N2) significantly increased MWD_w compared to the B0N1 and B0N2 treatments. An apparent increase of MWD_w, as reported by Jien and Wang (2013), might indicate that a biochar might more facilitate the formation of macro-aggregates. This explains the decreasing of WSA_{mi}. This effect is enhanced with addition of N (Table 2). As reported by Ma et al. (2016), applications of biochar and N-fertilizer contributes to a significant increase of SOC, which could be related to a higher absorption of cations (Liang et al., 2006), intensive cation exchange capacity (Yuan and Xu, 2012) and finally higher soil structure stability (Obia et al., 2016). In our case, a higher dose of biochar with both N fertilization levels resulted in a better structure state of the soil. Contradictory findings have been published as to how biochar affects soil structure. It is still unclear how combining biochar with N fertilization affects soil structure, but the major responsible factors include particle-size distribution of studied soils (Liu and Zhou, 2012), application rate of biochar, time after biochar application (Ruysschaert et al., 2016) its combination with other fertilizers (Ma et al., 2016) and biochar properties (Alburquerque et al., 2014). The surface of biochar particles after oxidation may contain hydroxyl and carboxylic groups which are able to absorb soil particles and clays and form macro-aggregates (Jien and Wang, 2013), however, this process requires a substantially longer times to take place. As reported by Herath et al. (2013), the formation of soil aggregates is a function of biological activity and time, and it is unlikely to occur immediately upon biochar application. In our study, applying biochar at rates of 10 and 20t ha^{-1} with no N fertilization did not affect the average contents of individual size classes of WSA_{ma} during studied period (2014–2016). On the other hand, a combination of both rates of biochar applied with both levels of nitrogen fertilizer showed a significant effect on the individual size classes of WSA_{ma} compared to the B0N1 and B0N2 treatments (Table 3).

Table 2. Parameters of soil structure (means and standard deviation).

Treatments	WSA_{ma}	WSA_{mi}	Sw	K_v	MWD_d	MWD_W
B0N0	73.4 ± 6.89^{a}	26.6 ± 6.89^{a}	0.85 ± 1.10^{a}	4.28 ± 1.10^{b}	2.98 ± 0.18^{a}	0.73 ± 0.25^{a}
B10N0	77.9 ± 8.51^{a}	22.1 ± 8.51^{a}	0.90 ± 0.10^{a}	3.22 ± 0.56^{a}	2.83 ± 0.61^{a}	0.89 ± 0.21^{a}
B20N0	75.2 ± 8.38^{a}	24.8 ± 8.38^{a}	0.87 ± 0.10^{a}	4.12 ± 1.70^{b}	2.82 ± 0.44^{a}	0.80 ± 0.33^{a}
B0N1	69.9 ± 9.11^{a}	30.1 ± 9.11^{b}	0.81 ± 0.11^{a}	4.66 ± 1.44^{b}	2.91 ± 0.58^{a}	0.68 ± 0.23^{a}
B10N1	75.0 ± 8.81^{ab}	25.0 ± 8.81^{ab}	0.87 ± 0.10^{ab}	3.60 ± 1.45^{a}	2.98 ± 0.50^{a}	0.93 ± 0.32^{b}
B20N1	77.5 ± 8.43^{b}	22.5 ± 8.43^{a}	0.90 ± 0.10^{b}	3.45 ± 1.33^{a}	2.78 ± 0.48^{a}	0.88 ± 0.28^{ab}
B0N2	67.3 ± 7.01^{a}	32.7 ± 7.01^{c}	0.78 ± 0.08^{a}	4.25 ± 1.22^{b}	2.58 ± 0.69^{a}	0.59 ± 0.13^{a}
B10N2	73.1 ± 8.15^{b}	26.9 ± 8.15^{b}	0.85 ± 0.10^{b}	4.12 ± 1.58^{ab}	2.60 ± 0.56^{a}	0.69 ± 0.19^{a}
B20N2	78.7 ± 6.65^{c}	21.3 ± 6.65^{a}	0.91 ± 0.08^{c}	3.26 ± 0.91^{a}	2.82 ± 0.51^{a}	0.91 ± 0.23^{b}

WSA_{ma} – content of water-stable macro-aggregates, WSA_{mi} – content of water-stable micro-aggregates, Sw – stability index of water-stable aggregates, K_v – vulnerability coefficient, MWD_d – mean weight diameter of aggregates for dry sieving, MWD_W – mean weight diameter of water stable aggregates.
Different letters (a, b, c) between lines indicate that treatment means are significantly different at $P<0.05$ according to LSD multiple-range test.

Table 3. Contents of individual size fraction of water-stable macro-aggregates in % (means and standard deviation).

	Individual size fractions of water-stable macro-aggregates in mm					
Treatments	>5	5–3	3–2	2–1	1–0.5	0.5–0.25
B0N0	2.21 ± 1.34^{a}	3.90 ± 1.88^{a}	8.49 ± 3.25^{a}	15.8 ± 7.92^{a}	25.6 ± 4.22^{a}	17.4 ± 4.69^{a}
B10N0	3.26 ± 1.08^{a}	5.41 ± 2.96^{a}	10.7 ± 3.40^{a}	20.1 ± 7.91^{a}	23.1 ± 3.45^{a}	15.3 ± 4.24^{a}
B20N0	2.47 ± 1.43^{a}	4.94 ± 2.21^{a}	9.51 ± 3.67^{a}	16.3 ± 6.68^{a}	25.5 ± 5.87^{a}	16.5 ± 4.54^{a}
B0N1	2.26 ± 1.58^{a}	3.75 ± 2.91^{a}	7.30 ± 3.61^{a}	13.9 ± 4.68^{a}	24.4 ± 4.01^{a}	18.4 ± 3.90^{b}
B10N1	4.22 ± 2.26^{b}	6.60 ± 2.11^{b}	10.6 ± 4.82^{b}	15.6 ± 6.27^{a}	21.2 ± 3.46^{a}	16.8 ± 5.13^{ab}
B20N1	3.40 ± 2.74^{ab}	5.26 ± 3.12^{ab}	10.0 ± 3.80^{ab}	19.3 ± 7.54^{a}	24.5 ± 5.62^{a}	15.0 ± 3.41^{a}
B0N2	1.94 ± 1.07^{a}	3.06 ± 1.57^{a}	5.78 ± 2.14^{a}	10.5 ± 4.52^{a}	27.2 ± 4.88^{a}	18.9 ± 3.64^{b}
B10N2	2.19 ± 0.98^{a}	3.45 ± 1.70^{a}	7.65 ± 3.67^{a}	14.4 ± 6.12^{a}	26.0 ± 5.24^{a}	18.6 ± 4.22^{b}
B20N2	2.81 ± 1.75^{a}	5.86 ± 2.37^{b}	11.5 ± 3.56^{b}	20.3 ± 6.70^{b}	24.2 ± 5.85^{a}	14.8 ± 4.91^{a}

Different letters (a, b, c) between lines indicate that treatment means are significantly different at $P<0.05$ according to LSD multiple-range test.

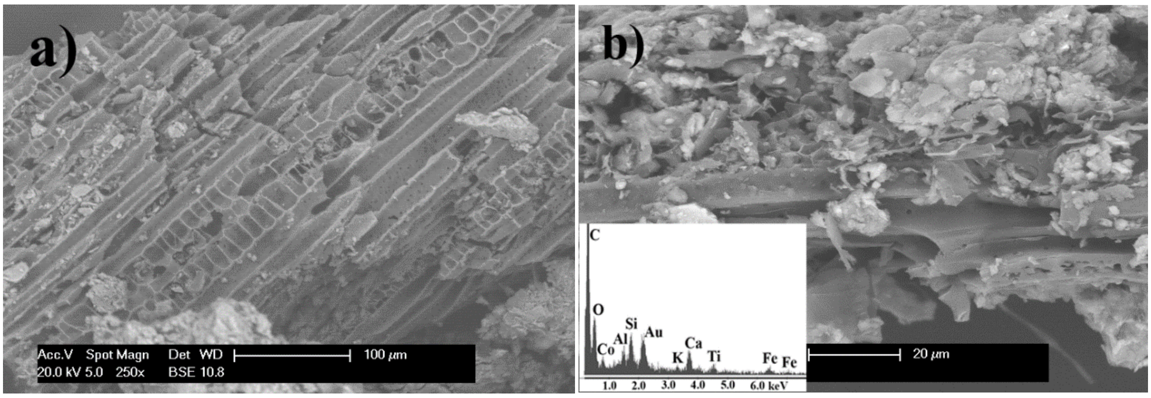

Fig. 2. a) Visualised high surface area and presence of micropores in biochar-related structure, b) Soil aggregate consists of biochar (in the center) with original surface lamellae texture and structure associated with various minerals. Incorporate minerals pose particular size, morphology, and sticking coefficients with biochar (Scanning electron microscopy); inset: chemical analysis of aggregate contained dominant element C (come from biochar), and elements O, Al, Si, Ti, Ca, K, Fe, Co which related to others soil minerals. Gold dispersed over the sample provided better conduction properties (Energy Dispersive Spectrum). This is one possible composition of elements to take the heterogeneity of soils into account.

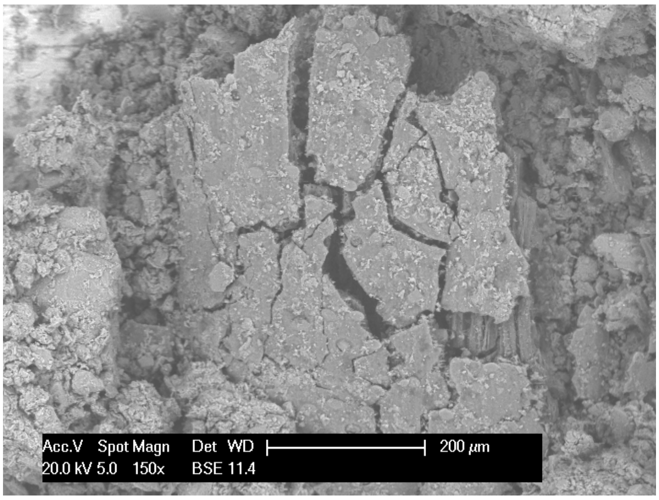

Fig. 3. Biochar incorporated into soil. Surface disruptions are visible. Large pore sizes increase the surface-to-volume ratio (Scanning Electron Microscopy).

In the case of the B10N1 treatment, the contents of *WSA_{ma}* in the size classes >5 mm (87%), 5–3 mm (76%), 3–2 mm (45%) were higher than in B0N1. We observed a considerably higher content of WSA_{ma} 2–1 mm and lower content of WSA_{ma} 0.5–0.25 mm, probably due to application of biochar at a rate of 20 t ha^{-1} together with first level of N fertilization as compared B0N1. Both doses of biochar as well as N fertilization levels did not have a noticeable effect on WSA_{ma} 1–0.5 mm as compared to the B0N1 and B0N2 treatments. The dosage of 20 t biochar ha^{-1} combined with second level of N fertilization had an significant positive effect on WSA_{ma} in size classes 5–3 mm (92%), 3–2 mm (99%) and 2–1 mm (93%) compared to the B0N2 treatment. The lower level of N combined with lower dose of biochar as well as the higher level of N with higher dose of biochar resulted in positive effects on average contents of higher classes of WSA_{ma}. Biochar as a soil amendment has a large specific surface area (Chintala et al., 2014) with the presence of micropores (Jones et al., 2010; Mukherjee et al., 2011). Fig. 2a shows the original texture and structure of the source material. Fig. 2b shows soil aggregate with biochar being in the center. This biochar is in connection with mineral particles which are present in the soil. Apparently, the surface of biochar (Fig. 3) gradually degraded, as indicated by the visually detected disruptions, large pores size distribution, and the increased biochar surface-to-volume ratio with high specific surface area. On the other hand, the portion of extractable biochar could support soil aggregate creation and physical aggregate stability as several authors reported (Chan et al., 2007; Lehmann et al., 2011; Spokas, 2010). Minerals constitute the main components in the formation of aggregates. The X-ray diffraction analysis (Fig. 4) revealed that the dominant mineral components were α-quartz (SiO_2), anorthite $Ca(Al_2Si_2O_8)$, and muscovite ($KAl_2(AlSi_3O_{10})(OH)_2$). The chemical analysis of aggregates (Fig. 2b) showed the presence of O, Al, Si, Ti, Ca, K, Fe, Co and C. The stability of aggregates can be influenced by the presence, content and the type of clay minerals (Bai et al. 2013). As presented by Lehmann (2007), adding nitrogen to soil in combination with biochar may improve microbial activity, increase the intensity of biochar mineralization processes (Cross et al., 2016) and increase the cation exchange capacity (Yeboah et al., 2009; Yuan and Xu, 2012) and active surface area (Liang et al., 2006; Mukherjee et al., 2011) with positive effects on soil aggregation (Bronick and Lal, 2005).

Contents of soil organic carbon and labile carbon in water-stable aggregates

The results indicate that the biochar became part of the soil aggregates (Fig. 2b, Fig. 3) after its incorporation to the soil, since the soil particles tend to form aggregates with occluded biochar (Brodowski et al., 2006). This explains the elevated carbon content in the aggregates (Blanco-Canqui and Lal, 2004) and the mechanism of carbon sequestration (Six et al., 2002). On the other hand, biochar is very stable compared to other organic matter amendments (Fischer and Glaser, 2012; Kuzyakov et al., 2009; Lehmann et al., 2008; Lopez-Capel et al., 2016) with insignificant increase of SOC levels even though the biochar had a high TOC content (Jien and Wang, 2013).

Our study showed that different application rates of biochar alone, and biochar combined with N fertilizer affected the distribution of SOC and C_L concentration in the analysed WSA (Table 4). The largest fractions of WSA_{ma} contained higher SOC and C_L in all treatments. The smaller fractions of WSA_{ma} and WSA_{mi} contained lower SOC and C_L. These findings are consistent with Tisdall and Oades (1980) and Six et al. (2004) who found higher concentrations of organic C in macro-

Table 4. Contents of soil organic carbon and labile carbon in the individual size fractions of water-stable aggregates (means and standard deviation).

Treatments	Individual size fractions of water-stable aggregates in mm						
	>5	5–3	3–2	2–1	1–0.5	0.5–0.25	<0.25
	Content of soil organic carbon in water-stable aggregates in %						
B0N0	1.46±0.25^{a}	1.35±0.12^{a}	1.29±0.10^{a}	1.35±0.12^{a}	1.23±0.09^{a}	1.15±0.09^{a}	1.03±0.07^{ab}
B10N0	1.41±0.24^{a}	1.28±0.17^{a}	1.26±0.15^{a}	1.30±0.22^{a}	1.21±0.15^{a}	1.15±0.13^{a}	0.97±0.15^{a}
B20N0	1.55±0.22^{a}	1.57±0.34^{b}	1.49±0.17^{b}	1.58±0.26^{b}	1.36±0.14^{b}	1.28±0.15^{b}	1.09±0.11^{b}
B0N1	1.33±0.18^{a}	1.22±0.10^{a}	1.21±0.10^{a}	1.28±0.13^{a}	1.18±0.09^{a}	1.13±0.06^{a}	0.97±0.06^{a}
B10N1	1.34±0.16^{a}	1.39±0.16^{b}	1.32±^{b}	1.44±0.17^{b}	1.28±0.09^{b}	1.20±0.11^{ab}	1.01±0.11^{b}
B20N1	1.43±0.13^{b}	1.45±0.19^{b}	1.36±0.13^{b}	1.48±0.18^{b}	1.35±0.14^{b}	1.23±0.11^{b}	1.09±0.10^{b}
B0N2	1.37±0.15^{a}	1.34±0.12^{a}	1.27±0.09^{a}	1.39±0.13^{a}	1.25±0.09^{a}	1.18±0.05^{a}	1.01±0.08^{a}
B10N2	1.52±0.24^{ab}	1.50±0.23^{b}	1.42±0.16^{b}	1.47±0.26^{a}	1.30±0.12^{ab}	1.17±0.09^{a}	1.04±0.13^{ab}
B20N2	1.57±0.26^{b}	1.44±0.18^{ab}	1.41±0.11^{b}	1.48±0.21^{a}	1.34±0.09^{b}	1.27±0.07^{b}	1.11±0.10^{b}
	Content of labile carbon in water-stable aggregates in g kg^{-1}						
B0N0	1.47±0.32^{a}	1.39±0.27^{a}	1.24±0.26^{a}	1.19±0.19^{a}	1.19±0.19^{a}	1.28±0.20^{a}	1.12±0.10^{a}
B10N0	1.65±0.30^{a}	1.40±0.14^{a}	1.29±0.15^{ab}	1.30±0.20^{a}	1.32±0.21^{a}	1.28±0.20^{a}	1.12±0.19^{a}
B20N0	1.72±0.44^{a}	1.55±0.35^{a}	1.40±0.20^{b}	1.48±0.23^{b}	1.28±0.19^{a}	1.30±0.16^{a}	1.16±0.18^{a}
B0N1	1.62±0.41^{a}	1.51±0.38^{a}	1.37±0.28^{a}	1.35±0.26^{a}	1.28±0.23^{a}	1.21±0.20^{a}	1.10±0.18^{a}
B10N1	1.68±0.33^{a}	1.32±0.31^{a}	1.43±0.20^{a}	1.46±0.25^{a}	1.38±0.26^{a}	1.31±0.26^{a}	1.20±0.17^{a}
B20N1	1.67±0.39^{a}	0.15±0.33^{a}	1.39±0.23^{a}	1.42±0.24^{a}	1.37±0.23^{a}	1.34±0.20^{a}	1.25±0.26^{a}
B0N2	1.63±0.33^{a}	1.44±0.23^{a}	1.33±0.27^{a}	1.24±0.14^{a}	1.19±0.17^{a}	1.13±0.25^{a}	1.04±0.14^{a}
B10N2	1.60±0.39^{a}	1.57±0.32^{a}	1.40±0.16^{a}	1.45±0.18^{b}	1.30±0.22^{ab}	1.20±0.15^{a}	1.10±0.18^{a}
B20N2	1.82±0.43^{b}	1.52±0.30^{a}	1.46±0.28^{a}	1.47±0.29^{b}	1.38±0.22^{b}	1.38±0.26^{b}	1.24±0.23^{b}

Different letters (a, b, c, d) between lines indicate that treatment means are significantly different at *P*<0.05 according to LSD multiple-range test.

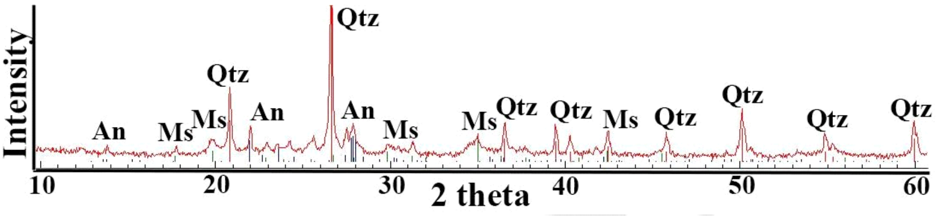

Fig. 4. X-ray powder diffraction analysis reveals the presence of soil aggregate minerals with dominant content of α-quartz (Qtz), anorthite (An), and muscovite (Ms).

aggregates than in micro-aggregates. Elliott (1986) suggested that macro-aggregates show elevated C concentrations because of the organic matter binding micro-aggregates into macro-aggregates, and because this organic matter is "qualitatively more labile and less highly processed" than the organics stabilizing micro-aggregates. In our study, the SOC in WSA significantly increased in all size classes (except >5 mm) after applying biochar at a rate of 20 t ha^{-1} compared to the B0N0 as well as B10N0 treatments. C_L in WSA significantly increased only in WSA_{ma} 3–1 mm and in B20N0 (Table 4). The application of 10 and 20 t ha^{-1} of biochar combined with both N fertilization levels significantly affected the re-distribution of SOC and C_L in WSA during the investigated period. The highest increase of SOC in WSA_{mi} and WSA_{ma} were determined in the B20N1 treatment compared to B0N1 (Table 4). A significant increase of SOC in WSA_{mi} and WSA_{ma} 5–0.5 mm in the B10N1 treatment as compared to B0N1 was observed. The same trends (increase of SOC in WSA) were observed in both application rates of biochar combined with the second N fertilization level. The significant effects were found in B20N2 for size classes of WSA_{ma} 1–0.25 and >2 mm as well as WSA_{mi}; however, in the B10N2 treatment the trend was observed only within size classes of WSA_{ma} 5–2 mm. Adding N to biochar might be responsible for intensive mineralization processes of biochar resulting in a higher increase of microbial activity in the soils (Cross et al., 2016; Jien and Wang, 2013; Liang et al., 2006) which might be related to higher contents of labile C in WSA and with enhancement aggregation (Bronick and Lal, 2005). The contents of C_L in WSA_{mi}, WSA_{ma} 0.5–0.25 mm, 1–0.5, 2–1 mm and >5 mm in the B20N2 treatment were higher by 19, 21, 16, 18 and 11%, respectively compared to the same size classes of WSA in the B0N2 treatment.

CONCLUSIONS

The results lead us to a conclusion that adding lower amounts of biochar with no N fertilizers might be more beneficial for soil structure vulnerability than higher rates of biochar. On the other hand, biochar combined with both N fertilization levels improved soil structure. Biochar facilitates the formation of macro-aggregates from micro-aggregates. This process is consequently accompanied with a lower content of water-stable micro-aggregates upon adding nitrogen. The higher content of soil organic and labile carbon in the aggregates is a result of biochar its combination with N fertilization. It can be concluded that the higher content of soil organic carbon and labile carbon delivered to the soils through biochar led to more water-stable aggregates.

The results of this study confirmed the effectiveness of biochar alone or combined with N in improving the soil structure and carbon sequestration in water-stable aggregates. In terms of sustainable agriculture, applying biochar with a nitrogen fertilizer appears to be a promising practice offering a chance to increase carbon sequestration rates.

Acknowledgement. This study was partially supported by the Slovak Research and Development Agency under the project No. APVV-15-0160, and the Scientific Grant Agency (VEGA) – project No. 1/0604/16 and 1/0136/17. Many thanks to Dr. Ľubica Puškelová from Earth Science Institute of the SAS, Slovak Academy of Sciences, Slovak republic for minerals identification using XRD analysis, and Dr. Gabriela Kratošová from VŠB- Technical University of Ostrava, Nanotechnology Centre, Czech republic for aggregate and biochar SEM and EDX analysis.

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Received 16 August 2017
Accepted 20 June 2018

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