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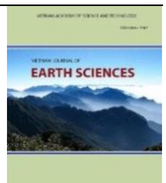

Soil structure and soil organic matter in water-stable aggregates under different application rates of biochar

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ABSTRACT

The effects of biochar and biochar combined with N-fertilizer on the content of soil organic matter in water-stable aggregates were investigated. 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), 1^{st} and 2^{nd} levels of nitrogen fertilization on silt loam Haplic Luvisol (Dolna Malanta, Slovakia), in 2014. The N doses of level 1 were calculated on required average crop production using balance method. Level 2 included additional 100% of N in year 2014 and additional 50% of N in year 2016. The effects were investigated during the growing seasons of spring barley and spring wheat in 2014 and 2016, respectively. Results indicate that the B20N2 treatment significantly increased the proportion of water-stable macro-aggregates (WSA_{ma}) and reduced water-stable micro-aggregates (WSA_{mi}). Aggregate stability increased only in the B20N1 treatment. The B20N2 treatment showed a robust decrease by 27% in the WSA_{ma} of 0.5-0.25 mm. On the other hand, an increase by 56% was observed in the content of WSA_{ma} with fractions 3-2 mm compared to the B0N0 treatment. The effect of N fertilizer on WSA_{ma} was confirmed only in the case of the B10N2 treatment. The proportion of WSA_{ma} with fractions 3-2 mm decreased by 42%, while the size fraction of 0.5-0.25 mm increased by 30% compared to the B10N0 treatment. The content of WSA_{ma} with fractions 1-0.5 mm decreased with time. On the contrary, the content of WSA_{ma} with particle sizes above 5 mm increased with time in all treatments except the B10N2 and B20N2 treatments. A statistically significant trend was identified in the proportion of WSA in the B10N2 and B20N2 treatments, which indicates that biochar with higher application levels of N fertilizer stabilizes the proportion of water-stable aggregates. In all treatments, the content of soil organic carbon (SOC) and labile carbon (C_L) in WSA_{mi} was lower than those in WSA_{ma}. A considerable decrease of SOC in the WSA_{ma} >5 mm and an increase of SOC in WSA_{mi} were observed when biochar was applied at the rate of 10 t ha^{-1}. Contents of SOC in WSA_{mi} increased as a result of adding biochar combined with N fertilizer at first level. C_L in WSA significantly increased in all size fractions of WSA.

Keywords: soil structure; soil organic carbon; labile carbon; aggregate stability; biochar; N fertilizer.

1. Introduction

Soil structure depends on the organization of mineral and organic particles with an active involvement of microorganisms and soil fauna (Bronick and Lal, 2005; Six et al., 2004). Soil aggregates are the key elements of soil structure. They play an important role in the accumulation and protection of soil organic matter

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(SOM), the optimization of soil water and air regimes, and the storage and availability of plant nutrients (Von Lutzow et al., 2006). Soil aggregates are also the basic units of soil structure (Lynch and Bragg, 1985). From the agronomical point of view, water-stable micro-aggregates and mainly macro-aggregates are essential.

One of the most important characteristics of soil aggregates is their stability. Aggregate stability refers to the ability of soil aggregates to resist disruption induced by external forces (Hiller, 1982). Aggregate stability is often regarded as a reflection of soil structure and soil health, because it depends on the balance between chemical, physical, and biological factors (Bronick and Lal, 2005; Brevik et al., 2015). Aggregate stability is affected by soil intrinsic factors such as the strength of electrolytes, types of exchangeable cations (Paradelo et al., 2013), type and abundance of clay minerals (Bronic and Lal, 2005), content of carbonates (Vaezi et al., 2008), SOM (Saha et al., 2011; Simansky and Jonczak, 2016), and geochemical barriers such as Fe, Mn and Al oxides and hydroxides (Barthes et al., 2008). All of these factors depend on the climate conditions, soil formation processes (wet-dry and freeze-thaw cycles), biological factors and soil management practices (Balashov and Buchkina, 2011; Kurakov and Kharin, 2012). It has been already observed that aggregate stability increases with the content of SOM (Kodesova et al., 2015; Simansky and Jonczak, 2016). Soil aggregates are of particular importance for processes of soil carbon sequestration (Chenu and Plante, 2006; Six et al., 2000).

Soil management plays an important role in the formation of soil structure (Balashov and Buchkina, 2011). It is already well known that soil management practices influence the content of SOM (Simon et al., 2009), which is one of the essential factors in WSA formation (Krol et al., 2013). Over the last decade, biochars have been in the focus of agricultural research due to their positive effects on soil pH (Jeffery et al., 2011). Since biochar has the surface-to-volume ratio with the high specific surface area (Glaser et al., 2002), nutrient regimes in soils are usually improved after its application. Applied biochar improves soil physical properties such as retention water capacity, total porosity (Kammanm et al., 2011) and soil structure (Barrow, 2012). Biochars associate with soil particles resulting in stable soil aggregates with favorable structure (Jien and Wang, 2013). Biochar is a stable source of organic carbon (Fischer and Glaser, 2012). Applying biochar into soil can also immobilize P, Ca and N nutrients (Rees et al., 2015). Therefore, incorporating biochar into soils requires that other organic and mineral fertilizers are artificially supplemented.

As for agriculture sustainability, combining biochar with a N fertilizer appears to be a promising practice offering a possibility of higher carbon sequestration rates. Since the interaction between biochar, mineral fertilizer and soil is a complex process, additional research is necessary.

The objectives of this study were to (i) quantify the effects of biochar and biochar in combination with N fertilizer on the soil structure parameters, the proportion of water-stable aggregates (WSA) and SOM in WSA, and (ii) evaluate the dynamic changes of proportion of WSA and SOM in aggregates in relation with doses of biochar and biochar with N fertilizer.

2. Material and Methods

Description of study site

The field experiments were conducted at the experimental site of the Slovak University of Agriculture Nitra, Dolna Malanta Nitra (48°19′00″N; 18°09′00″E). The site has a temperate climate, with a mean annual air temperature of 9.8°C, and the mean annual precipitation is 540 mm. The geological substratum consists of little bedrock materials such as biotite, quartz, diorite, triassic quartzites with phyllite horizonts, crinoid limestones and sandy limestone with high quantities of fine materials. The young Neogene deposits consist of various clays, loams and sand gravels on which loess was deposited during the

Pleistocene epoch. The soil at this site is classified as Haplic Luvisol according to the Soil Taxonomy (WRB, 2014). The soil has 9.13 g kg^{-1} of soil organic carbon, pH is 5.71 and the texture is silt loam (sand: 15.2%, silt: 59.9% and clay: 24.9%).

Experimental design and field management

The soil had been cultivated for over 100 years classic conventional agriculture techniques before the experiment. The experiment was established in March 2014 and experimental field is shown in Figure 1. As is shown in Table 1 the experiment consisted of seven treatments. The study was set up in the field research station as a total of 21 plots each with an area of 24 m^2 (4 m × 6 m). Each set of seven plots was arranged in a row and treated as a replication, and the interval between neighboring replications was 0.5 m. To maintain consistency, ploughing and mixing treatments were also performed in control plots where no biochar and N fertilizer were applied. A standard N fertilizer (Calc-Ammonium nitrate with dolomite, LAD 27) was used in this experiment. The doses of the level 1 were calculated on required average crop production using balance method. The level 2 included additional 100% of N in the year 2014 and additional 50% of N in the year 2016. The biochar used in this study was acquired from 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 has particle sizes between 1 to 5 mm. On average, it 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 sample is 53.1 %, while the total N content is 1.4 %, the C:N ratio is 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 spring barley (*Hordeum vulgare* L.) and spring wheat (*Triticum aestivum* L.) were sown in 2014 and 2016, respectively.

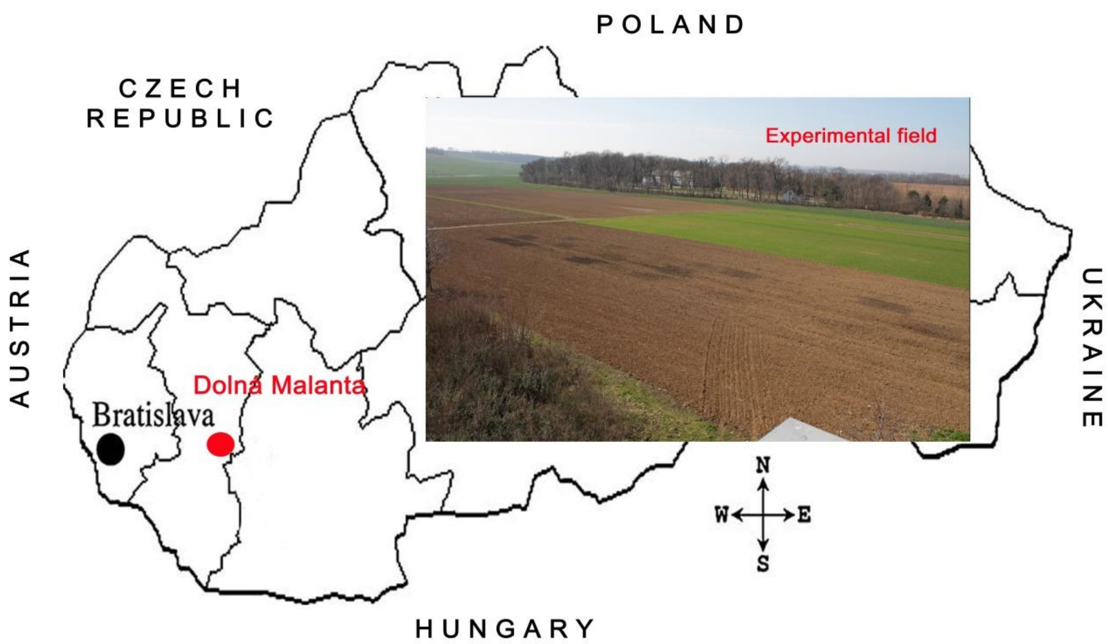

Figure 1. Field site location and an areal view of experimental plots

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}
B10N1	biochar at rate of 10 t ha^{-1} with N: dose of N were, 40 and 100 kg N ha^{-1} in 2014 and 2016, respectively
B20N1	biochar at rate of 20 t ha^{-1} with N: dose of N were, 40 and 100 kg N ha^{-1} in 2014 and 2016, respectively
B10N2	biochar at rate of 10 t ha^{-1} with N: dose of N were, 80 and 150 kg N ha^{-1} in 2014 and 2016, respectively
B20N2	biochar at rate of 20 t ha^{-1} with N: dose of N were, 80 and 150 kg N ha^{-1} in 2014 and 2016, respectively

Sampling and measurements

Soil samples were collected from the topsoil (0-20 cm) in all treatments. Sampling of soil was conducted monthly to cover the whole growing season of spring barley (sampling dates: 17 April, 15 May, 16 June, and 13 July in 2014) as well as in 2016 to cover the whole spring growing season of wheat (sampling dates: on 20 April, 17 May, 22 June, and 18 July). Thus, for the 2014 treatments, sampling was conducted at one, two, three and four months after biochar application, while for the 2016 treatments, sampling was conducted at 26, 27, 28 and 29 months after biochar application.

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. The collected soil samples were transported to the laboratory and large clods were gently broken up along natural fracture lines. The samples were air-dried at laboratory temperature 20°C to obtain undisturbed soil samples. We used the Baksheev method (Vadjunina and Korchagina, 1986) to determine the water-stable aggregates (WSA). The soil organic carbon (SOC) and the labile carbon (C_L) were analyzed in all fraction sizes of the WSA (Loginow et al., 1987; Dziadowiec and Gonet, 1999). The indexes of aggregate stability (S_w), mean weight diameters of aggregates for dry (MWD_d) and wet sieving (MWD_W), as well as vulnerability coefficient (K_v) were calculated according to following equations (1-4):

$$Sw = \frac{WSA - 0.09sand}{silt + clay} \quad (1)$$

where: S_w denotes aggregate stability and WSA is the content of water-stable aggregates (%).

$$MWD_d = \sum_{i-1}^{n} x_i w_i \quad (2)$$

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.

$$MWD_W = \sum_{i-1}^{n} x_i WSA \quad (3)$$

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

$$K_v = \frac{MWD_d}{MWD_w} \quad (4)$$

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 WSA (mm).

Statistics

The data was analyzed by ANOVA tests using a software package Statgraphics Centurion XV.I (Statpoint Technologies, Inc., USA). Comparisons were made using the method of least significant differences (LSD) at the probability level P = 0.05. The Mann-Kendall test was used to evaluate the trends in the proportions of WSA and the contents of SOC and C_L in the WSA.

3. Results and discussion

Proportion of water-stable aggregates and soil structure parameters

Parameters of soil structure such as MWD_w, K_v, S_w, as well as WSA_{ma} and WSA_{mi} as a result of biochar amendment are shown in Table 2. Our findings confirm the results of Atkinson et al. (2010) i.e. biochar exerted positive effects on soil structure. However, the effects of biochar on soil structure largely depend on the properties of biochar that may vary considerably due to the variations in feedstock materials, pyrolysis conditions, etc. (Purakayastha et al. 2015; Heitkötter and Marschner 2015). In our case, the proportion of WSA_{ma} decreased in the following order: B20N2 > B10N0 > B20N1 > B20N0 > B10N1 > B0N0 > B10N2. The index of aggregate stability increased in the following order: B10N2 < B0N0 < B10N1 = B20N1 < B10N0 = B20N0 <B20N2. The one-way ANOVA test did not show any significant differences between the treatments in terms of K_v and MWD_w (Table 2). Compared to the B0N0, only the B20N2 treatment significantly increased the proportion of WSA_{ma} and reduced the proportion of WSA_{mi}. Furthermore, our results suggest that biochar did not enhance the formation of WSA_{mi}, since the particle sizes of the biochar were within the range of 1 to 5 mm. These findings agree with those of Herath et al. (2013) who also observed that biochar applied after 295 days of incubation did not enhance the formation of micro-aggregates. Brodowski et al. (2006) stated that incorporation of biochar into soil contributes to the formation of micro-aggregates. Generally, organic amendments added to soil are accompanied with an increase in microbially-produced polysaccharides (Angers et al., 1993), especially those from fungi (Tiessen and Stewart, 1988) which can increase the stability of aggregates and the content of WSA_{ma} (Herath et al., 2013; Soinne et al., 2014). In our study, a statistically significant effect on S_w was observed in the treatment with 20 t biochar ha^{-1} combined with 2nd level of N fertilization. The reasons for a higher aggregate stability can be explained by the application of higher doses of biochar together with nitrogen. Fertilizer application generally improves soil aggregation (Munkholm et al., 2002). An improved nutrient management increases biomass and enhances the growth of roots and their activity (Abiven et al., 2015). The increased aggregate stability can be explained by the enhanced root activity and the direct effect of biochar acting as a binding agent of soil particles (Brodowski et al., 2006). The higher root biomass through exudates and moving soil particles help aggregate formation (Bronick and Lal, 2005).

The effects of various rates of biochar and biochar with various levels of N fertilizer on the individual size fractions of the WSA_{ma} are shown in Table 3. The B20N2 treatment showed a robust decrease (by 27%) in WSA_{ma} between 0.5 and 0.25 mm, but on the other hand, the content of WSA_{ma} with particle sizes between 3 and 2 mm increased by 56% compared to B0N0. Formation of soil aggregates is a function of biological activity and time, and it is unlikely to occur immediately upon biochar application (Herath et al., 2013).

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

Treatments	%WSA_{ma}	%WSA_{mi}	Sw	Kv	MWDd	MWD_W
B0N0	72.0±6.78^{ab}	28.6±6.78^{bc}	0.82±0.08^{a}	4.29±0.90^{ab}	2.97±0.69^{a}	0.72±0.21^{ab}
B10N0	75.6±9.70^{abc}	24.4±9.70^{abc}	0.88±0.11^{ab}	3.33±0.62^{a}	2.90±0.37^{a}	0.90±0.24^{b}
B20N0	75.4±10.5^{abc}	24.6±10.5^{abc}	0.88±0.12^{ab}	3.99±1.92^{ab}	2.85 ±0.13^{a}	0.87±0.38^{ab}
B10N1	75.2±6.54^{abc}	24.8±6.54^{abc}	0.87±0.08^{ab}	3.48±0.90^{a}	3.07±0.43^{a}	0.94 ±0.28^{b}
B20N1	76.3±8.68^{bc}	23.7±8.68^{ab}	0.87±0.13^{ab}	3.37±1.31^{a}	2.73±0.43^{a}	0.90±0.31^{b}
B10N2	68.0±6.93^{a}	32.0±6.93^{c}	0.79±0.08^{a}	4.75±1.68^{b}	2.74 ±0.48^{a}	0.62 ±0.17^{a}
B20N2	80.3±7.40^{c}	19.7±7.40^{a}	0.93±0.09^{b}	3.05±0.69^{a}	2.88±0.43^{a}	0.98±0.25^{b}

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

Table 3. Percentage contents of individual size fraction of water-stable macro-aggregates (mean and standard deviation)

Treatments	Individual size fractions of water-stable macro-aggregates in mm					
	>5	5-3	3-2	2-1	1-0.5	0.5-0.25
B0N0	2.44±1.58^{ab}	3.81±1.23^{ab}	7.87±3.41^{ab}	15.0±7.75^{ab}	25.5±5.03^{a}	17.5±4.60^{bc}
B10N0	3.62±1.22^{ab}	5.91±2.10^{ab}	11.0±4.23^{bc}	17.4±7.35^{ab}	22.5±3.52^{a}	15.2±3.90^{ab}
B20N0	3.19±1.02^{ab}	5.42±1.98^{ab}	11.1±5.89^{bc}	16.3±7.16^{ab}	23.9±5.14^{a}	15.5±4.87^{abc}
B10N1	4.70±1.30^{b}	6.16±3.16^{ab}	10.5±4.16^{abc}	15.7±5.25^{ab}	21.0±3.31^{a}	17.2±5.65^{bc}
B20N1	4.10±1.13^{ab}	4.50±1.35^{ab}	10.6±4.35^{abc}	19.3±7.65^{b}	23.4±5.32^{a}	14.3±3.12^{ab}
B10N2	2.06±0.93^{a}	3.29±1.09^{a}	6.34±3.29^{a}	11.7±7.30^{a}	24.9±4.95^{a}	19.7±4.19^{c}
B20N2	3.421.23^{ab}	6.66±2.39^{b}	12.3±4.64^{c}	21.8±7.13^{b}	23.5±5.58^{a}	12.7±2.80^{a}

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

The biochar in our experiments has rather coarse particle sizes with diameters ranging from 1 to 5 mm, which may pose limitations to the soil-microbe-biochar interactions. Furthermore, the conversion to WSA_{ma} with particle sizes 0.5-0.25 mm might therefore be difficult and can happen only after a certain amount of time. Applying biochar with no N fertilization at the rates of 10 and 20 t ha^{-1} did not affect the proportion of WSA_{ma}. A combination of biochar applied at 10 t ha^{-1} with both levels of N fertilizer had no significant effect on the proportion of WSA_{ma} compared to the B0N0 treatment. The effect of N fertilizer on the WSA_{ma} was confirmed only in the case of the B10N2 treatment. The proportion of WSA_{ma} with particle sizes ranging from 3 to 2 mm decreased by 42%, and increased by 30% for the size fraction 0.5-0.25 mm compared to the B10N0 treatment. The Mann-Kendall test identified a significant trend in the WSA (Table 4). The proportion of WSA_{ma} with particle diameters of 2 to 1 mm did not change during the growing season in 2014 and 2016.

The content of WSA_{ma} with particle sizes between 1 and 0.5 mm decreased, whereas the content of WSA_{ma} with particle sizes above 5 mm increased during the investigated periods in all treatments except the B10N2 and the B20N2 treatments. The proportions of WSA_{ma} with particle sizes between 5 and 3 mm and between 3 and 2 mm increased in the B20N0, B10N1 and the B20N1 treatments over the growing seasons.

Our findings show that sole biochar and biochar with the combination of N fertilizer do not explain the changes in the WSA_{ma} with particle sizes between 2 to 1 mm. The proportion of the WSA_{ma} with larger particle sizes increased over the investigated periods. On the contrary, the proportion of the WSA with small size fractions decreased during the growing periods. A stable trend was observed in the proportion of the WSA in both the B10N2 and B20N2 treatments. This means that biochar with a higher N fertilizer content may be responsible for the stabilized proportion of WSA (Table 4).

Table 4. Dynamics of individual size fraction of water-stable aggregates and soil organic carbon and labile carbon in water-stable aggregates during investigated period (Mann-Kendall test)

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
B0N0	increased	increased	increased	stable/no trend	decreased	stable/no trend	stable/no trend
B10N0	increased	stable/no trend	decreased	stable/no trend	decreased	stable/no trend	stable/no trend
B20N0	increased	increased	increased	stable/no trend	decreased	stable/no trend	decreased
B10N1	increased	increased	increased	stable/no trend	decreased	decreased	stable/no trend
B20N1	increased	increased	increased	stable/no trend	decreased	stable/no trend	stable/no trend
B10N2	stable/no trend	stable/no trend	stable/no trend	stable/no trend	stable/no trend	stable/no trend	stable/no trend
B20N2	stable/no trend	stable/no trend	stable/no trend	stable/no trend	stable/no trend	stable/no trend	stable/no trend
Content of soil organic carbon in water-stable aggregates							
B0N0	stable/no trend	decreased	stable/no trend	stable/no trend	increased	stable/no trend	stable/no trend
B10N0	decreased	stable/no trend	stable/no trend	stable/no trend	stable/no trend	stable/no trend	increased
B20N0	stable/no trend	stable/no trend	stable/no trend	stable/no trend	stable/no trend	stable/no trend	stable/no trend
B10N1	increased	decreased	stable/no trend	stable/no trend	stable/no trend	increased	increased
B20N1	stable/no trend	stable/no trend	stable/no trend	stable/no trend	increased	increased	increased
B10N2	stable/no trend	stable/no trend	stable/no trend	stable/no trend	stable/no trend	stable/no trend	stable/no trend
B20N2	stable/no trend	stable/no trend	decreased	stable/no trend	stable/no trend	stable/no trend	stable/no trend
Content of labile carbon in water-stable aggregates							
B0N0	increased	increased	increased	increased	increased	increased	increased
B10N0	increased	increased	increased	increased	increased	increased	increased
B20N0	increased	increased	increased	increased	increased	increased	increased
B10N1	increased	increased	increased	increased	increased	increased	increased
B20N1	increased	increased	increased	increased	increased	increased	increased
B10N2	increased	increased	increased	increased	increased	increased	increased
B20N2	increased	increased	increased	increased	increased	increased	increased

Contents of soil organic matter in water-stable aggregates

Organic amendments are known to increase the content of SOC (Agegnehu et al., 2016). Soil particles tend to form aggregates accompanying with occluded biochar (Brodowski et al., 2006). This could be the main reason of the elevated C content in the aggregates (Blanco-Canqui and Lal, 2004). Results of our study showed that different rates of biochar and biochar with different levels of N fertilization affected the distribution of SOC and C_L content in aggregates (Figure 2 and 3), ranging from 8.80 to 15.8 g kg^{-1} and from 1.11 to 1.65 g kg^{-1} for biochar treatments, and from 9.70 to 15.6 g kg^{-1} and from 0.99 to 1.81 g kg^{-1} for biochar with N fertilization treatments. In all treatments, the content of SOC in WSA$_{mi}$ was lower than WSA$_{ma}$. The SOC in WSA$_{mi}$ were 10.5, 8.80, 10.6, 9.70, 11.1, 10.4 and 11.5 g kg^{-1} of SOC in the B0N0, B10N0, B20N0, B10N1, B20N1, B10N2 and B20N2 treatments, respectively. The largest size class of WSA (> 5 mm) contained the largest C$_L$ in all treatments, with 1.54, 1.54, 1.65, 1.57, 1.59, 1.66 and 1.81 g kg^{-1} of C$_L$ in the B0N0, B10N0, B20N0, B10N1, B20N1, B10N2 and B20N2 treatments, respectively, while the smallest size class of WSA (< 0.25 mm or WSA$_{mi}$) contained the lowest C$_L$ pool in all treatments (Figure 3).

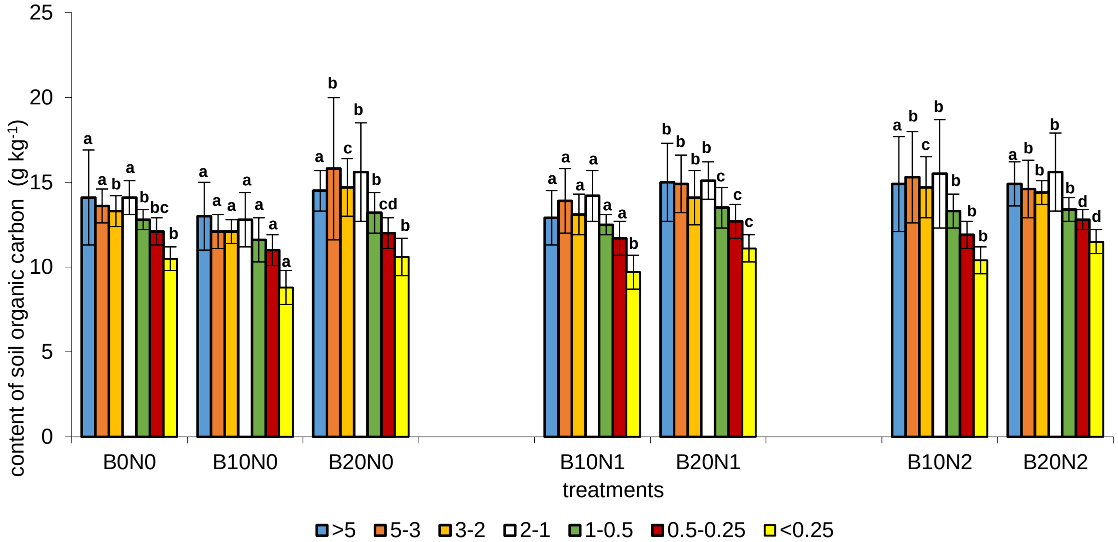

Figure 2. Contents of soil organic carbon in individual size fractions of water-stable aggregates (mean and standard deviation); Different letters (a, b, c, d) between columns (the same color) indicate that treatment means are significantly different at *P*<0.05 according to LSD test

Generally, the higher content of SOC is accompanied with a higher occurrence of WSA$_{ma}$ and WSA$_{mi}$. The importance of SOC content in the formation of aggregates is well known (Kodesova et al., 2015). In the study of Liu and Zhou (2017), macro- and micro-aggregation was significantly improved by using organic amendments. The large aggregates contained the largest pool of C in manure treatments (Simansky, 2013). Tisdall and Oades (1980) and Six et al. (2004) found higher concentrations of organic C in macro-aggregates than in micro-aggregates. Decomposition of roots and hyphae occurs within macro-aggregates. Elliott (1986) suggested that macro-aggregates have elevated C concentrations because of the organic matter binding micro-aggregates into macro-aggregates and the organic matter is "qualitatively more labile and less highly processed" than the organics stabilizing micro-aggregates. Based on Mann-Kendall test, the temporal behavior of SOC in WSA in relation to application of biochar or biochar with N fertilizer was different during the investigated period (Table 4). A considerable decrease in SOC with WSA$_{ma}$ >5 mm and an increase in SOC with WSA$_{mi}$ when 10 t ha^{-1} of biochar was applied. During the investigated period, the application of 20 t ha^{-1} of biochar as well as 10 and 20 t ha^{-1} of biochar combined with the second level of N fertilization had no effect on the redistribution of SOC in WSA. The SOC in

WSA_{mi} gradually increased after applying biochar combined with the first level of N fertilization during the investigated period. C_L in WSA significantly increased in all size fractions of WSA and in all treatments (Table 4) during the investigated period. The dynamic of C_L changes significantly due to different soil management practices (Benbi et al., 2012). Therefore, the C_L is used as a sensitive indicator of changes in SOM (Benbi et al., 2015) and aggregate stability (Simansky, 2013). As a result, the decomposition of the organic matter increases C_L, eventually enhancing aggregation (Bronick and Lal, 2005).

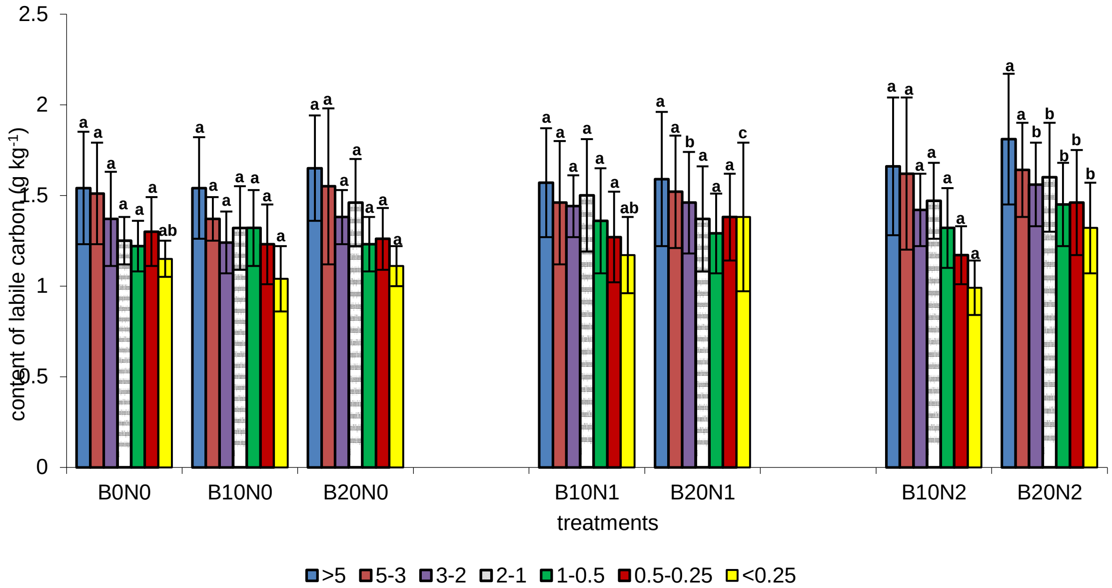

Figure 3. Contents of labile carbon in individual size fractions of water-stable aggregates (mean and standard deviation); Different letters (a, b, c, d) between columns (the same color) indicate that treatment means are significantly different at $P<0.05$ according to LSD test

4. Conclusions

Elevated doses of biochar with a higher level of N fertilizer application significantly increased the index of aggregate stability and the proportion of water-stable macro-aggregates, especially in the size fractions from 3 to 2 mm. On the other hand, less water-stable macro-aggregates within the fraction from 0.5 to 0.25 mm were observed. Application of N fertilizer at a higher level significantly decreased the proportions of water-stable macro-aggregates within the size fractions of 3-2 mm. On the contrary, increasing rates of N application increased the proportion of water-stable aggregates with sizes from 0.5 to 0.25 mm. During the investigated period, the proportion of larger macro-aggregates increased, while the proportion of smaller macro-aggregates 1-0.5 mm decreased.

Our findings show that the effect of SOM in the WSA can be significantly enhanced. Dosing biochar at higher rates resulted in a higher content of soil organic carbon and labile carbon in the WSA. It can be concluded that the higher content of SOM delivered through biochar led to more WSA_{ma} and WSA_{mi}. The temporal dynamics of C_L in WSA is more pronounced than in SOC. The content of C_L measured within all size fractions of the WSA increased in all treatments.

Water-stable aggregates are a significant pool of SOM. The rising content of C_L during decomposition of biochar enhances the aggregation processes. Our findings confirmed the fact that biochar is responsible for carbon sequestration within the WSA.

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